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FOREWORD

Soybean has been and will continue to be a major source of 'well being' of populations in different regions of the world. Soybean use has increased for purposes, including human nutrition and health, table oil, livestock feed, and many other industrial uses.

The scientific and technological developments in most regions have increased soybean production and yield, and all the sectors involved with the entire soybean production and processing chain have responded accordingly to comply with the demands of a globalized economy. Furthermore, there have been many initiatives towards soybean production systems that are also ecological and socially sustainable to counteract environmental degradation.

The many benefits of soybean, such as its use for human nutrition and health, have been widely explored in many countries in Asia, but, as a whole, have been underexplored in other world regions. The scientific and technological developments in this area and in other areas of soybean utilization have also increased exponentially in the last decade towards exploiting the full benefits of this "golden grain" and expanding the use of these benefits to a greater number of persons in many countries

This publication provides the papers presented as plenary lectures and those presented in symposia by invited speakers at the VII World Soybean Research Conference (WSRC VII), IV International Soybean Processing and Utilization Conference (ISPUC IV), and III Brazilian Soybean Congress (BSC III). Although papers related to the BSC III are in Portuguese, an abstract in English is provided for participants whose native language is not Portuguese. We hope that all the up to date information provided in this document will have a positive impact on the further improvement of soybean production, processing and utilization and that it is used and disseminated by the participants to

their different countries, for the benefit of humankind.

Flavio Moscardi
Chair, WSRC VII, ISPUC VII, and BSC III

Mercedes C.C. Panizzi
Chair, Continuing Committee for ISPUC

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PLENARY

MARCH 1

Status of the soybean industry in the North American Region

H. Kauffman[1]

Abstract

The North American region has been the leading producer of soybeans for the last half century. Although South American production surpassed North America for the first time in 2003, the United States continues to be the leading producer of soybeans and is the global center for innovation in research, training, and development. New technology that is generated in U. S. laboratories and fields is rapidly tested and implemented in many segments of the soybean industry. Many scientists in the public and private sector from the United States and other countries in the region will discuss their cutting edge research at this WSRC VII and others will share their experiences in working in various segments of the soybean industry.

Introduction

In this paper I will be giving a brief overview of the status of the soybean industry in the North American region. This region consists of the two major producing countries, the United States and Canada, both of which are in the temperate region. However, since the United States produces more than 95% of the soybeans in the region, much of the discussion will focus on the U. S. industry. Comments will be made about the tropical areas consisting of Mexico, Central America and the Caribbean where consumption of soy for feed and food is growing rapidly, but production has declined during the past decade.

Production - an overview

In the late 1800s the great prairies of the North American Midwest were found to be well adapted to soybean varieties introduced from China and other East Asian countries. As a result of excellent production conditions (good rainfall and rich soils) and high market demand, soybean production increased rapidly until the North American region became the leading global producer of soybeans during the 1950s. That leadership was maintained until early in the 21st century.

Across these vast rich prairies, soybean became a mainstay of the crop rotation system with corn (maize). During the past several decades the

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maize / soybean cropping system expanded towards the west, and north, areas that have less rainfall and shorter growing seasons. Improved genetics and innovative production practices have made this possible. Soybean production has also been established in pockets of the southern U. S. where special cropping systems have been profitable. For example in the state of Arkansas, soybean is grown in rotation with rice.

The growing of soybeans has been tried in a number of tropical areas of Mexico, Central America, and the Caribbean but yields have tended to remain low, thus making beans produced in these tropical regions less competitive than imported beans from the temperate north. For example, in Mexico since the North American Free Trade Agreement was implemented ten years ago, soybean production in Mexico declined approximately 75 percent (Table 1) while imports of soybeans from the north increased dramatically.

Table 1. Comparison of soybean production (mmt) in the North American region to that in the South American region from 1960 - 2002 (FAOSTAT)

	1960	1970	1980	1990	2000	2002
USA	18.5	30.7	48.9	52.4	75.1	74.3
Canada	0.2	0.3	0.7	1.3	2.7	2.3
Mexico	0.02	0.2	0.3	0.6	0.1	0.1
C. America and Caribbean	0.0	0.0001	0.002	0.04	0.03	0.04
N. A. Region Total	18.7	31.1	49.9	54.3	77.9	76.7
South America Total	.3	1.7	19.5	33.1	57.3	76.5

As new technology has become available, U. S. and Canadian farmers have quickly incorporated the technology into their production practices. The most recent example is the adoption of the new genetically modified roundup ready varieties beginning in 1996. The adoption rate of roundup ready varieties quickly reached more than 80 percent. During the past decade there has also been a substantial movement towards growing specialty soybean varieties to meet niche markets in the area of food and other targeted markets. The food and other specialty soybeans are identity preserved and are segregated into non-genetically modified (non-gmo) channels while most of the commodity soybeans are genetically modified.

Among some of the factors that will influence future soybean production trends in the United States and Canada are the following:

1. Level of productivity enhancement: If soybean yields can continue to increase and production costs are lowered as we go forward, total production in the region should continue to increase at a modest rate.

2. Strength of market prices: If market prices remain strong like they have been in 2003, U. S. farmers may slightly expand the acreage they devote to growing soybeans. However, should prices recede to pre 2003 levels the area gains will likely not take place.

3. Development of markets for specialty soybeans: The pace of development of markets for specialty soybeans will impact the ratio of specialty soybean production vs production of commodity soybeans. This in turn may impact overall yields of the industry as specialty varieties often have lower yields and higher production costs than commodity varieties.

4. Markets and price for maize and other crops grown in rotation with soybean: Economics plays a major role in how farmers determine how to portion out their crop rotations. Since most soybeans are grown in rotation with maize, the price of maize will continue to influence that decision. Industrial demand for maize (led by ethanol for fuel) is growing fast, thus some believe that long term maize prices will be better than soybeans so that the area devoted to maize will increase at the expense of soybean.

Marketing and processing

Historically, marketing of soybeans from the farm gate in the U. S. and Canada have been through normal commodity based channels. The construction of new modern crushing plants and the upgrading of transportation and storage systems for moving grain has been a key to keeping North American soybeans competitive on the global front. The building of modern, efficient infrastructures has also played an important role in the successful movement of soybeans into western and northern areas of the U. S. and Canadian prairies.

During the past two decades there as been a gradual growth of markets for specialty soybeans which has spawned a number of small scale processors. As specialty markets have developed, there has been a growing trend for small businesses, individual farmers, or cooperatives to process their own soybeans in extruders or in combination with cold press expellers. The trend towards segregating channels for soybeans has been accelerated since 1996 when U. S. and Canadian farmers began growing genetically modified soybeans. Identity preserved systems have been developed to handle the growing demand for specialty varieties.

In recent years there has been major consolidation of the companies involved in buying, transporting, storing, and processing soybeans in the region. Historically there was a mix of private companies and farmer owned cooperatives, but the numbers have been significantly reduced and four or five private companies now dominate a large share of the market.

Consumption and use

The crushing of soybeans for animal feed and vegetable oil has historically dominated overall usage of the crop in North America. All countries in the region have had dramatic growth in the use of soybean meal for animal feed. Thus major demand growth for animal feed has been the driving force behind soybean production increases. Consumption of soybean oil in food has also continued to increase steadily in all countries of North America (Table 2).

Table 2. Consumption (mmt) of soybean cake (animal feed) and oil (food and other uses) from 1960 - 2001 in the North American region (USDA / BICO database)

	1960	1970	1980	1990	1995	2001
USA						
feed (cake)	8.0	12.3	17.6	20.6	25.2	28.8
oil (food)	1.3	2.6	3.8	4.8	5.5	6.4
oil (other uses)	0.15	0.21	0.16	0.76	0.85	1.5
Canada:						
feed (cake)	0.39	0.62	1.1	1.3	1.7	2.3
oil (food)	0.07	0.1	0.1	0.1	0.06	0.15
Mexico, C. America & Caribbean:						
Feed (cake)	0.045	0.3	1.3	2.4	3.1	3.5
oil (food)	0.01	0.08	0.3	0.5	0.6	0.8

Today in North America there is a large and growing use of whole soybeans for direct human food. Although per capita consumption is small compared to Asia, the growing market for soy protein products and soyfoods - a combination of traditional and modern uses - is helping to create a new value-added market for soybeans beyond crushing. A variety of soy protein ingredients are used for food such as soy flour, soy protein concentrates and isolated soy proteins. Soyatech reports that soyfoods have successfully crossed over into mainstream food outlets and are now being marketed by America's largest food companies such as Kraft, Kellogg, ConArga, General Mills, Heinz, Unilever, and Dean Foods. The annual growth rate in the U. S. is more than 10% and the annual sales will soon exceed $4 billion. They project that the growth in soybeans for food use will increase annual per capita consumption of soybeans by more than 50 percent during the current decade. Soyatech estimates that the use of crushed soybeans for food ingredients is growing six times faster per year than whole soybean use (Soyatech).

Consumer products made with soybean ingredients are expanding in the U. S. A range of products are now available in the market place that include all-purpose lubricants, alternative fuels, animal care products, auto care, bar chain oils, building products, candles, cleaning products, crayons, furniture, hair care, hand cleaners, home and lawn, hydraulic fluids, paint strippers, and personal care products.

Industrial uses of soybean products are also beginning to grow in the United States. The United Soybean Board coordinates research and development in the commercial application of the following products: adhesives, agricultural adjuvants, concrete supplies, dielectric fluids, dust suppressants, fuel oil emulsifiers, hydraulic fluids, industrial cleaners, industrial lubricants, metalworking fluids, odor reduction, paint strippers, printing inks, printing supplies, and saw guide oils, diesel additives, and engine oils (United Soybean Board).

Trade

Historically more than one third of the soybeans grown in the United States have been exported to other countries. The U. S. soybean growers, working in partnership with the Foreign Agricultural Service of the USDA, have aggressively promoted U. S. soybeans exports within North America as well as to traditional markets in Europe, Asia, and other parts of the world. Traditional markets in Europe, Japan, Taiwan, and Korea have remained strong, but growth in markets to China and the North American Region has accelerated during the past few years (Table 3).

Table 3. Value ($ million) of soybean exports from the United States to selected regions in 1998 and 2002 (USDA / BICO database)

Regions / Countries	1998			2002		
	beans	meal	oil	beans	meal	oil
North America:	774	193	73	929	296	148
Canada	(20)	(165)	(8)	(137)	(209)	(50)
Mexico	(754)	(28)	(65)	(792)	(86)	(98)
C. America	42	96	32	50	97	31
Caribbean	11	70	43	45	108	58
European Union	1,534	283	12	1168	59	5
China & Hong Kong	273	160	463	996	.5	4
Japan	862	60	2	826	25	4
ASAEN region	310	210	28	471	275	5
Other	1035	564	276	1195	402	222

Research, development, and promotion

Soybean research and development in the United States and Canada is a joint effort of public and private organizations in association with various industry groups. Historically, public funding from the federal and state governments provided the largest share of research money in the United States, but recently funding from private companies and soybean growers organizations has exceeded that of public funds. There is a strong coordinated effort among the various organizations to direct research towards specific research and market goals for domestic consumers and users as well as toward foreign markets around the world.

Massive investments are being made in biotechnology research by both the private as well as the public sector. National projects are underway to determine the soybean genome and to diversity the genetic makeup of soybean varieties available to farmers. Research and development continues to focus on yield increases as well as yield stability to reduce losses caused by nematodes, insects, and diseases. Recently there has been increased attention given to combating exotic insect pests (such as. the Asian soybean aphid) that have recently appeared in the U. S. or preparing to combat foreign diseases such as rust that have not yet appeared. There is also extensive research devoted to developing new food and industrial products.

International collaboration

A number of different types of collaborative activities are directed by institutions in the United States. The USDA and many U. S. universities promote international research activities in soybean science and related areas. Soybean germplasm collection, maintenance, and evaluation, disease and insect control studies, as well as feed, food, and industrial product development are some of the more common areas addressed in these activities. Also students from many countries study for their graduate degrees at U. S. and Canadian Universities with soybean being the focus of their research.

U. S. soybean growers fund activities of the American Soybean Association which has offices in seven countries around the world. These offices promote education and collaboration trials on the use of soybeans. Growers also fund the World Initiative for Soy in Human Health (WISHH) Program which promotes the use of U.S. soy protein products in developing countries where deficiency of protein in human diets is acknowledged to be a serious problem. WISHH activities range from developing higher-protein breads that are suitable for Afghanistan relief efforts to identifying new uses for soy-fortified noodles in Indonesian school lunches. In addition, WISHH is

demonstrating the role of soy-based foods in providing essential nutrition to the millions of people affected by HIV/AIDS and much more (WISHH).

From time to time other international activities are organized by various segments of the U. S. soybean industry. For example, in 1999, the State Soybean Associations of Illinois and Iowa joined the University of Illinois to organize and host the Global Soy Forum in Chicago. The Sixth World Soybean Research Conference was part of the Global Soy Forum. As a follow up to the Global Soy Forum the University of Illinois was asked to work collaboratively with Indian and Chinese institutions to organize national soybean forums in their respective countries. These forums promoted exchanges and the strengthening of research and development in these important countries.

In the future it is hope that international collaboration can be enhanced to speed up the utilization of soybean products for the health and economic well being of human kind.

Summary

In summary, the state of the soybean industry in North America remains strong, dynamic, and proactive. Various segments of the industry will continue to aggressively promote the expansion of the role for soybean and soy products around the world throughout the 21st century.

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Current status of soybean production and utilization in Brazil

A. Dall'Agnol[1]

Commercial production

The first introduction and evaluation of soybean germoplasm in Brazil was made in the state of Bahia, at 12°S, in 1882. The germoplasm came from the United States of America (USA), was not adapted to such low latitudes and did not succeed in the area. Ten years later (1891), new varieties were introduced and tested in the state of São Paulo (SP), at 23°S, with reasonable production of hay and beans. In 1900, soybeans were tested at 30°S in Rio Grande do Sul (RS), the southernmost state of Brazil, where climatic conditions are similar to those prevalent in Southern USA, origin of the introduced varieties.

Soybean remained a marginal crop in southern Brazil until 1960 and was grown in small properties to produce hay for dairy cattle and beans to feed pigs within the properties. The first reference of commercial production in Brazil was in 1941 (Bonato, E.R and Bonato, A.L.V): an area of 651 hectares and production of 457 metric tons (MT). However, the real stimulus for large commercial production was in the mid 1950's, following a government decision to provide official incentives to grow wheat, aiming at self-sufficiency of this cereal. Soybean was equally benefited by the program, since it was the best option for a summer crop following wheat, under both a technical (a legume following a grass) and economical (use of same machinery and farm infrastructure) viewpoint.

In 1960, the soybean area was 177.000 hectares and production around 206.000 MT, with yields of less than 1.200 kg/ha. By the end of the 1960's, the area had increased to 906.000 hectares and production reached 1,056,000 MT, but no yield increase was observed during that decade. The South of Brazil, represented by the states of RS, Santa Catarina (SC), Paraná (PR) and SP claimed 99.5% of the production (respectively, 70,2%, 3.0%, 20.5% and 5.8%). Genetic material was entirely introduced from the USA.

In spite of the five fold increase in production during the 1960's, the real *boom* of national production occurred during the first half of the 1970's, consolidating soybean as the lead crop of Brazilian agriculture. Production increased from 1.5 million MT in 1970, to 12.5 million MT in 1977. The

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increment was due to the incorporation of new areas (1.3 to 7.1 million hectares), as well as to increase in yield (1.14 to 1.77 kg/ha). In 1977, production outside of the Southern Region was restricted to 7% (0.89 million MT).

The good profits obtained by farmers growing soybean in the South during the 1970's, turned cropland very expensive and unavailable in the region. Excited about the possibility of making more money by planting larger areas, many soybean growers made good profit by selling their small properties in the South and buying larger areas in the savannas (Cerrado) of central Brazil (Figure 1), where land was cheap and plenty, but unfertile. Existing varieties grown in the South were not adapted to the lower latitudes of Midwest Brazil and research organizations had to go after those pioneers, looking for the technologies they were demanding to farm the new soybean frontier. The initiative was successful, turning the cheap, unproductive and unpopulated Cerrado land, into the most important cropping area of Brazilian agriculture. Despite being chemically poor, the soils have very good physical characteristics, flat topography and good rainfall distribution during the soybean growing season.

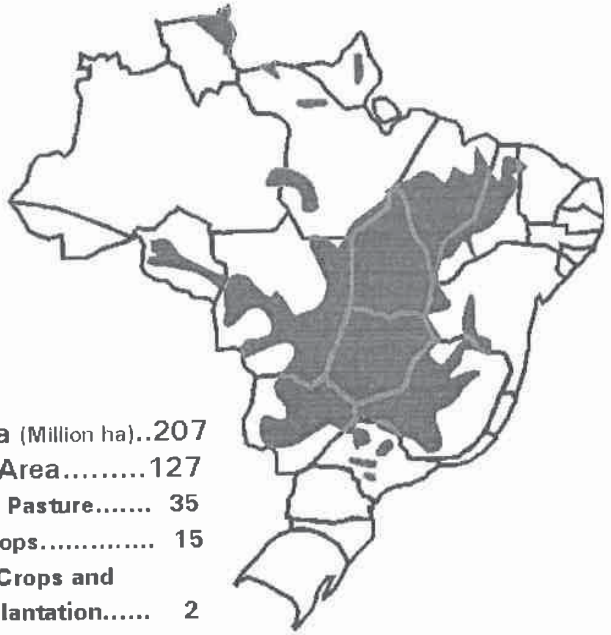


Figure 1. Brazilian Cerrados

The Cerrado region is still drawing new farmers into the area. Land is no longer cheap, but still cheaper than in the South. As a result, soybean production in Cerrado increased from 2% in 1970, to 20% in 1980, 40% in 1990, and claimed more than 60% of the 52 million MT harvested in 2003, despite the simultaneous increase in the traditional producing zone (Figure 2). Trends indicate more concentration of soybean in the Cerrados, in the future.

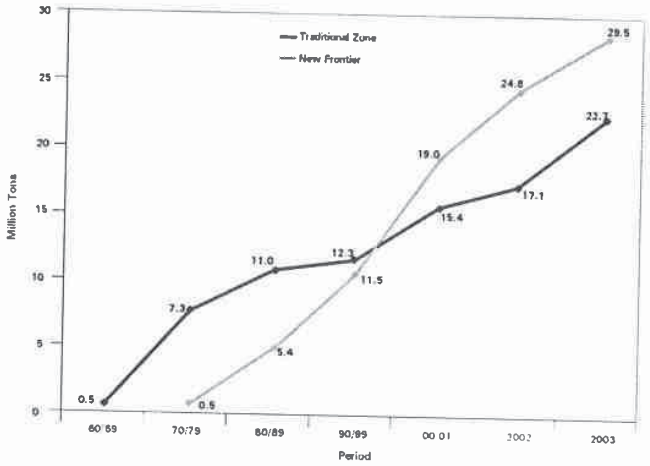


Figure 2. Evolution of soybeans in the South and Midwest Brazil

Reasons for the expansion

Several factors contributed to promote soybean expansion in the South during 1960's and 1970's and on the savannas of central Brazil from mid 1970's. Among those factors, a few deserve to be pointed out:

- similarity of the Southern USA climate and that of the Southern Brazil, enabling Southern Brazilian farmers to succeed in the establishment of soybeans in the 1950's and 1960's, using American technologies, particularly varieties;
- fiscal incentives, beginning in mid 1950's until the late 1970's, to promote wheat production, that also helped promote soybean;
- increased market demands for soybean as a protein component for animal rations and as cooking oil;

- high market prices, particularly during the early 1970's;
- establishment of an important industrial park, including processing, farm machinery and agricultural supplies;
- improvements in the transport, export and communication systems;
- construction of Brasília, the new Brazilian Capital, in the center of the Cerrados, improving the regional infrastructure, thus facilitating access and communication;
- low value of Cerrado lands, compared to prices in the South;
- development of proper technologies for the Cerrados, particularly varieties adapted to low latitudes; and,
- good economic and technology levels of farmers migrating from the South to central Brazil, as well as the previously mentioned good soil physical characteristics, flat topography and rainfall regime.

Soybean processing

In 1977, Brazilian soybean processing capacity was 12 million MT, similar to the Country's production. Crushing plants were concentrated in South Brazil (RS, SC, PR, SP). Also, small plants, processing up to 600t/day were responsible for more than half of the crushed beans. Five years later (1982), following government incentives given to the processing industry, capacity increased to 27 million MT and more than 70% of the beans were processed by plants larger than 600t/day (Figure 3). The Country's present crushing capacity is around 40 million MT, but beans processed do not exceed 30 million MT and around 35% is being crushed in plants located in the new soybean frontier.

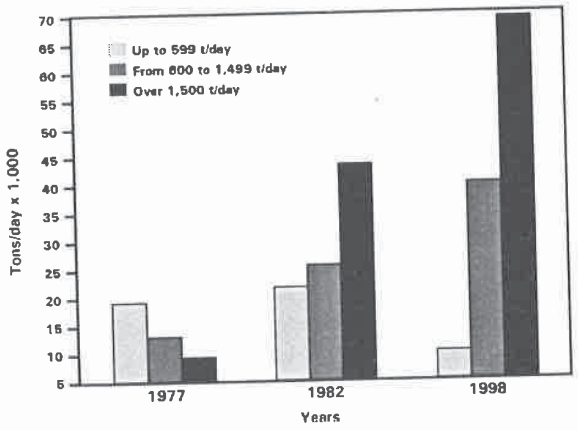


Figure 3. Evolution of processing capacity by plant size

Soybean impacts in Brazil

The explosive increment of soybean production since 1960, of about 260 times in four decades (Figure 4), determined paramount positive transformations in the Brazilian agribusiness. Soybean was responsible for or was a main driving force in the development of commercial agriculture in the Country. They, also, accelerated farm mechanization, increased technification of other crops (mainly corn), supported modernization of ports and transport systems, expanded the international trade and the agricultural frontier, supported Brazilian authorities in the effort to move population to the West, as well as decentralized the agroindustry, sponsoring the expansion of poultry and swine industries.

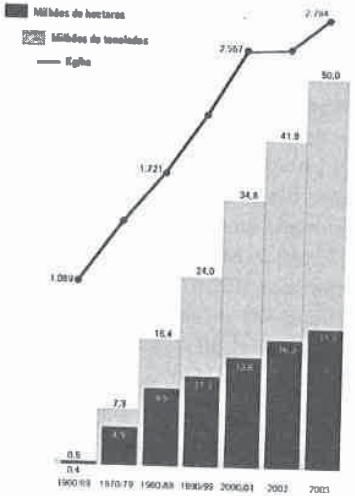
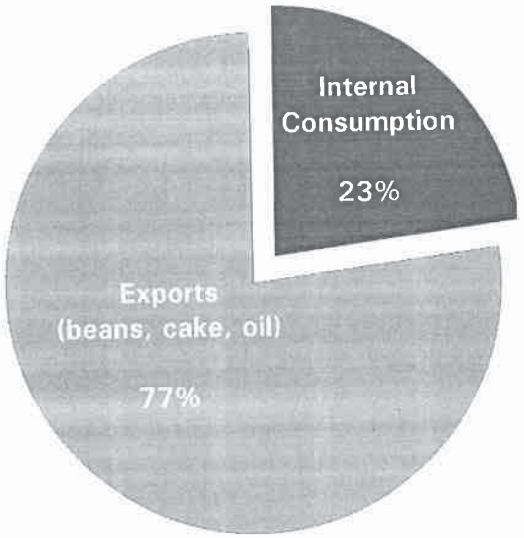


Figure 4. Evolution of soybean production in Brazil

Total exports of beans (22 million MT), cake (14,5 million MT) and oil (2,4 million MT) represented 77% of soybean produced in Brazil in 2003 (Figure 5), responsible for US$ 8.2 billions of direct external revenues (Figure 6) and 12% of total Brazilian income from exports (Figure 7). Along the last 30 years, as the leading crop of Brazilian agriculture, soybean commanded the implementation of a new civilization in Midwest Brazil, carrying the progress and the development to the Cerrados, thought to be wastelands before soybean development took over the area. The regional economy is based on the soybean complex, that greatly improved regional infrastructure. Mato Grosso, presently the leading soybean producing state in Brazil (13,5 million MT), was a marginal producer 23 years ago (117,000 MT in 1980).



Source: Abiove, Conab

Figure 5. Total Exports of Beans, Cake and Oil

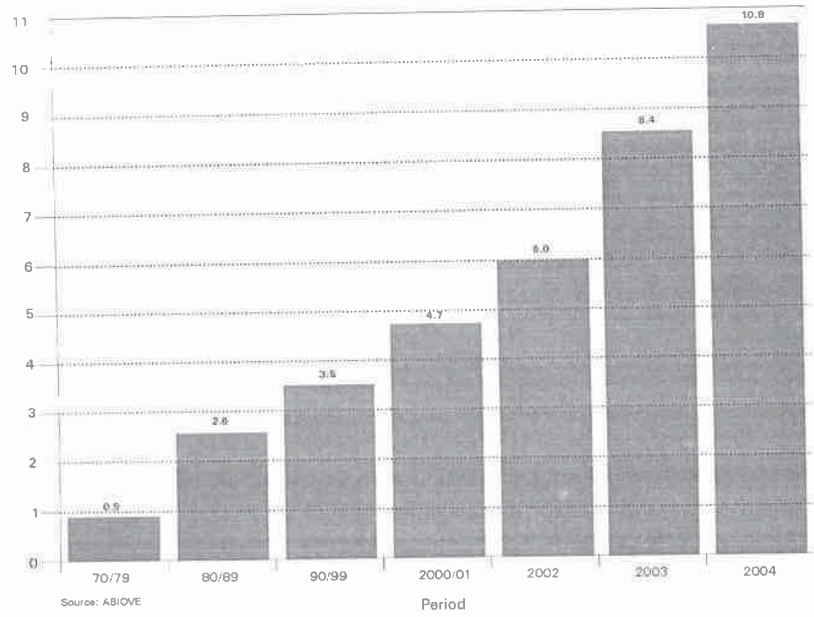


Figure 6. Soybean Complex – External Revenues in US$

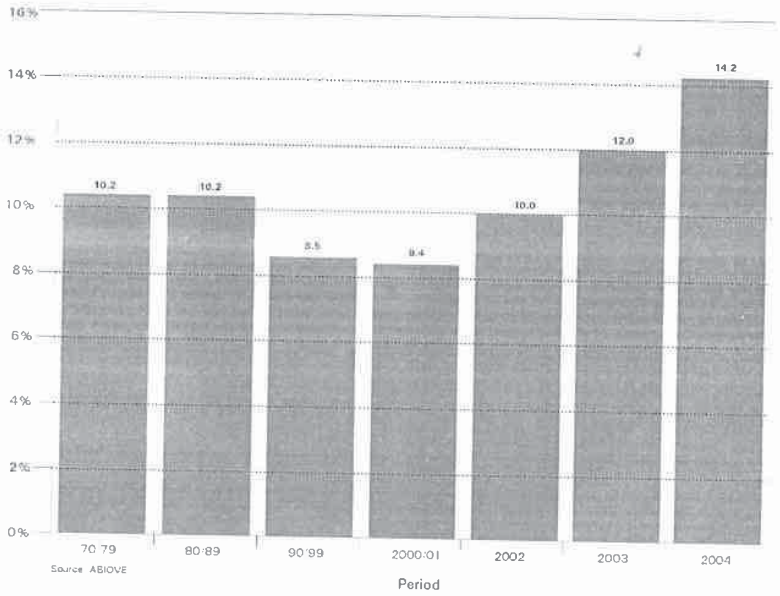


Figure 7. Soybean Complex – Participation over total Brazilian Exports

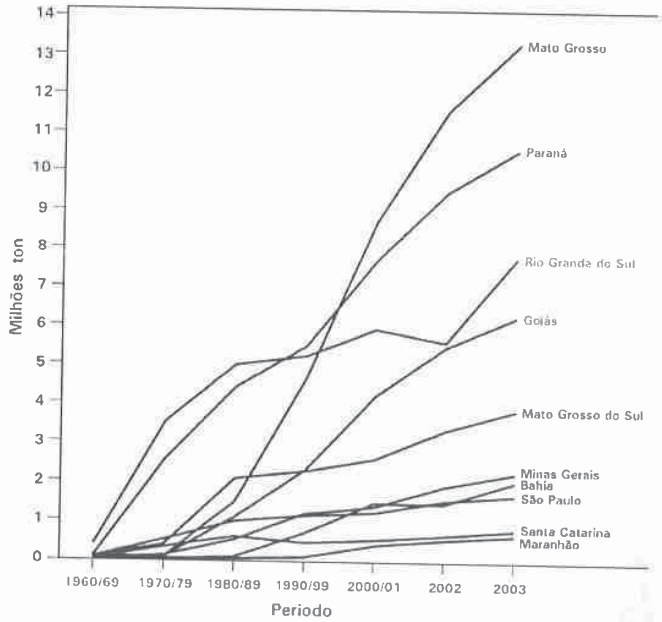


Figure 8. Evolution of soybean production by state

Perspectives of Brazilian soybeans

We see clear skies for soybean in this country, because:

- world demand and consumption will continue to expand, since human population will continue to increase;
- average income is expected to increase, particularly in Asian countries, where soybean is largely consumed;
- new industrial uses of soybean will increase global demands;
- fears to consume beef, due to the Mad Cow disease, will continue to promote the consumption of pork and chicken, fed mainly with soybean proteins;
- internal consumption in Brazil shall increase, due to the increase in poultry and swine industries, as well as to expected governmental policies promoting soybean as a human food;
- subsidies provided to growers of Developed Countries and the heavy taxes falling on the soybean chain in Brazil are expected to decrease, promoting more internal production due to better market prices, and
- new areas of major soybean producers (USA, Argentina, China and India) are restricted, giving Brazil the opportunity to supply future additional demands, by easily incorporating large uncultivated Cerrado areas, into crop lands. Less than 10% of the total of 207 million hectares of Cerrado are under cultivation with annual crops. At least 50 million hectares of wild Cerrado are suitable and available for soybean production and could be easily transformed into croplands, in case market demands so require. Keeping up with the present pace of world consumption, Brazil is expected to respond with more production, becoming the leader in soybean production by the end of the present decade.

Trends also indicate that Brazilian soybean will concentrate on more competitive large commercial operations in the Midwest, leaving to the southern small family farms the option of migrating to other profitable activities such as milk, poultry, swine, fruits and vegetables, that are ideal for smaller areas with abundant labor force, as typical of family operations, and where land is scarce.

To guaranty future competitiveness of Brazilian soybean in the global market, farmers will need to keep on investing in modern technologies to increase yields. Governmental support is also needed to reduce transportation costs, much higher than those of our competitors, due to the predominance of truck transportation (67% in Brazil and 16% in USA).

Research and development in Brazil

The increase in production and competitiveness of Brazilian soybean was always associated with scientific advances and the adaptation of modern

technologies by farmers. Soybean research was poor and concentrated in the South until the late 1960's, operating by introducing and adapting American technologies.

The rapid development of soybean began in the decade of 1960, and gave rise to an aggressive soybean producing sector, demanding technologies that research programs were not prepared to offer, thus urging government research organizations to increase research activities, particularly in the latitudes below 20°S. The establishment of Embrapa Soybean in 1975, was a result of such an effort, complemented with new public and private programs located mainly in the new soybean producing states of central Brazil.

As main achievements of soybean research programs, it is worthwhile to point out the development of soybean varieties adapted to low latitudes (up to 0°), the incorporation into commercial varieties of resistance to main soybean diseases, the implementation of an Integrated Pest Management Program, the development of biological control for the velvetbean caterpillars and stink bugs, the improvement in soybean fertilization and inoculation management, the promotion of crop rotation and no till practices (greatly supporting sustainability of production systems) and the agroclimatic zoning of the Brazilian growing regions, that indicated the more adapted cropping areas for soybean production.

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Current status of soybean production and utilization in China

R. Z. Chang[1] ; L. J. Qiu[1] and S. T. Guo[2]

As you know, soybean originated from China. It is one of the largest crops that ranked following rice, wheat and corn in China. It was domesticated from wild soybeans, and become the main source of protein for Chinese. In some sense, soybean has been fostering Chinese people. In recent five decades, soybean production cannot meet the needs of economic development, although it has been improved step by step, and now China still needs to import a great amount of soybeans every year.

Review of soybean production in China

In 1930's, soybean production in China came to the highest level and in 1936 the total yield reached to 11.3 million tons. During this period, a lot of soybeans, seed oil and bean cakes exported continuously via ports of Dalian and Yingkou. Since then, the soybean production descended because of war, the total products of soybeans only was 5.09 million tons in 1949. Soybean yield and total product kept increasing step by step since 1949(Table 1), although the planting area didn't change much. In short, soybean production started to resume in 1950's. The planting area come to 12.748 million hectares and the total products achieved 10.05 million tons in 1957. The planting area descended since the increase of population resulted in foodstuff in short supply in 1960's, and the yield and total products also kept on a low level. Profiting from the reforming and opening policy, during 1970's, although the planting area descended, the yield improved greatly, and achieved to the highest in 1998. In recent years the yield and total products steadily kept on a high level, this means that soybean production in China has stridden into a new step and has a booming foreground.

There are three main soybean production regions in China, the largest is Northeast spring sowing soybean production area, including Heilongjiang, Jinlin, Liaoning and east part of the Inner Mongolia Autonomous Region. In this region soybean was sowed in Spring (early and mid May) and harvest

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in Autumn (late September), only one crop one year. Northeast spring sowing soybean has high commodity rate. These soybeans are mainly used for oils besides protein food. Northeast spring sowing soybeans have large and round seed, light color of hilum, white flower and gray pubescence. Soybean seeds not only have excellent appearance but also have good quality and high oil. In 2000, the planting area was 4.5 million hectares, taking 48.4% of the total planting area in China, and the total product was 7.04 million tons; in 2001, both planting area and the total product were increased, which were 4.98 million hectares and 7.44 million tons, respectively.

Table 1. Area, yield and the total products in China

Year	area (million ha.)	Total products (million ton)	Yield (kg/ha)	Year	area (million ha.)	Total products (million ton)	Yield (kg/ha)
1949	831.67	510.0	615	1998	850.00	1515.2	1782
1950	960.20	745.0	780	1999	796.00	1425.0	1795
1960	934.80	640.0	690	2000	930.00	1540.9	1699
1970	835.60	920.0	1110	2001	900.00	1530.0	1700
1980	722.60	795.0	1110	2002		1650.0	
1990	755.96	1110.0	1470	2003			

The second production region is Huanghuai summer sowing soybean production region, including Heibei, Henan, Shangdong, the northern part of Jiangsu , Anhui and the southern part of Shaanxi and Shanxi. In this area soybean was sowed in early and middle June after winter wheat, and harvest from the end of September to early October, then plant wheat again. Soybeans in the northern part of this region contain high oils and in the southern contain high protein. Huanghui summer sowing soybeans have elliptic seed and brown hilum. Soybeans with high protein are often utilized to process foodstuff and with high oil are utilized to squeeze. At one time, the planting area of Huanghui summer sowing soybeans exceeded 6 million hectares. But the planting area decreased for the development of summer sowing corn and peanut. The planting area was 2.9 million hectares and the total production was 5 million ton in both 2000 and 2001.

The third production area is multi-harvesting area locating on the south of Changjiang River, including all provinces of south China. Soybean in each of these provinces, Hubei, Hunan and Guangxi have been planted over 0.2 million hectares. It is always cultivated in upland especially in hilly area because rice is the major crop in this area. Since the long growing time and complex cultivate system, soybeans can be sowed in spring, summer and autumn. Southern spring sowing soybeans are sowed from late March to early April, and harvested from the end of June to middle of July. Since soybeans mature under high temperature and humid weather, its seeds usually tolerate to high temperature and humid through artificial and natural

selection. However when northeast spring sowing soybeans are introduced to in this area, the vitality of seeds decreases and the rate of sick seeds or shriveled seeds increase. Southern summer sowing soybeans are planted after winter wheat or rapeseed, and will be harvested in October. Southern autumn sowing soybean is planted from late July to early August after early rice, and are harvested in middle of November. Since autumn sowing soybeans are sowing under high temperature, seeds need to have higher vitality and only cover with a little of soil after sowing. The soybeans in this area contain high protein. Southern soybean planting area is about 1.5 million hectares and the soybeans are processed as foodstuff.

Improvement of cultivars and development of cultural technology for soybeans in China

Improvement of cultivars of soybean in China started in 1913 when Gongzhuling Agricultural Experimental Station in Jilin Provance was founded. The researchers in this station collected some landraces of soybean in northeast region. By directly utilizing these materials and pure line selection, a number of cultivars were developed, such as Huangbaozhu, Fengdihuang, Xiaojinghuang1 and so on. Artificial crossing in 1930s also had developed some cultivars, such as Mancangjin, Mandijin and Huangjinbao. Among these cultivars, Mancangjin were planted in larger area than the other cultivars. Its planting acreage exceeds 1.0 million hectares a year. In the South, Prof. Wang Sui at Jinling University developed the cultivar 'Jinda 332'.

Since 1949, soybeans breeding developed rapidly. Up to now, more than 800 cultivars have been developed and have been utilized in production. The distribution area of each of the cultivars including Dongnong4, Jilin 3, Tiefeng 18, Yuejin 5, Ludou 4, Hefeng 25 and Aijiaozao kept several hundred thousands hectares, and some cultivars were planted even exceeds millions of hectares. For example, the accumulative total acreage of Hefeng25 has exceeded 10 million hectares.

A total of 76 cultivars have been released by national uniform test since 1984. These cultivars spread in the northeast, Huanghuai and south production area. These cultivars have the characters of resist main diseases, high yield and strong adaptability. Moreover, some cultivars have good quality. For example, the oil content of Jindou19 — a cultivar with high oils exceeds 23%, the oils of other cultivars such as Heinong 26 and Jinlin 3 is also very high. Besides, there are some high protein cultivars over 45% such as Heinong 35, Zhechun 2, Tongnong 10, Jidou 12 and so on.

As to disease resistance, most of the new cultivars are resistant to soybean mosaic virus. Northeast soybean cultivars are resistant to frogeye leaf spot.

Some cultivars resistant to soybean cyst nematode, drought and salt tolerance are also have developed.

During the early years of 21th century, soybean breeders pay much attention to developing cultivars with good quality and make great progress. Some cultivars with high oil or high protein have been released for the production. Oil content of those cultivars exceed 22%, such as Dongnong 46, Hefeng 42, Heinong 44, Jiyu 67, Jiyu 68, Liaodou 11, Jihuang 13, Zhonghuang 20 etc. Cultivars with high protein are Yudou 25, Zheng 92116, Qihuang 26, Tongnong 13, Heisheng 101 and so on. Some cultivars have developed, which are absent in lipoxygenase or trypsin inhibit, or high isoflavone.

On cultural technology, Planting and management developed from rough cultivation to refined cultivation and mechanized level is advanced step by step. Now we mostly use machine to sow and more and more farmers use machine to harvest. Each region has their cultural model respectively, for example "the ridge three"— planting technology on Northeast region. The mechanic working condition of this technology reserves water and enhances temperature by loosing soil deeply, layered fertilizing and refined sowing. The soybean yield increased more than 20% through chemical weeding and increasing fertilizer. In addition, the yield of soybean also increased 20% by using dwarf cultivars and dense planting on ridge and narrow row.

Soybean processing and utilization

With the whole development of the national economy, the industrial chain of soybean is continually extending. The soybean products are changing from the traditional rough soybean oil and meal (feed) into products with high supplementary value, such as grease, feed, food, functional food and medicine. The soybean processing industry has completed a historic conversion from the pattern of workshop into modernization. The main usages of soybean are for processing oil and meal. In 2001, the actual processing ability of soybean is only about 21 million ton in China. But in late 2002, there are 49 oil factories that can squeeze oils more than thousand tons each day and their processing capacity exceeds 25.86 million tons each year. Including both small and moderate size factories, the total processing ability can reach to 38.1 million tons one year. During recent years, the distribution of soybean processing enterprises transfer from the main producing region to dominantly consumptive area located at the harbors along the coast. The soybean processing scale becomes more and more larger and modernized. In the decade of 1990, most enterprises have processing capacity lower 1000 tons/d, but now there are 72 companies whose processing capacity exceeds 1000 tons/d, and 28 companies exceeds 2000 tons/d, 13 companies exceeds 3000 tons/d, 2 companies' processing

capability even exceeds 7000 tons/d. In 2003, the whole soybean processing capacity nearly reaches up to 50 million tons. The large enterprises not only adapt the advanced equipments for oiliness and extractor, but also make diverse products. For example, the lower temperature degrease soybean meal, high temperature metamorphic soybean meal, salad oil, diversified consonance oil and phospholipid meet the need for feed, food processing, health food and medicine. Furthermore, the enterprises take the modern strategies for selling.

In resent years, the consumption of soybean increased 0.15 million tons/ year and now the consumption each year is 7-8million tons. China devoted a great deal of finance on developing novel soybean food, such as soy milk, soy milk meal, separating protein, condensing protein and tissue protein, and processing technical of functional food, such as phospholipid, saponin, isoflavone, edible fiber etc. Both application fields and soybean processing chain are extremely extended.

The types and consumption amount of soymilk meal are continuously increased in recent years. The companies for soymilk meal production mainly distribute in soybean planting region in the past, but now there are a total of 30 soymilk meal companies scattering all over the country.

The production of isolating protein and condensing protein steadily increased since 1980, when the extraction technology was introduced from abroad. There had more than 30 productive lines and 0.1 million tons of productive capacity in 2000. Recently the factories with 3000 tons production per year have been founded. There probable has about 40 companies producing isolation protein of soybean. The structure of products have broadened from the single gelation meat to many kinds of emulsification and solvent products, such as meat, milk, flour, instant, fast-frozen, candy and drink. The extraction of components with high additional value, such as phospholipid, lower polysaccharide and isoflavone, has been industrialized, and the productions can be seen in the market as healthy food.

Except above new modern products, some traditional soybean foods such as 'tofu', originated from China, has laid down packing and sanitation rules for quality examination.

General situation about import and export of soybean in China

Soybeans were once traditional export commodity in China. The total amount of exporting soybean was 0.9 ~ 1.1 million tons from 1949 to 1960. After this period, the amount of export continuously decreased. From 1961-

1975,the total amount f export decreased to 0.4-0.6 million tons, and from 1976-1983, the amount of export only was 0.1-0.3 million tons, but from 1984 to 1994 the exports kept at 1.066 million tons every year and 1.714 million tons in 1987, because in this period, the yields and total soybean product has been greatly increased.

In 1996, China became the net importing country (Table 2). And since then the amount of import of soybean increased rapidly. Now China has become the largest soybean importing country. From 2000 the amount of import keeps over 10 million tons every year and be expected to exceed 18 million tons in 2003. The rapidly increase of import result from the increasing need for feeding protein firstly. At present, China can process feed 100 million tons and needs soybean meal 20 million tons. In this way, we need about 30 million tons of soybeans each year. Now 16 million tons soybeans can be produced in China, therefore 14 million tons of soybeans are still be needed. The second reason is that the ability of soybean processing increased very fast and now has exceeded 50 million tons. Therefore processing industries need much material and accelerate the soybean-import.

Table 2. Import and export of soybean in China

Year	area (million ha.)	Total products (million ton)	Yield (kg/ha)	Year	area (million ha.)	Total products (million ton)	Yield (kg/ha)
1949	831.67	510.0	615	1998	850.00	1515.2	1782
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1990	755.96	1110.0	1470	2003			

Prospect of soybean production in China

In 21th century, soybean production in China will make progress. The planting area will keep at 9 million hectares and the total production remains 16 million tons. In order to develop soybean in China, it is needed to stabilize planting area, increasing yield, decreasing cost and improving quality. At the same time un-transgenic soybeans should be produced to meet the need of foodstuff, processing and exporting, especially export of organic soybean or 'green soybean'. For example, Hulunbeier City in Inner Mongolia have exported 45000 tons green soybean.

Now soybean yield in China is only about 1700kg/ha, so there still is a big potential in increasing yield. By popularizing new cultivars, spreading high

yield cultural technology and necessary economic and substance investment, Chinese soybean yield would come to the average level of the world, and the total production in China will exceed 20 million tons. If the yield increase up to 2700～2800kg/ha, the supply of native soybean would greatly increase and import would decrease accordingly, but China still need to import about 10 million tons soybeans.

Current status of soybean in production and utilization in India

P. S. Bhatnagar[1] and O.P. Joshi[2]

Introduction

Ancient records of soybean cultivation in Indian subcontinent dates back to 1000 A. D. Black seeded soybeans locally referred *as Bhat, Bhatmas, Kalitur* etc were being cultivated in Himalayan foot hills (Kumaon and Ghadwal), Sikkim, Manipur Naga hills, Khasi hills and scattered pockets of central India (Anon1956, Hymowitz and Kaizuma, 1981). The resurrection of soybeans began with reactivated interest in soybeans in 1963 at G.B. Pant University of Agriculture and Technology, Pantnagar (Uttaranchal). and J. N. Agricultural University, Jabalpur, (M.P.) Trials conducted with yellow seeded varieties from North America, at these Agricultural Universities during the late sixties established agricultural feasibility and economic viability of soybeans in India with yield levels up to 3.0 to 3.5 tons /ha. The reduction in the maturity period from 134 - 139 days in the U.S to 100 - 120 days. was of significance to fit in the cropping systems prevalent in India.

Realizing the potential of soybeans in alleviating oil and protein shortages in India, the Indian Council of Agricultural Research (ICAR) launched an inter-disciplinary, multilocation All India Coordinated Research Project on Soybean (AICRPS) in 1967. Subsequentl, for providing centralized support to production systems research with basic technology and breeding material,. National Research Centre for soybean (NRCS) was established by ICAR at Indore, (M.P.) in 1987. The research efforts in the country resulted identification / release of 80 improved varieties and location specific appropriate production technology.

With the establishment of first soybean processing plant in 1972 at Indore, there had been virtually race in creation of processing factories. With the installed capacity of over 17.4 mt per annum, the area (5.99 m ha.) and production (4.99 m t) of soybean in 2002 was left way behind (Fig.1). This situation has led the Indian Soybean sector at the cross road to warrant concerted efforts by all concerned for exploiting production potential, estimated at about 12 m tons, by vertical as well as horizontal increase.

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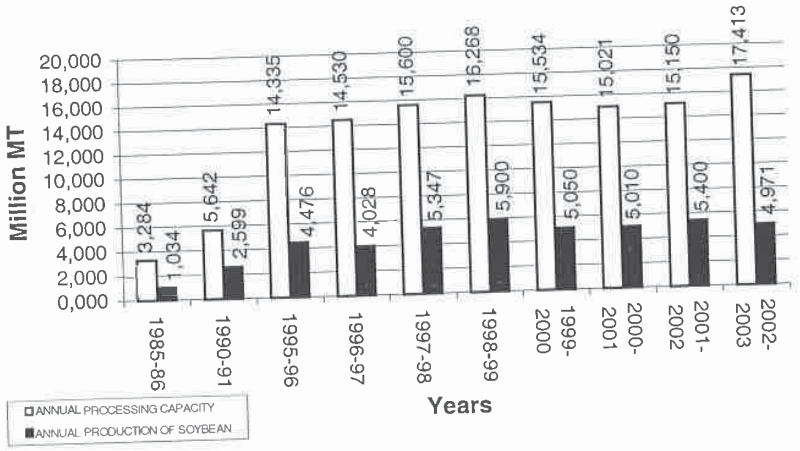


Figure 1. Growth of annual soybean production and processing capicity in India

Economic importance

Soybean farming has revolutionized rural economy of India. Coupled with the soya-based agro industry, it has generated conspicuous employment opportunities. There has been notable improvement in socio - economic status of rural folk in general and soybean farmers in particular. Malnutrition and sufferings due to squalor and poverty in villages are now notably reduced. Soybean farming resulting increased farm income has changed entire complex of the rural folk in India, specially women and children freeing many from labour intensive, low productivity farm work .

With the mission mode approach through GOI Technical Mission on Oilseeds (now pulses and maize added), soybeans have significantly contributed to the increase in oilseeds production from 11 million tons in 1986 to about 23 million tons in 1996 - 97. On the basis of average of 5 years annual production (1995 - 96 to 1999 - 2000), soybeans rank second only to groundnut among the nine conventionally grown oilseeds crops in India. At present, the crop contributes about 25% and 10% to the national oilseed and edible oil production. Soybeans have also resulted foreign exchange earnings in the tune of Rs.18, 000 million (about US $ 400 million) per annum (Fig.4). Today, soybeans have occupied important place in Rural / agricultural economy, edible oil economy and national economy of India. On the world map of soybean, India has occupied 5th place for area and production.

Growth of soybean in India

Location specific production research, coupled with development efforts and ensured market due to continuous establishment of soybean processing factories has resulted unparallel growth of soybean area and production (15%) in India (Paroda, 1999). Starting from 3,000 hectares in 1969, soybeans have demonstrated steady increase in area over the years, to 6.22 m ha in 2001 (Fig. 2).

Compound growth rate during the decade 1981-1991, for area, production and productivity of soybean was 17.89, 20.51 and 2.22, which slowed down to 6.61, 7.72 and 1.04, respectively in the next decade (1991-2001) (Mruthyunjaya and Singh, 2003). The production of soybean continued to increase till 1999 largely due to rapid growth in area (82 %) and modest yield enhancement (18%) (Tiwari and Joshi, 1999), however, it declined in 2000, due to poor yield and again in 2002 due to shrinking in area as well as very poor yield on account of consecutive and unusual drought. Nevertheless, soybean continued recording positive growth rates, where as other oilseed crops like groundnut, linseed, rapeseed, safflower, sesame and safflower recorded negative growth rate during 1991-2001. Sumptuous monsoon during 2003 has restored upward trend in soybean production. Today, the area under soybean is above 6.5 million hectares, which is likely to produce above 7.0 m t. The predominant soybean growing states commanding 98 per cent and 90 per cent of national soybean area and production are Madhya Pradesh, Maharashtra and Rajasthan in that order.

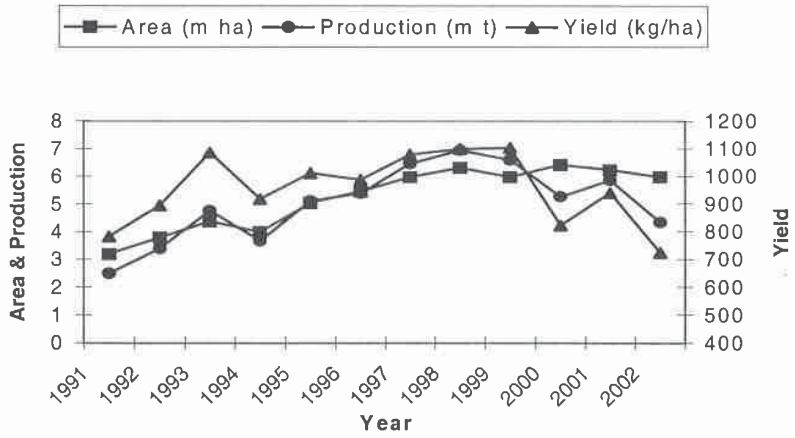


Figure 2. Area, production and yield of soybean in India

While, soybean area and production has more than doubled from 3.19 m ha and 2.49 m t in 1991-92 to 6.22 m ha and 5.86 m t in 2001, the productivity has remained low, hovering around 1 t per hectare. To put this into context, average yields of soybean in India (0.9 t/ha) is about half of those in China (1.77 t/ha) and one third of those in the Western Hemisphere particularly Brazil (2.70 t/ha). India, however, has only two decades of history of commercial cultivation of soybean. On the other hand, some of the Western Hemisphere countries have a century of experience while China has 20 centuries. Not only the growing conditions for soybean in India as rain-fed crop during monsoon are harsher than those in the countries under reference, the duration of the crop on an average in India is 96 days compared to the average of 120 to 170 days in these countries. Further, the small farm size, the large number of farmers, the low incidence of mechanization and the poor infrastructure in India make the task of overcoming the yield constraints more difficult (Table 1).

Table 1. Extent of adoption of production technology and environmental stresses in leading soybean-producing countries in the world

	Adoption of technology				
Technology	USA	Brazil	China	Argentina	India
Mechanisation					
Tillage	100	100	50	100	50
Planting	100	100	50	100	50
Harvesting	100	100	<30	100	5
Chemical inputs					
Fertilizer	100	100	50	100	40
Herbicide	100	>70	<30	>80	<10
Pesticides	?	>70	<30	>95	<30
Irrigation	1	0	?	0	1
Drought	50 %	40 %	<30 %	75 %	95 %

In addition to Technology gap, seed gap and information gap broadly encompass the factors limiting soybean yield in India. As has been demonstrated by the results of Front Line Demonstrations conducted with appropriate production technology over 14 years on farmers field under real farm conditions, the yield potential of soybean in India is 2 t/ha. Many farmers in the country are harvesting soybeans in the tune of 2.5 to 3 tons per hectare.

Cropping systems

Being an efficient legume, soybeans occupy an important place in existing and prospective cropping sequences for sustainable cropping systems in India. Grown during monsoon season (June – Oct.), soybeans usually follow

bread wheat (*Triticum aestivum* L.) cultivated during winter (Nov. to April), in regions where adequate/partial irrigation is available. Under rainfed conditions, soybeans are grown after chickpea (*Cicer arietinum* L.). While soybeans are largely cultivated as sole crop, in some fields, soybeans are also grown as mixed or inter crop or companion crop with pigeon pea (*Cajanus cajan* [L] Milsp.). and corn (*Zea mays* L.). The other cropping sequences like soybean-safflower (*Carthamus tinctorius* L.), soybean- garlic (*Allium sativa* L.) and soybean – potato (*Solanum tuberosum* L.), soybean - cotton (*Gossypium hirsutum* L) soybean - finger millet (*Eleusine coracana* Gaertn.), are in vogue to a limited extent. (Bhatnagar and Joshi, 1999). When intercropping is followed, the fertilizer is applied only to soybean.

Components of production system

Production technology: Research carried out in the last two and half decades has led to the standardization of appropriate agronomic practices of soybeans for different agro-climatic regions of the country. These include balanced fertilization, weed management, integrated pest management and disease management etc. in addition to conventional methods of extension being used to narrow the technology gap the recent attempts include use of IT, as in Soya Choupal, Agriwatch, India Agronet etc.

Varieties: To suit location-specific agro-climatic and growing conditions, a total of 80 varieties have been developed / identified under the aegis of All India Coordinated Research Project on Soybean (ICAR) and released for cultivation by the Central/State Committee of Crop Standards, Notification and Release during 1969 to 2002 (Fig. 3). Besides, genetic potential for high yield, the requirement in India calls for shorter duration, long juvenile period, and tolerance against biotic as well as abiotic stresses. Shorter duration varieties are usually preferred as they escape rigors of moisture stress often caused due to early withdrawal of monsoon, when soybeans are at most critical stage of plant growth for water requirement. The maturity duration of the varieties developed indigenously ranges between 85 to 112 days. Among the biotic stresses, 5 – 6 insect pests namely *Obereopsis brevis* (Swedenbord), *Melanogromyza sojae* (Zehntner), *Chrysodexis acuta* (walker), *Dichrysia orichalcea* (Fabricius), *Spodoptera litura* (Fabricius), *Helithis armigera* (Hubner) and diseases viz. Charcoal rot, Collar rot, Rhizoctonia blight, myrothecium leaf spot, soybean rust, Bacterial pustules/ blight and Yellow mosaic virus are of major economic importance.

Some of the recently released varieties show yield potentials up to 3.0 to 3.5 t/ha. Apart from the varieties (Bragg, Lee, Improved Pelican, Hardee, Monetta, Shilajeet, Co 1, Gujarat Soy 1, Gujarat Soy 2, VL Soy 2 and JS

71-05) which owe their evolution to direct selection from exotic and indigenous material, bulk of the Indian varieties were developed through hybridization and/or mutation breeding. It has been estimated that between 1969 and 1993, the annual genetic gain in seed yield of soybean varieties has been to the tune of 22 kg/ha (Karmakar and Bhatnagar, 1996). For increased resilience, three or four varieties, which suit the location and cropping system, are recommended for cultivation. There is definite need of broader genetic base in the cultivars.

Management of biotic stresses: Soybeans being rainy season crop are seriously threatened by weeds for higher yield. Chandel and Saxena, 1988, Tiwari and Kurchania, 1990 and others have reported yield losses from 30 to 77% due to weeds. Presently, weed management is being mostly attempted by cultural practices. However, due to uncertainty of rains, manual weed control in this rainy season crop is generally not effective. Use of herbicides is hardly 7 per cent, which results yield erosion in many fields. The suggested integrated approach involves summer ploughing, use of weed free seed, cultivation of more than one variety, weed free land surroundings, use of any one of the recommended herbicides in rotation and one need based inter-culture operation (Joshi, 2001).

The yield losses due to insect-pests and diseases, if not managed are estimated up to 32 per cent (Sharma and Shukla, 1997). While diseases, usually play limited role in reducing yield, insect pests are of major concern. Hence, they must be more effectively controlled. The integrated pest management approach involves summer ploughing, planting of resistant/ tolerant varieties, land incorporation of phorate before seeding, seed treatment with recommended bio-agent (*Trichoderma viride*)/ fungicides, crop hygiene, mechanical removal of affected plant parts in early stages of infection, use of light traps, and use of bio-pesticide/insecticides at economic thresh hold value of the pest. Reliable information regarding repeated occurrence of specific disease and pests in the regions of soybean growing and plant stage vulnerable to pest / disease incidence is available for working out schedules to effectively manage them. The management schedules for new emerging biotic stresses like soybean rust (caused by *Phakopsora pachyrhizi*), no podding syndrome and defoliators have been recommended. Use of Information Technology for fore-warning and timely control being recently attempted through the concepts like Soya Choupal, Agriwatch, India Agronet etc. are needed to be further strengthened.

Balanced nutrition: Constraint analysis and survey of major soybean growing regions have indicated that soil problems due to the wide range of soil types and management conditions as well as lack of balanced nutrition is

one of the reasons for low productivity of soybean in India (Kauffman *et al.* 2001 and Tiwari, 2001). The fertilizer application is not only on lower side, but is also skewed (N:P:K consumption ratio is 6.7:2.5:1 against the ideal 4:2:1) resulting sub optimal and unbalanced nutrition. By and large balanced nutrition (4.2:2:1) in Maharashtra since last couple of years (Anonymous, 2003), has perhaps been one of the reason for higher yields of all the crops including soybean in the state (1.4 t/ha). Awareness is needed for adoption of integrated approach in nutrient management with due emphasis on crop residue/ manure recycling and correcting the faulty use of fertilizer. Increased use of bio-fertilizers must be encouraged to reduce dependence on chemical fertilizers.

Utilization and processing

Earliest known record of soy foods in India dates back to 1600s. According to Ichiyama, (1968), Shoyu (soy sauce) were imported from Japan to the Coromondal coast in the vicinity of Madras now Chennai. This indicates that Shoyu (soy sauce) was used in India but the consumers were mainly Britishers. Historically, the soy utilization efforts in India were started by none else but the Father of the Nation, Mahatma Gandhi in thirties. He advised the use of soybeans in food in his published papers in the magazine named "*Harijan*" in 1935. Since then, there had been series of efforts to popularize soy-food. Several cook books were brought out after Kale's monumental work in 1936. These were: Handbook of Soybean Recipes (1969) by the Home Science Wing of the upgraded Gramsevaks Training Centre at Harward, Mysore published by Govt. of Mysore; Technical Bulletin

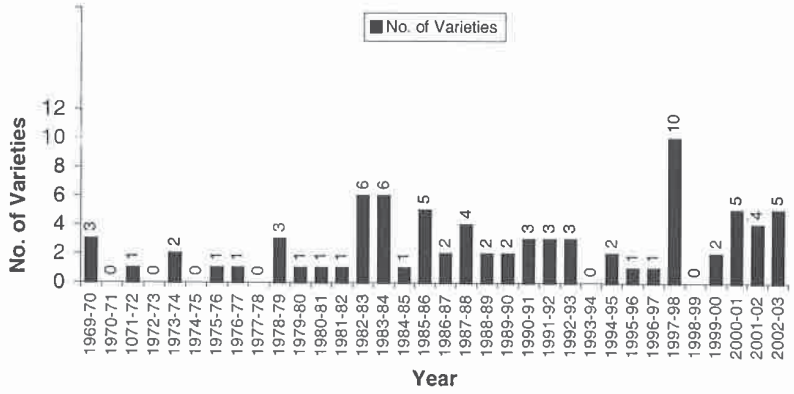


Figure 3. Soybean varieties identified/released/notified for cultivation in India

by CFTRI (1951-52); Soy Ahar: Indian Recipes of soybean (260 pages) edited by Mr. R. Singh at GBPAU&T, Pantnagar (1970); Tasty Recipes from soybean (1970) by Ms. S. Kantamani from Jabalpur; Danty Dishes of soybean by Vaidehi, USIS, Bangalore; The Indian Soy Cook Book by Kavith Reddy (2002). Recently, in 2002 a conference "Soybeans for Health and Nutrition Security in India" organized by SOPA with Mr. O. P. Goel as its Chairman and Dr. Prem S. Bhatnagar as Conference Convenor was inaugurated by Union Minister for Women and Child Development.. Experts of repute from within and out side the country participated and deliberated in the meeting under the Conference Chair of Prof. M.S. Swamitahan, Dr. Mark Messina and Harold E. Kauffman.

After the setting up of Agricultural Universities in India, more systematic work on soybean food uses and utilization was done with the active support of University of Illinois (USA). In late 60s a company named Soybean Production and Research Association (SPRA) was formed at Bareily, Uttaranchal as a joint venture of Nave Technical Institute and GBPUA&T, Pantnagar. SPRA did play key role in pioneering soy foods in India. Few more companies in private sector started making different forms of TVP. These were Ganesh Flour Mills in Delhi, Ruchi Soya of General Foods in Indore and Mysore Snack Foods in Bangalore. However, soy-based foods could not be popularized in real sense.

Commercial utilization of soybeans in India and thus their future in India remained in suspense till the first soybean processing plant came into existence in 1972 at Indore (M.P.). Subsequently, soybean processing plants were set up in succession to provide the economic viability and sustainability to Indian soybean sector in general and farming segment in particular. Today, there are 160 solvent extraction units with 58,045 TPD and 17.41 million tons annual capacity of 300 working days (Fig. 1)

While soybeans have attained vital status in agricultural economy, edible oil economy, rural economy and national economy of the country, only recently 10% of its national production is used for food and feed, another 10% is used as seed for planting and 70% of the total production of 5.86 million tons (2001) is used in solvent extraction plants. (Ali, 2001). Of the 4.2 million tons (approx.) De-oiled Cake produced, hardly 5% was used to be consumed in the country, mainly in poultry. Only recently, domestic utilization of DOC is steadily increasing to about 1.30 million tons. Notwithstanding, the export earnings from DOC rose from Rs. 2990 million (U.S. $67 million) in 1988-89 to over Rs. 24377 million (U.S. $ 542 million) in 2001-2002, (Fig 4) there had been drain of high quality lowest priced protein from the country. The situation is paradoxical on the face of rampant

malnutrition in India particularly among children, lactating and expecting mothers leading to infant mortality, low birth weight (LBW) and child hood diseases as well as adult chronic diseases in alarming numbers, specially in the weaker section of the society.



Figure 4. Export of DOC from India and Export earnings

Appreciating the need of domestic utilization of soybean for food in India, the Indian Council of Agriculture Research is continuing the, USAID collaborated project on Soybean Processing and Utilization (SPU) at Central Institute of Agriculture Engineering (CIAE), Bhopal. The project was initially launched in April 1985, in collaboration with USAID at CIAE, Bhopal and GBPUAT, Pantnagar. The SPU project has developed 17 soy-products, 20 soybean processing equipments and 5 limited scale pilot scale production facilities for full fat soy-floor, partially defatted soy-flour, soy-milk, soy-paneer and soya-fortified bakery products (Ali, 2000). However, soy food could not be so far popularized in India. Misconceptions, myths, information gap, lack of awareness about the nutritional and health benefits have perhaps been the cause for not harnessing the health and nutritional potential of soybean in India so far.

Summary

Location specific production research, coupled with development efforts and ensured market created by continuous establishment of soybean processing plants has resulted unparallel growth of about 15% for soybean area and production in India. During 2003, soybean coverage is estimated at 6.5 m ha. While, soybean production has more than doubled from 2.49 million tons in 1991-92 to 7.0 million tons (estimated) in 2003, the

productivity has remained low, hovering around 1 t per hectare primarily due to, a) environmental stresses namely inadequate soil moisture and vertisol soil of shrink swell nature; b) technology gap; c) seed gap and d) information gap.

Yield potential of about 2 t/ha demonstrated on farmers fields under real farm conditions during last 12 years can be harnessed on national level by overcoming the yield constraints.

With the present trend in growth, area and production of soybeans, in the tune of 8m ha and 12 m t in immediate future but 10 m ha and 20 million tons respectively in next decade is achievable. Urgent need of exploiting health and nutrition benefits of soybean calls for dogmatic efforts. Creation of awareness coupled with renewed interest of processors in the direction of value addition will result further sustainability of all the segments of Indian Soybean Sector, which provides enormous scope for private and public partnership from within and outside the country

The R&D efforts underway utilising modern tools of science like molecular breeding using marker assisted selection and quantitative trait loci (QTLs) will go a long way to breed/improve varieties with better yield potentials, resistance to biotic and abiotic stresses, better food grade characters and other useful traits. Similarly, the efforts to broaden the base of Indian soybean varieties, and graded production technology for resource poor and resource rich farmers will lead to sustainable and competitive production of the crop. The awareness and renewed interest of processors in the direction of value addition will further sustain the soybean farming and industry.

With the changed directives contained in the National Agricultural Policy 2001, the unison efforts made under National Agricultural Research System (NARS) shall make this crop more and more important among oilseeds in the future. To seek enhancement of production and productivity of crop, India has to further look for international cooperation in specific fields. Linkages with institutions within the country and abroad are therefore essential for better utilization of germplasm, sharing of available technology and human resource development in the areas of soybean research (Paroda, 1999). There exists vast potential of mutual benefits for partnership between public and private sectors, both from within and out side the country to enhance productivity, profitability, sustainability and competitiveness of all the segments of Indian soybean sector.

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Current status of the soybean production and utilization in Argentina

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Abstract

Argentina is the soybean oil and soybean meals number one exporter at a world level, showing an important growth in grain exports during the last years. All the complex strength is based on the permanent expansion of the planting area, which was doubled during the years 1995/6 (5,9 M hectares) and 2002/3 (12,4 M has), and on the production growth that grew from 11,7 Mt in the two-year period 1995/96- 1996/97 to 32,5 Mt in the two-year period 2001/2-2002/3, reaching 35,5 Mt the last season. (Cuadro I). The Argentine soybean grain crushing reached 21,5 Mt.; exporting grains by 8,5 Mt., what represents the 14% of the total world exports. Compared with USA and Brazil, that export 43% and 33% respectively, Argentina takes the first place in soybean meal and soybean oil exports. In the case of soybean meal, Argentina has 36,5% (16,8 Mt) of the annual export, followed by Brazil with 26% and USA with 14%. For soybean oil, Argentina exports 41% (3,9 Mt.) of the world market, followed by Brazil with 20% and USA with 10%. The sum of these regional situations provides the crop with certain possibilities to continue growing to a surface of 16 M has. by the year 2008. Taking in account average yields of 2.700 kg/ha., it is estimated a production of 44 M tm. Argentina has become a tidy, reliable, efficient, and versatile supplier of soybean grains and by-products for the demanding world.

Introduction

The purpose of this work is to let you know what has happened since the last years regarding production and industrialization and soybean uses in Argentina. A historic report was showed in the WSRC VI (Larreche H.J. et al, 1999).

The soybean complex

The Soybean Complex has had important changes in all levels and has acquired a crucial importance for the Argentine economics, based on the production and industrialization constant rising, with an export level that overcomes any other sector of the national economy. In Argentina, the

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Crushing Complex exports in 2002 for a value of $ 5.874 millions, which represents the 23% of all Argentine exports. Soybean, that is the main export sector, has a market share of 86%; formed in 2003 by $ 1.8 billions of soybean grain, $ 1.9 billions of soybean oil and $ 2.9 billions of by-products.

In this way, Argentina is the soybean oil and soybean meals number one exporter at a world level, showing an important growth in grain exports during the last years.

All the complex strength is based on the permanent expansion of the planting area, which was doubled during the years 1995/6 (5,9 M has) and 2002/3 (12,4 M has), and on the production growth that grew from 11,7 Mt in the two-year period 1995/96- 1996/97 to 32,5 Mt in the two-year period 2001/2-2002/3, reaching 35,5 Mt the last season. For the same comparison, unitary yields has showed average values of 2.000 Kg/ha and 2.745 Kg/ha respectively (2004 Soya & Oilseed Bluebook, SAGPyA & USDA Ag Report June 2003).

According to official estimates, (SAGPyA), in the season 2003/4 a new record is achieved: 13.6 M has. The estimates figures for the soybean complex for 2003 is close to $ 7 billions.

The most important **facts and the strengths** developed by the soybean complex, added to the excellent agroecological conditions that Argentina has for the crop, are detailed as follows:

- High competitiveness in soybean production related to others crops in the Argentine context.
- Soybean production has become the local producer "first choice crop", reason by which all efforts are involved to assure the crop success.
- Soybean crop expansion in all agricultural and cattle areas, with growing unitary yields in the global range.
- Favorable climatic situation, because of higher average rainfalls in submarginal and central areas.
- Dedicated activity of genetic breeding, which has allowed an increase in the yield potential and planting possibilities in new handling situations and production areas.
- Technology availability that allowed a high level of national and international competence: Soybean resistant to glyphosate and no-till planting.
- Many institutions and Non Governmental Organizations participation that has supported the crop expansion and other relate activities: National Institute of Agricultural Technology (INTA), Universities, Non Tillage Farmer Association (AAPRESID), Grain Boards, Argentina Oil Industry Chamber (CIARA), Breeders Associations (PROSOJA), Argentine Association for Soybean Uses and Development (SADESO) among others.

- Competitive and anxious farming producers, of high efficiency and capacity to develop new technologies and companies management related to different economic and production situations.
- Fluent commercialization of transgenic soybean production, which it is almost 100% of Argentine production.
- Great competitiveness of the installed Oil Industry, based on important investments in modern processing plants of high technology and scale and being an important competitor at a world level.
- Development of Ports and sailing ways that have diminished transport costs and activated exports.
- Closeness to the main production areas to ports and industry concentration in port areas.
- Increase of field storage capacity, what allows a moderate harvest release to its destination.
- Diversification of countries importers of soybean oil and soybean meal, like the Chinese demand appearance, to absorb the new and bulky exportable remnant.
- Soybean prices rise due to the stocks reduction, based on the highest world consumption per capita of soybean by-products.
- Growing demonstrated independence of the Soybean Complex, despite the changing and critical Argentine economic situation and the political and taxes scenery.
- Successful campaign of soybean consumption and usage diffusion in a humanitarian food aid program framework.
- Favorable interaction of all these elements that favored the rise in production and international positioning.

The list of **unfavorable internal elements** detailed below has not obstruct this development, but they would be a bar to grow up:

- Monoculture situation in more than 50% of the soybean surface and 25% of wheat-soybean double culture.
- Short resources and lack of adaptation in soybean research and development to the new crop regional structure.
- The soybean grain contain relative low protein percentaje compare with competitive countries.
- Delay in a suitable legal framework development regarding Intellectual Proprietary Rights in Biotechnology and lack of transparence in the Seed market.
- Low level in fertilizer usage that determines a lack of nutrient replacement.
- Return of Government production deductions in the year 2002 (23% of the price), after 10 years of having been eliminated. Hard tax structure that in many cases leads the profitability values to be negative.

- Reduction of provoked infrastructure investments because of the economic crisis and lack of definition on a farming policy.
- Lack of credits and the financial system crisis have been balanced due to extra-rural sectors have placed their capital in agricultural production.
- Strong Press controversy about the so-called Argentine *"soybeanization"*, mainly in city areas far of the farming reality.

Among the **external negative factors** of the last decade, we detail as follows:

- Strong protectionism of the local production on behalf of EC and USA.
- Growing protectionism to imports enforcing import rights (customs duties).
- Controversies regarding GMO's commercialization.

In spite of the above mentioned, Argentina has become a tidy, reliable, efficient, and versatile supplier of soybean grains and by-products for the demanding world.

The Argentine crushing industry and the soybean

The industry total capacity in the year 2003 was 29,3 Mt., with a daily capacity of 94.268 metrics tons. The latter is based in 85% in soybean and 13% in sunflower. The usage percentage is 88,73%. This industry is considered the most efficient and modern of the main competitor countries. Globally analyzing the Argentine Oily Complex participation, values are as follows: production: 12,1%, milling: 10,3%, grain exports: 14%, oils and fat productions: 5,3%, meal production: 9,9%, total oil and fat exportation: 13,2% and meal exportation: 31,9%.

The Argentine soybean grain crushing reached 21,5 Mt.; exporting grains by 8,5 Mt., what represents the 14% of the total world exports. Compared with USA and Brazil, that export 43% and 33% respectively, Argentina takes the first place in soybean meal and soybean oil exports. In the case of soybean meal, Argentina has 36,5% (16,8 Mt) of the annual export, followed by Brazil with 26% and USA with 14%. For soybean oil, Argentina exports 41% (3,9 Mt) of the world market, followed by Brazil with 20% and USA with 10%. Historically, Argentina is growing in exports of the 3 sections. The industry exportable coefficient is as follows: in soybean meal: 97% and in Soybean oil: 88%.

The list of countries where Argentina exports sums up more than 100, being for the Soybean meal, the EC the main destination (Holland, Italy, Spain, Denmark, etc) and Asia (India, China, etc) for soybean oil.

In the year 2002, the Soybean Complex export values were $ 4.674 millions and the estimation for 2003, $ 6.615 millions. The division is as follows:

soybean oil: $ 1.880 millions, soybean grain: $ 1.818 millions and soybean meal and by-products: $ 2.917 millions.

There is an important concentration of processing plants in the province of Santa Fe- 64%- situated close to the center of Argentinean production main area. Cordoba represent 11% and 23% is in Buenos Aires. The oily pole is concentrated in the bank of Paraná river. Near the city of Rosario. This Port has been recently developed with a Parana River Waterway Project at a depth of water of 32 feet. Better transport efficiency and low relative costs. The cost per Tm of load in the port is the 50% of the Brazilian cost. The current average capacity of the processing plants is 5.000 tm/day, compared to 1.500 tm/day in Brazil and 2.500 tm/day in USA. Processing costs are estimated in $ 6, $ 12 and $ 8 respectively. In the processing structure outstands a plant with 12.000 tm /day. The oily sector invested during the period 1994-2000 $ 780 millions. In the last 3 years, because of internal political and economic reasons, the sector has restrained investments.

The main grain mean of transport in Argentina is the truck, and in the second place, the railway. Costs compete with Brazil, but distances to processing plants and ports are appreciably shorter than in Brazil and USA. Argentina produces more than 75% of its soybean at an average distance of 270 km of the destinations.

Regarding storage capacity, the situation is pretty good. Argentina has a capacity near the total volume of all the grains and oilseeds harvest (65 Mt / 69 Mt: 94%), but it is lower than other competitive countries (values of 150%). The 38% belongs to private stocks and cooperatives, 22% to the industrial sector and 6% is stored in ports and export sectors. A 21% is stored by farmers in fixed constructions and in recent years the "silobag" has become popular. It is estimated that the latter sums a capacity of 12% of the total. This new situation has allowed to handle the grain delivery and sale more slowly during the whole year; and in this way it is determined a decisive farmers participation in determining prices.

Outstanding topics of the soybean situation in Argentina

Biotechnology: Regarding GMOs, the only deregulated event approved for commercialization is the event RR. Several papers analyzes the positive impact of Roundup Ready (RR) soybean in Argentina. In the year 2001, two Soybean events resistant to Ammonium Gluphosinate (LL soybean) were flexibylized. CONABIA, advisor commission of the Secretary of Agriculture, has been authorizing trials of field releases of many events regarding as follows: lepidopteran resistant, new herbicides resistant, oil

quality, high lysine, yield traits, etc. During the last years, the Market office was working more closely with the others offices responsible for the study of cases and approvals corresponding to biosafety, risk assessments, environmental impact and food security. This office deals with the risks to authorize an event in an unilateral way, which may lead to trading problems. In this way, the approval line takes in count on the EC and others countries decision.

In others aspects advanced have been produced regarding usage of Marker Assisted Selection as follows: parent selection, recovery of recurrent parent in backcrossing, screening disease resistance genes, and to study the effects of different breeding procedures in genetic diversity.

No till planting and its impact in production: The massive adoption of this true "production system", since more than 85% of soybean is planted according to this system, is based not only on soils conservation and fertility maintenance, but also on a better and more efficient water handling. This impact is more noticeable in the new soybean areas, where in almost 100% of the surface this system is used. All sources agree that synergy with RR varieties has provided this phenomenal surface increment, production and yields of soybean in Argentina. The Non tillage farmers association (AAPRESID) lead the research and extension in these matter, together with INTA.

Genetic development: Breeding programs have been active. After an average of 35 new varieties per year in 1998 and 1999, during the last two years the average diminished to 11 varieties per year. Private companies are responsible for more than 95% of the certified seed. This is an important difference with Brazil. With the new variety contributions, the Stem Canker disease has been almost eliminated. The outstanding regarding defensive aspects is that they have been obtained varieties resistant to many diseases and nematodes. Yield Genetic Gain studies demonstrated that for the different maturity groups they were in a range from 1,2% to 2 %.

Seed industry: In Argentina, almost the 70% of planted surface correspond to self pollinated varieties. The area of hybrids of corn, sorghum and sunflower still relative low.

The variety is protected by the Seed Law, which is based on UPOV 78. In the legislation enforced, plant varieties cannot be patented, although gene constructs and genetic alteration processes can. Self pollinated varieties current market situation is that only 20% of the seed consumed is sold by companies. The black market is almost 50% of wheat and soybean market. This situation risks the breeding programs to continue. Companies with a

high market share persist because of the size of their own market. It has been showed an important reduction in the quantity of multiplier seed companies and seed cooperatives in recent years. A world soybeans genetics leader decides to close activities recently. From year 2001 seed companies agreed to launch contracts for extended royalty payments as a mechanism to capture additional innovation rents. These contracts cover newly released varieties of soybeans and wheat, and require farmers to pay a certain fee for the use of farm-saved seeds.

Current production of soybean seed has suffered important changes in cycle shortenings or maturity groups. More than 45% of certified seed corresponds to groups III and IV.

Crop ecology and variety performance: they have been extended trials and proofs about planting dates of different maturity groups in all soybean areas, mainly in the new ones; what have allowed to achieve a pattern related to each area in particular. It has been extended the usage of simulating models for a better variety adjustment to planting date and management. INTA and private companies cooperated in the design of an important trial evaluation network called RECSO.

Fertilization: Traditionally, soybean is a crop of low usage of fertilizers. Good physical and mineral characteristics of the Pampeano soils have not provided the necessary answers to the farmer to adopt it. New studies have showed answers related to low phosphorus levels (less than 13 ppm) and response to sulfur. They have been strengthened the studies in response to fertilization in other crops that rotate with soybean. In the wheat-soybean system, this is very important. In the new soybean areas of Nor east and Mesopotamia, there is nutrient deficit and in many cases there are important answers, including micronutrients. During the last years, soybean fertilization growth led to fertilize 30% of the planted surface.

Soybean diseases and their controls: In Argentina, it has been completed a total sanitary list in comparison with other countries regarding diseases and nematodes. After the stem canker epiphytes in 1997 and 1998 in the North area of the country, the disease that has almost disappeared because of the resistant variety contribution, it has not been showed any other case of a new disease that has significantly depressed yields. In another way the Soybean Cyst Nematode identification in a great part of the central area has not become a yield depressor factor. There was a resistant variety release, but they have not taken an important share. The diseases that are causing problems at a farmer lot level are as follows: Sudden Death Syndrome, Brown stem rot, for which there is not good genetic resistance,

and Phytophthora root rot and Frogeye leaf spot, for which there are resistant varieties. The late season diseases complex and other endemic diseases have increased during the last years; some of them are as follows: Brown spot, Cercospora blight, Mildiu, Antracnosis, Bacterial blight, Rhizoctonia, Pod and stem blight and Damping off complex. Their appearances with moisture stress of Charcoal rot have been sporadic.

Fungicides usage has increased in order to control many of these diseases. A new threat, the Soybean rust, was identified in May of the year 2002 in a border area near Paraguay, but at the moment (January 2003) has not been spread to other regions. In the North of Argentina there are areas that could hold the disease, because of the conditions of humidity, rainfalls, temperatures, and hosts during the winter season and also for the wide planting date. Nevertheless, in the great Pampeana Region, the situation is contrasting. Programs for genetic resistance research have been activated. The Secretary of Agriculture release a National Program to detect and prevent mayor damage because the rust.

Insect plagues and their control: Insects have been a problem for soybean in Argentina; but during the last years, insect pressure has diminished and so the insecticide usage. One of the facts that has contributed is handling based on IPM. Farmers make periodical controls based on monitoring. There are alert systems generated at INTA. The new insecticides are more selective and in accordance with environmental rules. Nevertheless, the most common and harmful insects are *lepidoptera* and stink bug. Among them, an important plague in the central area is *Epinotia aporema* that attacks buds; also the defoliating worms *Rachiplusia nu* and *Anticarsia*. Two types of stink bugs are the prevailing ones and produce damages in all soybean areas; these are *Nezara viridula* and, the most harmful, *Piezodorus guildinii*. Extension of no till planting has brought new plagues that were less important as cut worm, mollusks, etc.

Status of mechanization. The highlighted points of this fact are the incorporation of: specific planter for direct planting, that have improved stubble section, better seed and fertilizer emplacement, high volume fumigators comanded by computers and with satelite control- GPS, new fertilizer equipments with "specific site" aplication, low preasure wheels, sophisticated and computerized combines with axial cilinders, etc. Many of these farm implements are Argentine made. The goverment is helping the local industry by tax returns. Local research lead by INTA results predict new achievements in the short term.

Soybean local consumption: efforts were developed during many years to increase the local soybean acceptance by the public and possible feed

users. In Argentina beef is the major supplement in people´s daily diet and the soybean acceptance was low. Farmers make efforts to face Food emergency during the last economics crisis. AAPRESID, SADESO and others organizations coordinate the strategies used to determinate food supply logistics and distributions from the farmers and the end users. A big educational program was implemented. The results obtained in such a short time as regards geographical coverage, nutritional education and solidarity initiatives are widely encouraging. Biodiesel is an important topic were the Argentinean are working very hard.

Current and future surface and production

In Argentina, the surface in the traditionally soybean areas has increased, like the Pampeana provinces of Cordoba, Santa Fe, and Buenos Aires. In 1996/7, 88.5% of the surface was concentrated in these provinces, and in 2002/3 is 75% of the total. These areas are the most productive ones and where soil quality and whether conditions are more suitable for the crop. There was a significant growth in the other areas, called submarginal, with lower quality soils and erratic rainfall regimen; except the Norwest of the country. These regions, represented by the provinces of Norwest, Nor east and Mesopotamia, have increased their surface 400%. Exceptional cases are showed in the provinces of Santiago del Estero, Chaco, and Entre Ríos. In fact, soybean has not taken the place of other crops, except cotton that was also affected by low international prices. On another hand, it used lands previously used for grazing and meat and milk production.

Among the 1992-93 season and the current one, soybean planted area grew 238% and grain production, 313% with country average yields in 28%.

There was a real expansion of the so-called "Agricultural Frontier", being the soybean crop the pivot which stimulated the change.

Nevertheless, the growth in no-traditional areas, with lower aptitude and in the same typically soybean areas, yields were higher. In the season 2002-03, Argentina reached an average yield record of 2.800 kg/ha. Management factors were changed, especially the massive adoption of no till planting, changes in the crop structure, planting dates, better weather conditions in a humid cycle with a prevailing "el niño", and the best transgenic varieties released by the seed industry.

Each of these regions has particular characteristics

In Argentina, soybean can be planted in low latitudes of 23° LS to 40°. This allows diversity in planting dates and possible crop rotations.

Possible expansion of soybean in Argentina

Crop growing possibilities in general are wide, but it is important to analyze each area particularly.

In **Pampeana Region** we include in the first place the **Province of Buenos Aires (2003: 2,5M has)**. The bigger area for development is in the west and southwest area of the province. There is a reserve near to a million hectares to grow in the next years. In the double crop system, soybean has more possibilities after wheat and barley. The location of soybean cultivars of short cycle - groups II and III that grow fast, together with branches plant structures, would provide a crop with an acceptable yield for attractive economic margins. Any possibility of forwarding the planting date would probably bring better yields. In the same region; if soybean advances over sunflower, will depend on the relative prices; since agronomically, soybean has acceptable and steady yields in relation to this crop and with cost reduction. In the southeast area, soybean will continue growing and become a rotation wheat/soybean - corn-soybean. Two essential elements for these regions will be to cover the necessities of fertilization and election of adapted varieties.

In the **Province of Santa Fe (2003: 3,35M has)**, the soybean surface seems to have reached the top. The crop and rotation current situation has remained steady in a unbalanced way in the South of the province. The higher growth may be in the north central area because of the cattle fields, but this would not demand an important surface. Nevertheless, in a great part of the north central area of the province, adoption of fertilization will contribute to higher yields. Another factor in the same sense will be the timely planting date, to escape the most important moisture deficit moments, with diversification of crop cycles. They outstand the recent releases of groups VII and VIII short of high yields, adapted to these northern regions.

In the **Province of Córdoba (2003: 3,5M has)**, there are more possibilities of soybean expansion. There are more possibilities in the southwest and noreast area. There are cattle fields and a possible substitution by sunflower and corn. Soybean in the double crop system will consolidate in the north area and soybean as the only crop, in the south. There are possibilities to provide 500 thousand has. in the next 5 years. Continuity of a favorable hydric situation will consolidate the tendency.

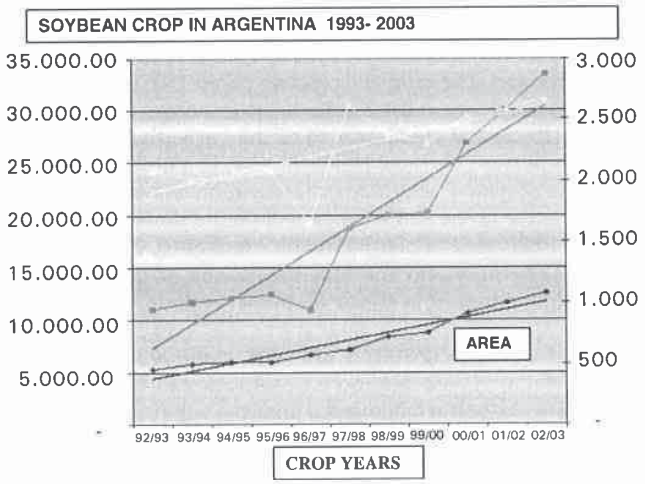
For the three Pampeana provinces, we estimate an increment of the area of 2 M has in the next 5years.

The province of **Entre Ríos (2003: 1 M has)** –Mesopotamia – has an important grazing surface available for agriculture. Soybean is the most competitive specie in colonizing the province soils. The current cultivars and the developing ones will make possible to sort and adapt to the special types of soils of heavy structure, called "vertisoles". In the south area, planting dated diversification and maturity cycles will make yields stable. In these situations,

it is necessary to discover the crop fertilization necessities. It is estimated a growth of 550 m has, summing up the province of **Corrientes,**

The **Norwest Region includes the provinces of Salta, Tucumán and Catamarca (2003: 590 thousand has)**. Most surfaces to increase are in Salta. The west central area, the most developed one, will continue increasing soybean in soils that incorporate agriculture. In the north area, the clearing of trees (controlled and supervised) will provide more surfaces in environments of very high yields and replace the dry beans in certain extension. A positive tool will be the use of better crops of long cycle that will allow diversifying risks and making good use of favorable conditions in late plantings; keeping for the very short cycle crops the areas at the foot of the mountain. The sanitary threats should be attacked with genetic breeding and with the reasonable usage of fungicides. It is estimated an increase of the surface of 200 m has.

Quadro 1



Most surfaces to incorporate the soybean crop are in the Noreast. The provinces of **Chaco, Santiago del Estero, and Formosa (2003: 1,4M has)**, have a situation of wide planting date: from September to February; abundant, but erratic rainfalls; soils of varied structure; extremely high temperatures that, summed up, they show an area of high climatic risk. For that, diversification in planting date and usage of undetermined growing crops in extreme dates is the necessary tools to overcome the climatic irregularity impact. In all cases, the crop viability would be more foreseeable in a framework of rotations with grasses, beside the case of wheat in the sequence, or the seasonal crop secuence, sunflower-soybean, corn–soybean, soybean-sorghum, etc. This region can increment the surface in 800 m has.

Conclusions

The sum of these regional situations provides the soybean crop with certain possibilities to continue growing to a surface of 16 M has. by the year 2008. Taking in account average yields of 2.700 kg/ha., it is estimated a production of 44 M tm. The oil industry will make important investments to efficiently process these new production level.

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Present state of soybean production and utilization in Europe

J. Daydé[1]; J.C. Chibarie[2] ; F. Labalette[2]; M. Berger[1]

Current characteristics of european soybean production and utilization

Due to the exceptional quality of its seed (40 % protein and 20 % oil contents), soybean production has been increasing tremendously over the five past decades: from around 20 million tons in 1955 to nearly 200 in the first years of the 21st century (Figure 1).

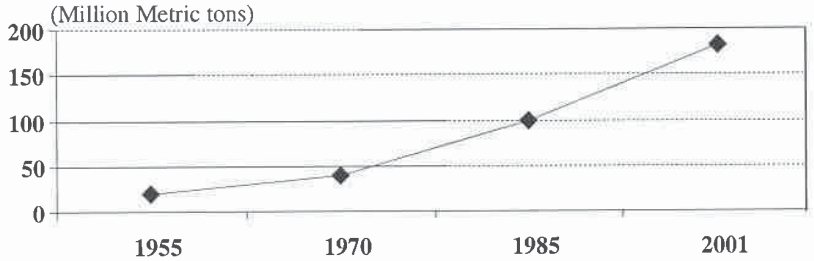


Figure 1. World soybean production increase

Moreover, an increase of 30 million tons (Mt) has been observed during the five past years. Three producing countries are roughly sharing 80 % of the world total production (Figure 2) : the U.S.A. are leading (74.3 Mt forecasted for 2002/2003), followed by Brazil (47.5) and Argentina (33.8) both for the same period.

This success story has pushed the soybean to the first rank among oilseeds and protein plants. In 2001, 184 Mt of soybean seeds were produced among a total of 303 Mt of oilseeds (more than 60 %), soybean meal represented also 60 % of the total amount of meal consumed in the world (121 Mt out of 198) and soybean oil (28 Mt) reached 24 % of total consumption (118 Mt).

Faced with this huge supply, the demand is mainly driven by the soybean meal market for animal feeding. In this field, the main consuming countries are the U.S.A. followed by the European Union (E.U.), China and lastly Brazil (Figure 3).

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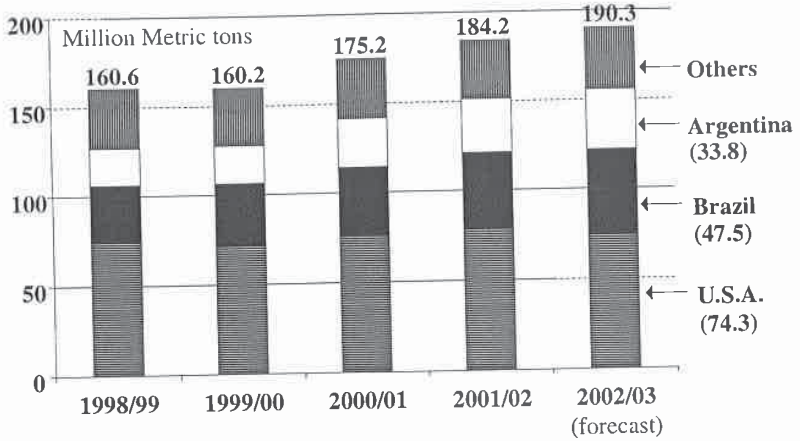


Figure 2. Soybean main producing countries (1999/2003)

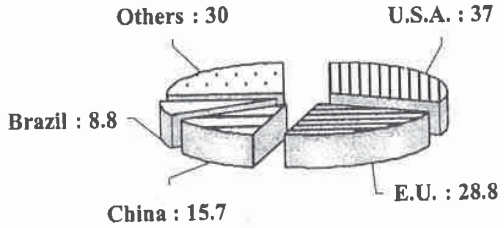


Figure 3. Soybean meal consuming countries in 2001 (Million Metric Tons)

Since the end of the fifties, the E.U. has been considered as the first consumer of meal in the world. The use of soybean meal carries an increasing (Table 1) and reached a new record in 2001 with a total consumption of 28.8 Mt.

Table 1. Soybean meal consumption in the E.U. (Million Metric Tons)

Year	1973	1981	1991	1996	1999	2000	2001
Meal Consumption	7.8	14.7	19.3	22.2	26.5	25.4	28.8

This amount represents 60 % of the total consumption of meal in the E.U. (49 Mt in 2001). Countries where milk and meat (pork and poultry mainly) production and the feed industry are strongly developed are all very heavy meal consumers (France, Germany, Spain, the United Kingdom, the Netherlands, ...). At the same time soybean meal and seed imports are showing a regular increase.

Even if soybean has been quite successfully introduced in Italy (plain of the Pô river) and in the south-west of France, the production is generally above 1.5 million tons per year, which represents 3 to 4 % of the meal consumption in the E.U. The acreages remain very low due to the competition of soybean with other crops and mainly due to the successive European agricultural policies – consequences of the world trade agreements – which have also significantly limited soybean cropping development.

As a consequence of this situation, the deficit in proteins used for animal feeding has been above 70 % over the five past years and reached 77 % in 2001/2002 (Figure 4).

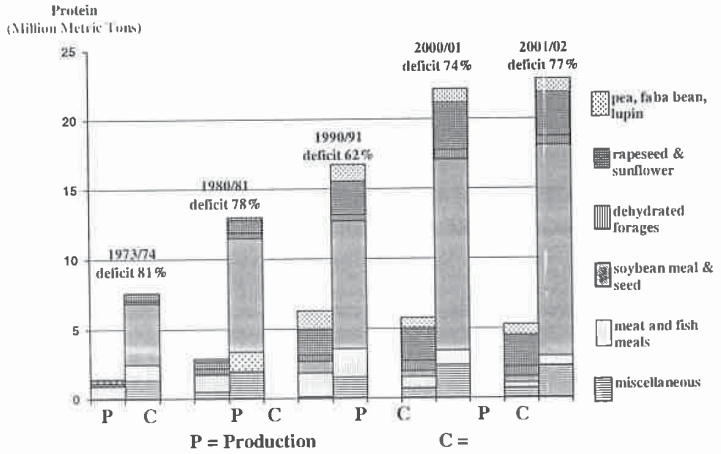


Figure 4. Production and consumption of protein used for animal feeding in the E.U.

This deficit is entirely covered by massive imports of soybean from the three major exporting countries : the U.S.A. (29 Mt of seeds exported in 2001), Brazil and Argentina (16.6 Mt of seeds – 11 Mt of meal and 7 Mt of seeds and 14.5 Mt of meal respectively in 2001). Since 1995, the huge emerging market of China both for oil and meal, has changed the market equilibriums. The political will of China to create added-value by

crushing imported soybean seeds increased the demand of seeds in the world market : 14 Mt imported by China in 2001 mainly form the U.S.A. and from Argentina. Meanwhile, the crushing industrial potential in the E.U. slowly decreased. Furthermore the demand for meal is still increasing (+ 30 % since 1995 and + 20 % forecasted up to 2005). Thus the total European imports of soybean (*Table 2*) are nowadays shared by Brazil (first soybean-selling country in the E.U for both seeds and meal), Argentina. and the U.S.A. (who used to be the leader on the European market over the past years).

Table 2. Soybean imports in the E.U. (2001)

(Million Metric tons)	U.S.A.	Brazil	Argentina	Total imports
Seeds	7.4	9.2	0.8	17.7
Meal	0.5	8.5	8.3	17.6

Brazil's leadership as far as soybean exports towards the E.U. are concerned, can be partly explained by its banning of planting GMO soybeans, but this rule was partly cancelled in 2003... Argentina is exporting practically exclusively meal to the E.U. while the U.S.A. are doing the same with seeds. Finally, 17.7 Mt of seeds and 17.6 of meal were imported in the E.U. in 2001. Seeds are imported in countries which have set crushing facilities – like Spain or the Netherlands – while France is the first soybean meal importing country of the E.U. (4.4 Mt in 2001). However, the E.U. is not able to consume the total amount of soybean oil it produces (3 Mt in 2001 and one third re-exported).

To conclude, the E.U. is clearly extremely dependant on a few exporting countries so as to meet its animal feeding needs. As we shall see hereafter, this will probably carry on being the case over the next years.

Major future trends in the European soybean industry

European Union Agricultural Policies and World Trade Organization

The former (1992) Common Agricultural Policy (CAP) moved the previous system (1956) based on price support to the principle of acreage subsidies. For oil crops – including soybean – an additional subsidy was introduced. The Berlin agreement (2000 – 2006) is very negative for oil and protein crops : oil seed subsidies are decreasing to the level of those of cereals. Furthermore, the midtime revue of this "Agenda 2000" (June 2003) did

not show any favorable evolution. The major part of subsidies will be provided regardless of the amounts of production, and the link to acreages –still not clearly defined – should be based on "historical references". Specific supports to oil and protein crops proposed by French and European farmers' lobbies like the "Protein Plan" are not supported by E.U. authorities. It appears that there is no political willpower and even no interest of the E.U. to reduce protein and oil seed deficits. In this field, the enlargement from 15 to 25 members in 2004 will not allow a real increase of production and will probably lead the E.U. to a higher protein deficit (more needs for animal feeding due to the development of meat production in many states among the new members).

The general feeling of farmers is nowadays uncertainty and fear to face the future. They will also need to fulfill many administrative and technical standards to receive the subsidies. These constraints will be progressively put in place in several fields :

- environment (underground water, nitrates, urban wastes, pesticides, bird protection, ...) in 2005 ;
- veterinary and food safety (identification of animals, hormone uses, pesticide residues in food, traceability, ...) in 2005/2006 ;
- animal welfare in 2007.

All these points are adding more and more pressure on farmers in the E.U. Many of them are thinking that two main ways of farming could appear in the E.U. if the Common Agricultural Policy continues to be driven by the World Trade Organization recommendations as it often appears to farmers' lobbies (poor research of an identity and no visible wish to protect the economic potential of European agriculture). The first way should be based on world competitiveness struggle and market driven choices for both crops and cropping systems ; this will concern very few producers in large size farms like everywhere around the world. The second way should concern farmers maintained only to act on the rural landscape as "Nature gardeners". In this new frame, oil crops among which soybean, are probably situated between those extremes with real assets as far as agronomic and environmental management issue are concerned. Unfortunately this kind of issue could be less and less driving farmer decisions as nothing is done to redirect the European agricultural policy.

GMOs and creation of added value issues

Facing E.U. needs, there is an obligation of a huge import of GMO soybean for animal feeding uses, just because there is no choice and this should increase due to the partial allowance of GMO soybean in Brazil. Consumers'

refusal of GMOs' consumption leaded producers and industrials to promote non-GMO certified chains. As an example, in France (2001) 98 % of the soybean produced on the 120 000 hectares cultivated were processed in 27 chains with total traceability (food or feed products). These chains were supported by a special subsidy (at national level) which was cancelled due to the midtime revue of the Common Agricultural Policy in 2003. As a matter of fact the low amount of these non-GMO outlets for soybean are decreasing rapidly (70 % of the 80 000 hectares in France in 2003). As the feed market is unable to give a real added-value to non-GMO soybean (as opposed to the food market but which is very low : no more than 5 % of French production, as an example) the farmers are wondering if this outlet is a long-term market or not ... Furthermore, most of them are feeling that the moratory and wait-and-see policies on GMOs in Europe put them at a disadvantage by comparison with other farmers, in the frame of the competitiveness of world agricultures.

Finally, the use of soybean for food and dietary supplements is the only field were added value could undoubtedly be created. Health benefit claims of soybean are more and more strategic issues and identity preserved chains dedicated to these outlets are promoted with great successes. Nevertheless even if these markets are exploding, the amount of soybean consumed in those industries is too low to consider these chains as a way to maintain a significant soybean production in Europe.

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Soybean production and utilization in Africa

B. B. Singh[1]; F. Hakizimana[1]; R. Ortiz[1];
E. A. Kueneman[2] and R. Ortiz[1]

Abstract

Soybean (*Glycine max* (L.) Merrill) is slowly but steadily getting becoming popular in Africa. It was introduced by missionaries Missionaries and explorers introduced the crop in early 1900s but it gained popularity from 1960 onwards. From a total of 191,000 ha and 72,000 t production in 1961, the total area and production of soybean in 2002 was 1,090,000 ha and 989,000 t in Africa. Small scale production of soybean is doneoccurs by in many countries but Nigeria, Zimbabwe, South Africa and Uganda have substantially increased the production of soybean in last 20 years. The average yield of soybean in Africa has also increased from 376 kg/ha in 1961 to 907 kg/ha in 2002. The major constraints to production are low soil fertility, diseases and lack of marketing. The Rrecent out break of rust has threatened soybean production in many countries. Concerted efforts are being made by the International Institute of Tropical Agriculture, in collaboration with national programs to develop resistant varieties and home level as well as industrial uses of soybean and this is driving intensive cultivation of soybean in several countries particularly in Nigeria which now has over 80 soya soybean-based industriesagro-processing businesses. With growing population and increasing malnutrition in Africa, soybean will play an important role in African agriculture. Its role as a rotation crop in cereal and root-crop based production is expected to increase.

Introduction

Soybean [*Glycine max (L.)* Merrill)] is a major source of vegetable oil and protein in the world. However, its large large-scale cultivation is mainly concentrated in a few countries such as Argentina, Brazil, Canada, China, India, Paraguay and United States of America, which together produce about 96% of the world soybean. Soybean is relatively a minor crop in Africa and its total production in 2002 was 989,000 tons which that accounted for only 0.50% of the global production of 179,917,000 tons (Table 1). Part of the reason for such a differential distribution of soybean cultivation is agro-climatic suitability and partly due to technological capacity to process soybean for enhanced utilization. An FAO study (1978) estimated

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that about 145 million ha in Africa is suitable for soybean cultivation ((Kueneman et al. 1985). The area where maize can be grown is normally suitable for soybean and there is a good potential of soybean to be a rotation crop with maize in Africa. It can also be grown as intercrop with cassava and sorghum. With declining per capita food production in Africa and increasing malnutrition, soybean can play a major role in increasing protein and oil intake for the people. Therefore, several countries in Africa are making concerted efforts to promote soybean cultivation and utilization. Soybean is presently being cultivated in Benin, Cote Cotê d'Ivoire, Ghana, Nigeria, Senegal, Togo in West Africa. Among these, Nigeria has the largest area and production.

Table 1. Global soybean area and production in 2002*

Region/country	Area x 10^3 ha	Production x 10^3 t	Yield kg ha^{-1}	% of world production
Africa	1,090	989	907	0.50
Asia	17,124	23,720	1,385	13.00
Central America	72	135	1,871	0.08
Europe	969	1,824	1,882	1.00
Latin America	30,021	76,689	2,554	42.62
North America	30,245	76,761	2,538	42.67
World	**79,410**	**179,917**	**2,265**	-
Argentina	11,405	30,000	2,630	16.60
Brazil	16,345	41,903	2,568	23.30
Canada	970	2,334	2,405	1.30
India	5,670	4,270	753	2.40
China	9,420	16,900	1,794	9.40
Paraguay	1,445	3,276	2,267	1.80
USA	29,202	74,290	2,544	41.00

* FAO STAT (November 2003).

The National Agricultural Research Systems (NARS) in these countries have strong links with the International Institute of Tropical Agricultural (IITA) and other international regional and national programs link IRAT and Brazil for to obtaining improved soybean varietiescultivars. In the Central Africa Africa, Cameroon, D.R. Congo D.R., Rwanda, and Burundi grow soybean in small scale at mid-elevations where rainfall and temperatures are adequate. In the East and Southern Africa Africa, Tanzania, Uganda, Zambia, Zimbabwe, South Africa have substantial program on soybean research and development. Soybean production in Zimbabwe and South Africa is mainly by large large-scale farmers.

Soybean production in Africa

Soybean was introduced into Africa by missionaries and explorers in at the beginning of the 20th century (Mayo 1945, Root et al. 1987, Naik et al. 1987) particularly in Nigeria (1910), Cameroon (1924), Congo DR (1915), Uganda and Southern Africa. However, soybean cultivation did not become popular until after 1960 and that also only in a few countries. The total area under soybean in Africa in 1961 was 191,000 ha producing about 72,000 tons with an average yield of 376 kg/ ha^{-1} (Table 2). This was limited to only 0 countries, of which Nigeria, Egypt, Zimbabwe and South Africa had a major share.

Table 2. Area, production and average yield of soybean in Africa overtime*

	Africa			
Year	Area	Production	yield	No of
	x 10^3 ha	x 10^3 t	kg ha^{-1}	countries
1961	191	72	376	10
1971	236	98	412	10
1981	453	358	789	16
2002	1090	989	907	19

* FAO STAT (November 2003).

Soybean production received greater attention in late 1960s and early 1970s when systematic soybean research and development programs were initiated by several national programs in collaboration with the International Soybean Program (INTSOY) of the University of Illinois and the International Institute of Tropical Agriculture (IITA) based in Nigeria (Leng, 1973; Kueneman et al., 1984 and, 1985). Since then there has been a slow but steady increase in soybean production particularly in Nigeria, South Africa, Uganda and Zimbabwe. From 191,000 ha in 1961, the area under soybean in Africa has risen to 1,090,000 ha in 2002 with corresponding increase in production from 72,000 tons in 1961 to 989,000 tons in 2002. The number of countries producing soybean also increased from 10 in 1961 to 19 in 2002 2002, and the mean soybean yield has also improved from 376 kg/ ha^{-1} in 1961 to 907 kg/ ha^{-1} in 2002 (Table 2). The actual soybean area and production for 1981 and 2002 in the 19 countries in Africa are presented given in Table 3. There has been a major increase in soybean production in all the countries except for Egypt. Nigeria is the largest producer (437,000 t) followed by South Africa (202,000 t), Uganda (166,000 t) and Zimbabwe (83,139 t) The maximum increase in Nigeria has been due to to a significant contribution of IITA in soybean research and-for- development activities and active participation of national researchers, traders, and the private industriesy.

Soybean production constraints

Physiological constraints

Photo-sensitivity, pod shattering, rapid loss of seed viability and poor nodulation without *Rhizobicum* inoculum are often limiting factors in selection of improved varietiescultivars.

Biotic constraints

Several diseases, insect pests and parasitic plants cause severe reduction of yields in soybean. The major and widespread diseases of soybean in Africa are soybean mosaic virus, bacterial pustule (*Xanthomonas campestris pv. phaseoli* (Smith) Dye, bacterial blight (*Pseudomonas syringe pv. glycinea*), *Pyrenochaeta leaf* spot (*Pyrenochaeta glycines*), frog eye leaf spot (*Cercospora sojina*), Alternania leaf spot (*Alternaia sp.),* target leaf spot (*Coryonespora cassicola*), web blight (*Rhizoctonia solani, Fusarium* wilt and root rot (*Fusarium sp.*), purple stain (*Cercospora kikuchii* and nematode (*Meloidogyne sp.*). Recently soybean rust (*Phakopsora pachyrhizi*) has become a wide spread problem throughout Africa.

Soybean does not have serious problem of insect pests in Africa except for pod sucking bugs like *Nezara viridula, Clavigralla* spp. and *Riptortus dentipes*. Adults and nymphs of these pests feed on pods and reduce yield as well as

Table 3. Soybean production in Africa in 1981 and 2002*

Country	Area x 10^3 ha		Production x 10^3 t		Yield kg ha^{-1}	
	1981	2002	1981	2002	1981	2002
Benin	0	6,532	0	6,541	-	1,001
Burkina Faso	0	2,278	0	2,533	-	1,111
Burundi	900	400	800	300	888	750
Cameroon	450	9,200	200	5,800	444	630
D.R. Congo	10,500	29,208	8,300	14,108	790	483
Cote d'Ivoire	1,977	2,500	1,903	3,000	962	1,200
Egypt	45,956	5,331	130,360	14,885	2,836	2,792
Ethiopia	6,630	7,000	19,180	25,000	2,892	3,571
Gabon	0	2,100	0	2,200	-	1,047
Liberia	5,000	7,500	1,900	3,000	380	400
Madagascar	65	50	75	50	1,153	1,000
Morocco	500	1,000	500	1,000	1,000	1,000
Nigeria	300,000	624,000	78,000	437,000	260	700
Rwanda	7,384	31,289	6,365	17,085	826	546
South Africa	22,000	124,150	27,600	202,395	1,254	1,630
Tanzania	5,000	5,600	1,080	2,100	216	375
Uganda	5,000	151,000	5,000	166,000	1,000	1,099
Zambia	4,900	3,900	3,673	2,400	749	615
Zimbabwe	37,000	77,150	72,881	83,139	1,969	1,077
Africa (Total)	453,262	1,090,186	357,817	988,542	789	907

* FAO-STAT, November, 2003.

quality. However, their incidence is seasonal and sporadic (Ezueh and Dina, 1980, Jackai and Singh 1987, Dashiell et al. 2001). The parasitic plant, *Alectra vogelii* (Benth.) is widespread in Eastern, Southern as well as West Africa and heavy infestation and severe losses in soybean have been observed in the northern Guinea Savanna of Nigeria.

Aboitic constraints:

Soybean is affected by early as well as terminal drought because the beginning and the end of the rainy season is very erratic in most parts of Africa. Also, soybean yields are highly affected by low fertility status of the savanna soils, which are low in phosphorus, organic matter and several other nutrients.

Progress in soybean breeding

Soybean research and –for-development work undertaken done from 1960 to 1980 in Nigeria, D.R. Congo DR, Cameroon, Ghana, Cote Cotê d'Ivoire, Senegal and other countries in West and Central Africa was reviewed by Root et al., (1987) and Kueneman et al., (1985), in Uganda by Rubaihayo and Leaky (1970) and Rubaihayo et al., (1973), and the work done in the Southern African countries including Zambia, Zimbabwe and South Africa was reviewed by Naik et al., (1987). These countries have evaluated and identified superior varietiescultivars and released them for general cultivation. However, since the 15 last years, the major soybean research and –for-development work undertaken was by IITAhas been done at IITA in collaboration with the National ProgramsAfrican NARS. The , whose progress made IS bethis article briefly reviewed in this paperbriefly provides.

1981 to 2000

IITA and collaborating NARS initially focused on genetic improvement for seed storability promiscuous nodulation and resistance to pod shattering using selected varietiescultivars from Indonesia like. Malayan was parent in the breeding program and several improved varietiescultivars were developed, which stored well and nodulated well without *Rhizobium* inoculum (Dashiell et et al., 1985, IITA- 1993). Bacterial pustrils pustules (*Xanthomonas campestris*), frog eye leaf spot (*Cercospora sojina*) and soybean mosaic virus were the major diseases, without much isignificant insect problems except for sporadic damage due to slink but, (*Nezera rizidula*) and other pod sucking bugs like *Clarigialla* speciesspp. Combining the resistance to these diseases with seed longevity, promiscuous nodulation, early maturity and resistance to pod shattering, IITA developed and distributed several improved varietiescultivars to national program for testing by African NARS (IITA 1993, Chanda et al., 1990, et al., Musiyiwa et al., 2001). After thorough testing in mutilation multi-location trials, many of these varietiescultivars have beenwere released in several countries including Nigeria, Ghana, D.R. Congo DR, Benin, Togo and Uganda (Table 4, Asafo-Adjei and Adekunle 2001). These cultivars have stimulated and catalysed catalyzed a rapid increase of soybean cultivation particularly in Nigeria, where over 10 improved varietiescultivars have beenwere released. Of these cultivars, TGX 1485-1D and TGX 1448-2E have become became very popular in the Nigerian middle - belt of Nigeria. Resistance to pod shattering in TGX 1448-2E has beenwas confirmed and it has been used as a parent for a genetic study (Tukamnhabwa, 2002) and, which indicated that 2 two genes were found to be responsible for this trait.

Table 4. List of improved IITA soybean varieties cultivars from IITA released in Africa

S/No.	Cultivar	Pedigree	Day to maturity	Country of release
1.	TGX 306-036C	TGm 107 x TGm 479	118-125	Nigeria, Ghana
2.	TGX 536-02D	TGX 376 A x TGm 737 P	100-110	Nigeria, Ghana
3.	TGX 297-192C	TGm 737 x TGm 479	105-112	Ghana
4	TGX 813-6D	TGm 1197 x TGm 618	105-110	Ghana
5.	TGX 814-76D	TGm 618 x TGm 7	110-117	Congo DR
6.	TGX 849-294D	TGX 182 x TGm 119	97-103	Congo DR
7.	TGX 849-313D	TGX x TGM 119	109-115	Nigeria
8.	TGX 923-2E	TGX 17 x TGX 849	118-122	Nigeria
9.	TGX 1019-2EB	TGm 1551 x TGX 536-100C	105-110	Nigeria
10.	TGX1019-2EN	TGm 1551 x TGX 536-100C	98-106	Nigeria
11.	TGX 1440-1E	TGX 536-02D x TGX 814-27D	115-120	Nigeria, Benin, Togo
12.	TGX 1448-2E	TGX 824-18D x TGX 814-27D	115-120	Nigeria, Benin, Togo
13.	TGX 1485-1D	TGX 316-02D x TGX 813-11D	85-95	Nigeria, Benin,Togo,
14.	TGX 1740-2F	TGX 539-5E x SIBLEY	95-100	Nigeria, Benin, Togo
15.	TGX1830-20E	TGX 1483-3D x TGX 1448-2E	95-100	Nigeria

2002 –2003 and Continuing

High biomass with high harvest index

With declining soil fertility and little or no use of fertilizer, soybean is playing a major role in maintaining soil fertility when grown as intercrop or in rotation with maize. (Sanginga et al., 2002, 2003, Carsky et al., 1997; Ogoke et al., 2001). Therefore, efforts are being made to develop improved soybean varietiescultivars with higher N-fixation, high biomass production and high harvest index. The increased production of soybean stover enhances soil organic matter if left in the field or returned as manure when fed to livestock. Combining these traits, a number of new improved early- and medium medium-maturing soybean varietiescultivars have been developed with yield over exceeding 2 t/ ha^{-1} grain and 2.5t/ ha^{-1} stover (Tables 5 and 6), (Dashiell et al., 2001). The best among early varietiescultivars is TGX 1905-5F with 2338 kg/ ha^{-1} grain and 2553 kg/

ha^{-1} fodder, and the best among medium varietiescultivars is TGX 1918-21F with 2423 kg/ ha^{-1} grain and 2875 kg/ ha^{-1} fodder. These varietiescultivars also have goodshow enhanced nodulation without inoculation and resistance to pod shattering and higher harvest index.

Table 5. Mean performance of the four best breeding lines and two improved cultivar checks (TGX 1448-2E and TGX 1844-18E) at Mokwa and Zaria, Nigeria in 2002.

Genotype	Grain yield (kg ha^{-1})	Stover + yield (kg ha^{-1})	Harvest index (HII	Nodule# score	%Ndfa**	Pod + + shattering score	Plant height (cm)	Maturity days
TGX 1905-5F	2338	2553	0.47	3.7	47	1.5	68	116
TGX 1910-14F	2257	2761	0.44	3.3	60	1.2	63	115
TGX1910-8E	2069	2834	0.41	3.1	53	1.8	73	118
TGX 1910-5F	2004	2467	0.45	3.4	60	1.4	62	116
TGX 1448-2E	2085	2386	0.45	3.1	55	1.3	68	116
TGX 1844-18E	1191	2407	0.44	3.4	57	1.2	67	117
Mean (18) varieties	1815	2282	0.45	3.3	53	1.6	64	117
SE	150	167	0.01	0.2	6.1	0.2	2.9	0.5

+ The air dry weigh of the above ground parts of the harvest minus the grain weight
# Nodulation rated on a 1-5 scale where 1 = no nodules seen and 5 = excellent nodulation, comparable to inoculation
** Percent of nitrogen derived from the atmosphere (2002 data for Zaria only)
+ + Pod shattering rated on a 1-5 scale where 1 = highly resistant and 5 = highly susceptible

Ability to stimulate suicidal germination of *Striga hermonthica*

Striga hermonthica is a major parasite on maize, sorghum and millet causing severe yield losses. Soybean stimulates suicidal germination of *Striga hermonthica,* and reduces the seed bank of this parasite in the soil (Carsky et al., 2002). Thus, breeding for improved soybean varietiescultivars with greater ability to stimulate *Striga* germination is anwas added recently by IITA as a breeding objective. There is a great deal of variability among soybean varietiescultivars for their ability to cause suicidal germination of *Striga hermonthica*. This iInitial observations from 209 soybean genotypes screened fort this traits recorded a range from zero 0 to 35% germination. The two best lines are TGX 1844-18E and TGX 1895-35F. These are , which are being used now in theIITA's breeding program. Umba (2002) studied reported the inheritance of the ability of soybean to cause suicidal germination of *Striga hermonthica* using TGX 1649-11 and Emgopa 305 as high stimulant parents and TGm 987 as low stimulant

parent. He observed high heritability and estimated 1 or 2 genes for controlling this trait.

Table 6. Performance of the five best breeding lines and two improved cultivars checks varieties (TGX 1448-2E and TGX 1884-18E at Mokwa and Zaria, Nigeria, in 2002.

Genotype	Grain yield (kg ha^{-1})	Stover + yield (kg ha^{-1})	Harvest index (HI)	Nodule# score	%Ndfa**	Pod + + shattering score	Plant height (cm)	Maturity days
TGX 1918-21F	2423	2875	0.46	3.5	52	1.5	80	111
TGX 1918-19F	2170	2972	0.42	2.9	46	1.6	78	108
TGX 1916-2F	2158	2181	0.50	3.3	53	1.0	78	113
TGX 1918-7F	2005	2167	0.49	3.2	46	1.5	64	108
TGX 1912-7F	1957	2417	0.45	2.6	48	2.0	75	108
TGX 11448-2E	1830	2028	0.46	2.7	60	1.7	67	114
TGX 1844-18E	1691	2223	0.42	3.1	48	1.3	74	114
Mean of 25 var.	1707	2224	0.44	3.1	48	1.6	75	110
SE	140	208	0.02	0.2	3.7	0.3	3.1	0.7

+ The air dry weight of the above ground parts of the harvest (at harvest maturity) minus the grain weight

Nodulation rated on a 1-5 scale where 1 = no nodules seen and 5 = excellent nodulation, comparable to inoculation

** Percent of nitrogen derived from the atmosphere

+ + Pod shattering rated on a 1-5 scale where 1 = highly resistant and 5 = highly susceptible

Breeding for rust resistance

Soybean rust caused by *Phakopsora pachirhizi* (H. & P. Sydow) was first noticed in Zimbabwe and by 1998 it had spread over several countries including Rwanda, Mozambique and Uganda. It was noticed in Nigeria in 1999 (Akinsanmi et al., 2001) in several states and has become a serious threat to soybean cultivation all over Africa. It was subsequently reported in Benin and Cameroon. In 2001, rust was detected for the first time in South Africa in northern Kwazuly – Natal (Pretorius et al., 2001) and as season progressed the disease spread to other parts of the province and epidemic levels were reached in many places. Symptoms first develop on leaves closer to the ground and progress upwards until all the leaves are infected causing premature defoliation, and yield losses up to 80%. (Akinsammi Akinsanmi et al. 2001, Kawuki et al., 2001).

In view of the serious and rapid spread of rust in Africa, IITA has identified a few hot spots in Nigeria and Uganda and to initiated systematic screening of soybean breeding lines as well as germplasm lines accessions for resistance to rust. From a total of 212 lines, including some reported to be resistant in other countries, TGX 1835-10E TGX 1903-12F, TGX 1903-5F and TGX 1913-3F were found regarded as moderately resistant. Additional lines have beenwere obtained from other sources and are now being screened.

Genetic gains

In Nigeria, seed yields increased due to the improved cultivars, cultural practices, and general management. To determine the contribution of improved genotypes to this increase, 13 cultivars in early maturity group, 10 cultivars in medium maturity group and 11 cultivars in late maturity group released from 1980 to 1996 were analyzed for grain yields (Hakizimana et al. unp. data). Other plant characteristics were assessed to determine if differences existed among cultivars and if these differences were correlated with year of release and grain yield. The regression of year on grain yield indicated that a positive significant ($P < 0.01$) genetic gain occurred. The genetic gain was 2.1% per year for the early maturity group, 3.2% per year for the medium maturity group, and 3.6% per year for the late maturity group. Averaged over the three maturity groups for the period 1980 to 1996, the genetic gain was 3%. For all maturity groups, there were associations between grain yield, fodder, and number of nodules. Significant genetic improvements ensued from hybridization and selection during almost the past 20 years of breeding in the three maturity groups by IITA breeders working in Africa.

Soybean utilization

Since soybean is a new crop in Africa, IITA started a systematic program to promote processing and utilization in collaboration with INTSOY and subsequently with the support from the International Development Research Center (IDRC, Canada). The focus has beenwas to develop recipies recipes for direct utilization of soybean as food at the household level, and to extract oil and process the meal through extrusion cooker for various snacks and baby foods (Weingartner et al., 1987). This has approach led to the development of a number of home level products such as soybean flour, and recipies recipes for making soya soya-soup, soya soya-akara, soya soya-moin-moin, soya soya-milk, soya soya-ogi, soya soya-dawa dawa, soya soya-tofu, and industrial products such as Nutrend baby food by Nestle (Nig Ltd) and Cerevap baby food by Victoria Associated Products in D.R. Congo DR (Weingartner 1987, and Osho 1999). IITA's work on fried soybean 'Tofu' or 'Wara' in Nigeria, in collaboration with Japanese scientists,

has beenwas very successful in to promoting promote large large-scale consumption of soybean as snack food and a source of income for women. These developments and mass training for home level use of soybean have led to rapid increase in soybean production and consumption as well as establishment of a number of soya soybean-based industries agro-processing businesses in Nigeria, Ghana and other countries (Table 7). Starting with 2 two industries businesses in Nigeria in 1986 and none in Ghana in 1987, there were 80 soya soybean-based industries businesses in Nigeria and 13 in Ghana in 2002. The soybean production in Nigeria has also increased from 78,000 tons in 1981 to 437,000 tons in 2002. It is expected that this will increase further in the coming years.

Table 7. Number of industries agro-processing businesses processing soybean in Nigeria and Ghana

	No of industries	
Year	Nigeria	Ghana
1987	2	0
1990	20	2
1995	50	6
1998	70	11
2002	80	14

Diffusion and social impact of improved soybean in Nigeria

A social impact assessment frame- work was used (Sanginga 1998, Sanginga et. al. 1999) to examine the level of adoption and impact of soybean on the lives of 203 resource poor farm households in Benue State of Nigeria. Among the household respondents, 37% were women and 63% were men. The results show that the status of soybean has changed from a traditionally male controlled minor export crop, to one of the most important crops cultivated by the majoritymany of male and female farmers. More women have become involved in soybean production and utilization as improved varietiescultivars and household utilization technologies have become readily available.

The study Impact research showed that all the local varietiescultivars like 'Tiv soybean' and old improved varietiescultivars like 'M351', 'Samsoy 1' and 'Samsoy 2' , which were released in the 1980s, have beenwere completely

replaced by the new soybean varietiescultivars, TGX 536-02D, TGX 923-2E and TGX 1440-2E. Farmers replied indicated that the new varietiescultivars are better than other varietiescultivars interms ofdue to their maturity time (85.5%), high yield (63.8%), performance under low fertility (61.6%) and ease of harvesting (45.6%). These new varietiescultivars have beenare being widely adopted, ; it beginning began with 9% of farmers in 1989 and reaching reached over 75% in 1997, and thesebut now occupy about 30% % of the total soybean land area depends on IITA bred-cultivars.

The adoption of soybean has had a clear positive impact on household income generation and distribution, material welfare, human capital development, gender relations, resource use, social equity, and other social processes in the community. In zones of production, Ssoybean was the most important source of income of 42% of men and 47% of the women. Income from soybean contributed greatly to the acquisition of radio, bicycles, livestock and metal sheets for houses. Increased soybean production also had great impact on household food security and nutrition as local foods like 'Akara', 'Moin-moin', 'Wara', and 'Dawa Dawa' could be easily made from soybean. Average comsumption of soybean per family ranged from 1.5 kg to 2.5 kg. Anthropometric studies showed that soybean had great beneficial effect on children's health. Soybean alone accounted for up to 34.4% protein intake of children. Many innovations in soybean utilization have been adopted, to the extent that soybean has become a staple food. The results further showed that the nutritional status of children was significantly better in households using soybean products and using households than in those that did not use soybean. Recent studies (unpublished) have indicated similar results in other areas in Nigeria where soybean has been adopted.

Future outlook

Soybean cultivation is well established in several countries in Africa particularly in Nigeria, Zimbabwe, Uganda and South Africa and the area as well as production is increasing from year to year (Table 3). However, further rapid increase will depend upon the availability of niche for soybean in the existing cropping system as a rotation with maize or intercropping with cassava and availability of new lands. But with the fast and emerging threat of soybean rust, there may be a set back unless soybean varietiescultivars resistant to rust are quickly developed. Sources of resistance to soybean rust caused by *Phakopsora pachyrhizi* have been earlier reported in India and Australia and therefore, there is a hope that resistance breeding would be able to contain this threat. IITA in collaboration withand African NARS in Africa isare well on its their way to develop rust resistant cowpeasoybean varietiescultivars. In addition to breeding improved soybean varietiescultivars, concerted and

continued work on promotion of household utilization of soybean would be needed to enhance soybean market and ensure its nutritional benefit to millions of under nourished people in Africa.

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PLENARY

MARCH 2

The Science behind soyafoods

M. Messina[1]

Abstract

Soyafoods and soyabean constituents, especially isoflavones, are being investigated in laboratories throughout the world. Soyafoods are hypothesized to reduce the risk of several chronic diseases including breast and prostate cancer, osteoporosis, and coronary heart disease (CHD). The estrogen-like effects of isoflavones have caused many health professionals to view soyafoods and isoflavones as alternatives to conventional hormone replacement therapy (HRT). However, there are also non-hormonal mechanisms by which isoflavones can exert physiological effects in vivo.

Introduction

Over the past decade soyafoods have become increasingly popular among Westerners because of their purported health benefits.(United Soybean Board, 2000) Sales of soyafoods have tripled within the past 10 years and are expected to increase at least 10% per year for the foreseeable future. The two components of soyabeans thought to be responsible for the hypothesized health benefits are the protein and isoflavones. In recognition of the high quality of soya protein in 2000 the U.S. Department of Agriculture (USDA) issued a ruling allowing soy protein to replace 100% of the animal protein in the National School Lunch Program (U.S. Department of Agriculture, 2000). Previously, soy protein could replace no more than 30% of animal protein. Indirectly, this change in policy occurred as a result of the protein digestibility corrected amino acid score replacing the protein efficiency ratio as the preferred assay for determining protein quality.

In addition to high quality protein soyafoods can be good sources of both essential fatty acids, linoleic acid and ±-linolenic acid (U.S. Department of Agriculture). The latter is an omega-3 fatty acid with possible coronary benefits (Marckmann, 2003). The diets of many populations are low in omega-3 fatty acids. Despite the importance of these nutritional attributes they have little to do with the dramatic increased popularity of soyafoods. Rather, it is because soyafoods are hypothesized to directly reduce risk of several chronic diseases.

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Brief review of isoflavones

Impetus for early investigation of soya

In 1990, the National Cancer Institute (NCI) in the United States organized a workshop to examine the potential role of soya consumption in reducing cancer risk (Messina and Barnes, 1991). The importance of this workshop cannot be overstated because as a result, the NCI issued a request for applications (RFA) for study of the anticancer effects of soya (Messina and Messina, 1991). The effects of this RFA were far reaching because it represented acknowledgement by a U.S. Federal agency that soya might reduce cancer risk and second, the availability of funding led to a new pool of investigators considering conducting soya research.

The NCI workshop was initiated in large part because of research published in 1990 which found that adding soya protein to the diets of laboratory rats inhibited chemically induced mammary cancer.(Barnes et al., 1990) The investigators of this study attributed the protective effects of soya to the soyabean isoflavones. Today, interest in isoflavones continues to provide much of the basis for investigation of the health effects of soya. Incredibly, there are now approximately 600 scientific papers published on isoflavones annually, compared with just 12 in 1985 (based on Medline search).

It is beyond the scope of this paper to review the relationship between soya intake and cancer risk so only a few brief comments will be made. Notably, in 1998, in a comprehensive review of chemopreventive agents the Chemoprevention Branch of the NCI in conjunction with researchers from the University of Illinois at Chicago concluded that of the 25 agents tested, genistein, the main isoflavone in soyabeans, was of four agents considered to possess superior chemopreventive properties (Lee et al., 1999). There is evidence to suggest soya may be protective against several different cancers (Messina et al., 1994) but most focus has been on breast and prostate cancer. The evidence for protection against prostate cancer is quite intriguing although it consists mostly of animal work (Messina, 2003). For breast cancer, arguably, the most promising hypothesis is that early (during the teenage years) intake reduces risk of developing breast cancer later in life (Lamartiniere et al., 2000).

Finally, the NCI is by no means the only U.S. Federal agency interested in soya. In 1999, the National Institute on Aging sponsored a two-day workshop on isoflavones (Lu et al., 2001) and in that same year the USDA in conjunction with Iowa State University created an online database of the isoflavone content of foods.

Background on isoflavones

Isoflavones are a subclass of a larger and more ubiquitous group of bioactive compounds called flavonoids. In comparison to most flavonoids however, isoflavones have a very limited distribution in the plant kingdom. The primary isoflavones in soybeans are genistein (4' 5, 7-trihydroxyisoflavone) and daidzein (4', 7-dihydroxyisoflavone), and their respective ß-glycosides, genistin and daidzein. Typically, more genist(e)in exists in soybeans and soyfoods than daidz(e)in (Murphy et al., 1999).

By the early 1950s isoflavones were established as weak estrogens based on their ability to stimulate uterine weight in young rodents (Carter et al., 1953; Cheng et al., 1953). In 1966, Folman and Pope were the first to conduct assays establishing the relative binding affinities of the soybean isoflavones for the estrogen receptor.(Folman and Pope, 1966) Ironically, they were also the first to demonstrate genistein could function as an antiestrogen under certain experimental conditions and as a result, suggested that the antiestrogenic effects of isoflavones may prove to be of greater importance than the "estrogenic" effects (Folman and Pope, 1966).

Although there are no definitive data showing isoflavones or soya exert antiestrogenic effects in humans, there are multiple mechanisms by which such effects could occur (Messina and Loprinzi, 2001). Still, unarguably, it was the discovery in 1987 that genistein was a specific inhibitor of tyrosine protein kinase (Akiyama et al., 1987; Ogawara et al., 1986), an enzyme frequently over expressed in cancer cells (Hunter and Cooper, 1985), that really drew widespread research attention to soya and isoflavones, especially in regard to cancer prevention. Because of the widespread effects of genistein on signal transduction this isoflavone is routinely used as a tool for understanding cellular processes. Finally, like many phenolic compounds, isoflavones also possess antioxidant properties (Rimbach et al., 2003).

The effects of genistein on signal transduction demonstrate that isoflavones are more than just phytoestrogens. Furthermore, the finding that isoflavones have a much greater binding affinity for estrogen receptor beta than estrogen receptor alpha (Barkhem et al., 1998; Kuiper et al., 1996; Kuiper et al., 1997; Kuiper et al., 1998) has led to isoflavones being viewed as selective estrogen receptor modulators and thus, as being especially important for the health of postmenopausal women (Brzezinski and Debi, 1999).

Soya and isoflavones as alternatives to conventional hormone replacement therapy

Osteoporosis

The first human trial that provided insight into the possible skeletal benefits of isoflavones was published by Potter et al in 1998 (Potter, 1998). In this six month study, postmenopausal women consuming an isoflavone-rich isolated soy protein experienced an increase in spinal BMD compared to women consuming soy protein containing a lower amount of isoflavones or casein. Similar results were found by St. Germain et al in a nine month trial involving perimenopausal women (St. Germain et al., 2001). Research using isolated soy isoflavones is limited, but results suggest that they are protective in postmenopausal (Chen et al., 2003; Yamori et al., 2002) but not premenopausal women (Anderson et al., 2002). Particularly noteworthy, is a one-year study which found isolated genistein was as effective as conventional HRT at increasing BMD at the hip and spine in postmenopausal women (Morabito et al., 2002). While the estrogen-like effects of isoflavones provide a mechanism by which soy can promote bone health, the non-hormonal effects of isoflavones may also play a role (Setchell and Lydeking-Olsen, 2003).

Soy protein and coronary heart disease

Cholesterol reduction

Investigation of the hypocholesterolemic effects of soya protein in humans has been underway for nearly 40 years (Hodges et al., 1967). However, this extensive body of literature was largely ignored until the publication of a meta-analysis in 1995, which summarized the results of nearly 40 clinical trials (Anderson et al., 1995). Trials included in this meta-analysis formed the bulk of the research upon which the U.S. Food and Drug Administration (FDA) based their decision in 1999 to award a health claim for soy protein (Food and Drug Administration, 1999). The hypocholesterolemic effects of soy protein are due to the protein itself, and not because soyfoods are low in saturated fat and cholesterol free.

The meta-analysis cited above found that the average reduction in low-density-lipoprotein cholesterol (LDL-C) was 12.9% (Anderson et al., 1995) but new data suggest a more likely reduction is about 4-5% (Weggemans and Trautwein, 2003). The reduction in cholesterol in response to soy protein has clinically relevant public health implications as each 1% reduction in cholesterol reduces CHD risk by as much as 3-4% (Holme, 1990; Law et al., 1994a; Law et al., 1994b). Furthermore, soy protein also appears to modestly raise high-density lipoprotein cholesterol levels (HDL-C)

(Weggemans and Trautwein, 2003). The authors of a recent meta-analysis estimated that the modest reduction and increase in LDL-C and HDL-C respectively, would reduce CHD by 20% (Weggemans and Trautwein, 2003).

Beyond cholesterol reduction

There are provocative data suggesting soya favorably affects multiple risk factors for CHD although in all cases the literature is inconsistent. The text below consists of a few brief comments about findings involving the effects of soya on several different CHD risk factors.

Blood pressure: Data are very inconsistent but a recent study which found that in comparison to dairy milk soymilk dramatically lowered systolic blood pressure in hypertensives has drawn attention to this area of research.(Rivas et al., 2002) A recent review concluded that soya lowered blood pressure in two-thirds of the randomized clinical trials conducted (West, 2003).

LDL-C- oxidation: In 1998, in a small study Tikkanen et al showed that an isoflavone-rich soya protein increased LDL-C oxidation lag time (Tikkanen et al., 1998). Several other groups have confirmed these findings (Jenkins et al., 2000a; Jenkins et al., 2000b). Some in vitro (Hodgson et al., 1996; Kapiotis et al., 1997) and in vivo (Tsai and Huang, 1999; Wagner et al., 1997; Wiseman et al., 2000) research by comparing isoflavone-rich ISP (ISP+) with ISP devoid or very low in isoflavones suggests isoflavones are responsible for the antioxidant effects. However, several studies have failed to show isoflavone supplements inhibit LDL-C oxidation (Hodgson et al., 1999; Nestel et al., 1997; Samman et al., 1999). Furthermore, not all studies have found that even ISP+ inhibits cholesterol oxidation.

Direct effects on coronary vessels: The most established effect of isoflavones is the enhancement of systemic arterial compliance (SAC) (Nestel et al., 1999; Nestel et al., 1997), which was first demonstrated in 1997 (Nestel et al., 1997). SAC, which is an indicator of vascular elasticity, is considered by some as an independent measure of CHD risk (Simons et al., 1999; van Popele et al., 2001). There are also studies indicating isoflavones enhance endothelium-dependent vascular response although data are conflicting on this point (Hodgson et al., 1999; Nestel et al., 1997; Samman et al., 1999; Simons et al., 2000; Teede et al., 2001; Walker et al., 2001).

Hot flashes

The estrogen-like effects of isoflavones in combination with the low reported frequency of hot flashes in Japan prompted investigation of the effect of soya on menopausal symptoms (Adlercreutz et al., 1992). In a recent

analysis of 19 trials Messina and Hughes concluded that soya and isoflavones are modestly effective in reducing hot flash frequency but only among women with frequent (e"5/day) hot flashes (Messina and Hughes, 2003). These authors also concluded that the evidence justified recommending women try soya/isoflavones for relief of hot flashes but not sufficiently strong to definitively conclude that the treatment is efficacious.

Conclusions

A large body of evidence indicates the consumption of soyafoods can potentially reduce risk of several chronic diseases although no firm conclusions can be made at this time. Still, the nutritional attributes of soyafoods alone – low in saturated fat, high quality protein, and source of both essential fatty acids – justify greater soyafood consumption by Westerners. There are multiple mechanisms by which soyafoods can exert physiological effects although most focus has been specifically on isoflavones. The main area of exception is cholesterol reduction, where it is thought protein is the hypocholesterolemic component of the soyabean. Isoflavones exert both hormonal and non-hormonal effects under a variety of experimental conditions. There are intriguing data indicating soyafoods can be especially beneficial to postmenopausal women.

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PLENARY

MARCH 3

Perspectives on the impact of biotechnology on soybean production and utilization

R. F. Wilson[1]

Abstract

This paper presents a perspective on the impact of biotechnology in meeting the World's demand for soybean and soybean products, both now and in the future. Following that theme, my comments will focus on the following points.

- First, there is overwhelming science-based evidence that genetically engineered soybeans on the market today are as safe to eat as their conventional counterparts.
- Second, the benefits of any new technology will be accompanied by risks. Finding and maintaining the appropriate balance between risk and benefit is a dynamic challenge.
- Third, National and International regulatory policies have profound impact on the rate of advancement in the biotechnological sciences, as well as the trade of biotechnological products. These policies may reassure consumers in the safety of biotech-foods, but also may be perceived to be barriers to trade.
- Beneficial innovation in the genetic sciences will be an outcome of the political and social debate on biotechnology. These innovations will embody breakthroughs that should significantly stimulate continued growth in soybean production and utilization.

Discussion

Benefits and risks associated with GE soybeans:
The rate by which GE crops have been adopted worldwide is an impressive story, growing at a linear rate of about 24 million acres planted in GE crops per year (Pew Initiative, 2003). Only 6 years after the introduction of RoundUp Ready ® soybeans in commercial agriculture, 16 countries now report total GE crop production on about 145 million acres (58 million ha), or approximately 22% of the cultivated global land mass. Currently, four countries dominate the production of transgenic crops. The U.S. leads the world with 96 million acres (39.0 million ha); Argentina follows with 33

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million acres (13.5 million ha); Canada has 9 million acres (3.5 million ha) and China reports 5 million acres (2.1 million ha).

Presently, two major transgenic traits have been deployed in commercial soybean production. These are GE herbicide-resistance (primarily to the compounds glyphosate & glyphosinate) and GE insect resistance derived from *Bacillus thuringensis*. In 2003, the USDA National Agricultural Statistics Service estimated that U.S. farmers planted 60 million acres of GE soybean which is 81% of U.S. soybean acres (Figure 1). Approximately 55 million of those acres were planted with RoundUp Ready ® soybeans (NASS, 2003). In comparison, U.S. farmers planted 32 million acres of GE corn. Obviously, the high level of U.S. adoption of GE soybeans suggests that this technology provides tangible net economic benefits at the farm level. Such benefits generally are attributed but not limited to: improved yield, better weed control, higher crop quality and reduced tillage costs.

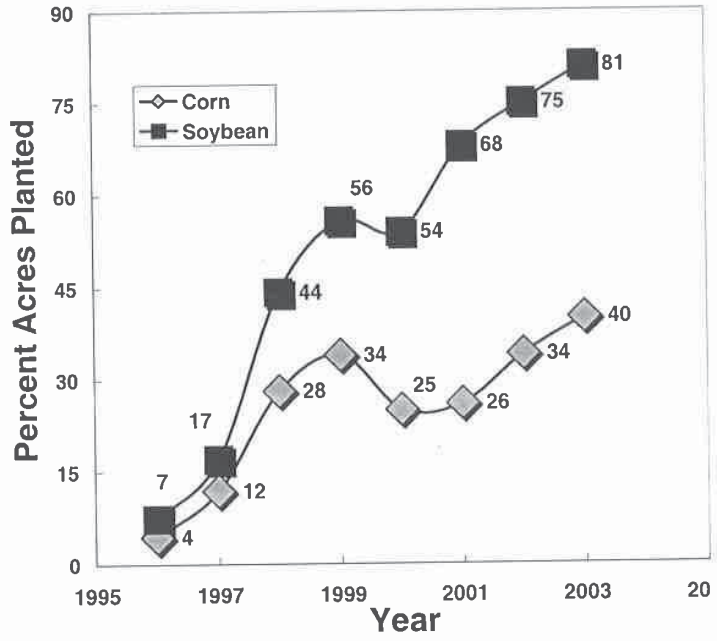


Figure 1. Adoption of Genetically Engineered Soybean and Corn in the United States.

The influence of GE and conventional soybeans on the global soybean supply may be inferred from Figure 2, which shows an upward inflection point in 1996 (the year RoundUp Ready® soybeans entered commercial markets). In the period from 1984 to 1996, world soybean supply (production plus previous year end-stocks) grew at a steady rate of 3.2 million metric tons (MMT) per year (FAS, 2003). After 1996, world soybean supply has grown at a linear rate of 11.6 MMT per year, nearly a 4-fold increase. It must be noted that global soybean acres planted also increased significantly during that period. However, the post-1996 global rate of increase in planted acres was only 2-fold greater than the pre-1996 rate. Thus, there is high probability that at least half of the post-1996 rate of increase in world supply may be attributed to genetic gain in yielding ability and GE soybeans.

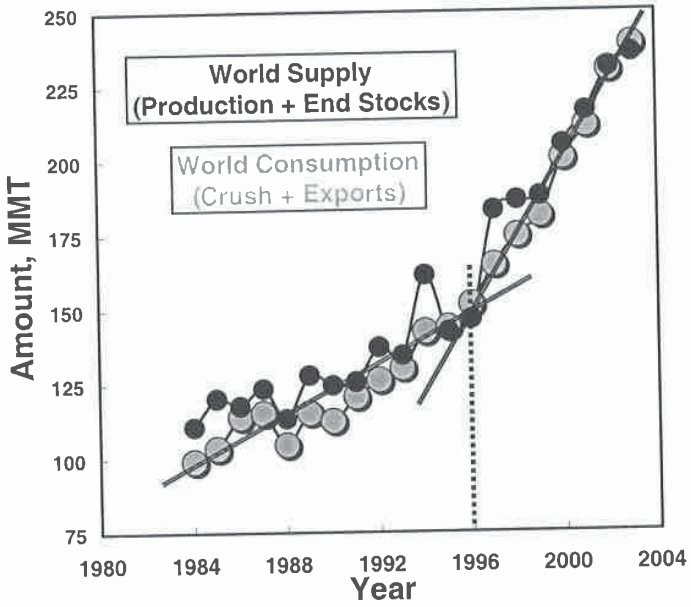


Figure 2. USDA Estimates of Global Supply and Demand for Soybeans

It also is important to note that the trend in global demand (crush plus exports) for soybeans tracks very close to global supply, especially in the past 3 years. Given the intensity of the biotechnology debate, one might have expected a significant divergence between supply and demand. Although wide fluctuation in soybean end-stocks may exist in certain countries, there apparently is little negative effect of GE traits on the global

trend in consumption of soybeans. In fact, global soybean exports doubled, from 32 MMT in 1995 to 65 MMT in 2003.

Yet, certain shifts have occurred in the destination of soybean exports (Figure 3). Annually since 1996, soybean imports by the European Union (EU) and Asia together have averaged 79% of the world's soybean exports (FAS, 2003). However, USDA Foreign Agricultural Service data shows that there has been a precipitous decline in the relative share of soybean imports by the EU. Fortunately, this trend has been compensated by the expansion of soybean markets in Asia; and the volatility of these relations appears to be stabilizing. Still, the reason for the relative decline in EU soybean imports may be attributed to the level of confidence that consumers have for transgenic agricultural products. Many surveys conclude that consumer confidence in GE crops remains a function of how people perceive potential risks posed by a new technology. A typical poll (Moon & Balasubramanian, 2001), suggests that U.S. consumers are ambivalent about the development of GE crops, but are apprehensive about GE food products. This may be understandable considering the strength of some of the opinions that have been expressed about GE foods.

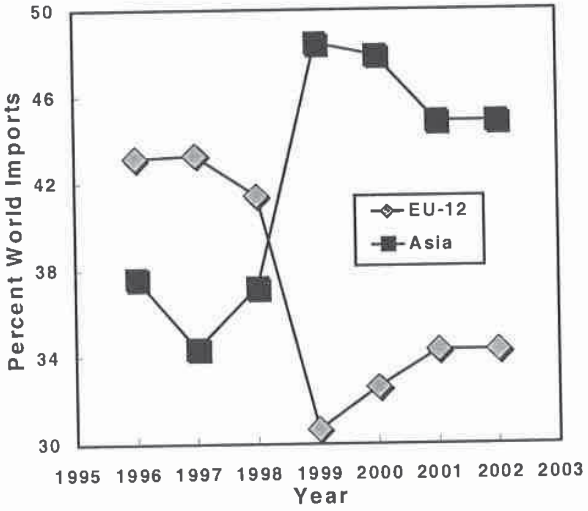


Figure 3. Relative Share of Global Soybean Imports in Asia and Europe.

However, the question of food safety is not taken lightly. Detailed studies to determine the safety of GE foods have been commissioned by many of the most reputable bodies of scientific knowledge around the world. These include: the National Academies of Science in the U.S., Brazil, China, India

and Mexico; the World Academy of Sciences; the Royal Society of London; the British and the American Medical Associations; and the International Council of Science (which is funded by the European Union Parliament). Each of these investigations has concluded that GE crops in commercial production today "are safe to eat" (NRC, 2000). In addition, the findings of the International Council of Science are endorsed by the Codex Alimentarius Commission, and the Organization of Economic Cooperation & Development.

Of course that overwhelming consensus invites the proposition: If GE soybeans *per se* are not a health risk, can GE make soybeans safer? Evidence is beginning to emerge that may support that hypothesis. Consider the case for hypoallergenic soybeans (Herman, et al., 2003). Soybeans may possess as many as 15 different proteins that show some cross-reactivity to human immune system proteins from sensitive people with ingestion allergy. USDA-ARS research has shown that these allergenic symptoms may be reduced via two different GE approaches: 1) altering the structure of the allergenic portion of the molecule via site-specific mutagenesis, and 2) complete removal of the allergen via co-suppression or RNAi technology. Comparative analysis of over 1400 different polypeptides plus other characteristics indicate that these GE hypoallergenic soybeans are substantially equivalent to conventional soybean. Research is in progress to test the efficacy of these soybeans in mitigating gastrointestinal problems in neonatal pigs, a model system to study food allergies in humans. Preliminary results on piglet growth rates have been very encouraging.
Such innovation may add a health benefit to the environmental benefits derived from GE soybeans, which include: improved weed control with fewer herbicides and little herbicide carryover, reduced tillage costs and less erosion. However, these agronomic benefits are not immediately obvious to consumers who may have concerns about the longer-term impact of current and future transgenic crops. The main environmental and ecological issues are 'gene-flow' (transmission of pollen from GE to non-GE plants or wild relatives), and potential development of pest-resistance in native weed and insect populations. These concerns are researchable problems, and peer reviewed findings should be the basis for credible governmental policy and actions for dealing with the 'perceived risks' that may be associated with GE technology. Thus, governmental regulatory systems that mediate these policies and actions are a legacy of biotechnology. As these regulatory systems continue to develop, they will have indelible impact on future commercialization, trade and scientific advances in global agriculture.

National and international regulatory policy:
One of the earliest established governmental regulatory systems is the U.S. Coordinated Framework for Regulation of Biotechnology Products

(initiated in 1986). Three federal agencies, under separate Congressional legislation, share distinct responsibilities in this undertaking.

- USDA-APHIS (Animal & Plant Health Inspection Service) administers permits to conduct field and greenhouse research with GE plants, and the transportation and commercialization of GE products. APHIS makes decisions on GE issues such as: changes in crop nutritional composition, gene-flow, and allelopathy.
- Environmental Protection Agency (EPA) administers permits for biotechnology derived pesticides or new chemicals (Experimental Use, Food Tolerance or Exemption), and is responsible for product registration of commercial pesticides and herbicides. EPA makes decisions on GE issues such as: tissue specificity of gene expression, toxicity to other organisms, and the environmental fate of the GE agent.
- Federal Drug & Food Administration (FDA) evaluates safety assessments for potential toxins or novel allergens before companies may market GE foods. FDA also is responsible for regulation of GE product labeling, which now is voluntary in the U.S.

It is essential that the public has confidence in the regulatory process for GE products. Therefore, many reviews have been conducted to assess the credibility of the U.S. 'Framework'. A prominent assessment was conducted by the U.S. National Academy of Science, National Research Council (NRC). In its second report, NRC found that the scope and adequacy of first generation GE crop regulation has been very effective for over 10 years (NRC, 2000). However, the report also made several recommendations to help the 'Framework' prepare for the emergence of new transgenic plant varieties and novel 'Next-generation' GE products.

The recommendations of the NRC report have initiated improvements in this system. As an example, the decision process at APHIS is now more 'transparent' or open to scientific and public input. The other agencies also have strengthened the rigor of review of applications, ensured transparency, adapted the scope of regulations as needed, and improved inter-agency interaction to reduce the cost of regulation. In addition, USDA has expanded its Biotechnology Risk Assessment & Mitigation research program (in place since 1992). This program is implemented jointly by the Agricultural Research Service (ARS), through competitive grants administered by the Cooperative States Research Education & Extension Service (CSREES), and formerly by the Initiative for Future Agriculture & Food Systems (IFAFS). In FY03 the ARS program was funded at $21.5 million; and the CSREES Biotechnology Risk Assessment Research grant program was funded at $3 million. Currently, USDA has approved about 138 on-going scientific investigations of key biotechnology issues. The mission of the program is

to provide regulatory agencies, and the public, with independent information to verify potential problems, and to develop approaches that minimize or eliminate potential GE related risks. The current focus of this research includes: long-term effects on non-target organisms, strategies to minimize pest resistance to GE technology; methods for precise transgene insertion in the genome; and determining how transgenes may effect the expression of other genes.

The integrity of the U.S. regulatory system has been tested and improved in recent years by its response to issues such as 'bio-pharming' (USDA, 2003). As of this writing, USDA has not banned 'bio-pharming', but USDA-APHIS has agreed, under existing authority, to increase field test site inspections, enforce buffer zones around field sites, require dedicated use of farm equipment and facilities for growing and handling 'bio-pharm' materials, and maintain a one-year moratorium on food crops in fields where 'bio-pharm' plants have grown. In addition, FDA, in the Department of Health & Human Services, has maintained a 'zero tolerance' policy on contamination of the food and feed supply by GE crops that are intended for bio-production of pharmaceuticals and vaccines. In an example of interagency cooperation, both APHIS and FDA aggressively pursued a company for permit violations when inspectors discovered soybeans in storage that had been harvested from an experimental field plot with volunteer corn plants that were reportedly designed to produce a type of swine vaccine. The company has paid a civil penalty of $250,000; will reimburse USDA for costs to retrieve and destroy 500,000 bushels of contaminated soybeans; and has destroyed 155 acres of corn growing around the experimental field site. Because of the regulatory actions taken in this incident, the contaminated seed never reached the human or animal food stream.
In other cases, EPA approved a new GE rootworm resistant type of corn, with the requirement that the company ensure that 20% of the surrounding corn acreage be planted in conventional corn varieties. Recent evidence suggests that this management strategy has been effective in minimizing the risk of insect populations which become immune to the resistance agent. As a final example, APHIS recently blocked approval of a new herbicide resistant type of 'creeping bent grass', on the basis that the species already was considered to be an invasive weed in many natural areas, parks and lawns where it was unwanted. Other examples could be cited as evidence of an active and effective U.S. Framework for regulation of biotechnological products.

Of course the U.S. is not the only country to develop a regulatory system for products of biotechnology. The Canadian Food & Drug Act, for instance, provides authority for regulation of Plants with Novel Traits (PNT; Plant

Biosafety Office, 2003). By definition, these are plants that have no obvious equivalent to distinct populations of cultivated species known to be present in Canada. For example, soybeans with very high-oleic concentration would be considered a PNT.

The framework for this regulatory system is the responsibility of two separate Canadian agencies: Canadian Food Inspection Service (CFIS) and Health Canada. CFIS has authority to regulate products under the Canadian Feeds Act, the Plant Protection Act, Health of Animals Act, Seeds Act and Fertilizers Act. Health Canada is responsible for setting standards for the safety of the food supply, including 'novel foods' developed from plants with novel traits.

This regulatory process begins with an import permit, and approval for unconfined release of the product in Canada. Then a Decision Document determines the approval status based on evidence presented in: variety registration materials, experimental trials demonstrating the product is safe for animal and, if applicable, human consumption.

What makes Canada's Plant Novel Trait regulations novel, is that Canada is the only country that requires regulatory approval of conventionally developed crops. In other words, plants with novel traits that are derived from natural plant selection, mutagenesis or genetic engineering are considered equivalent in terms of potential risk. Although 90+% of the PNT approvals to date are for transgenic events, phenotypic variation that transcends an accepted norm or Codex standard in any crop, regardless of how it was attained, is subject to regulation. The implications of is policy likely will constrain the development of enhanced germplasm by public institutions and private companies who wish to commercialize PNT varieties or PNT derived agricultural products in Canada. As reported by one company seeking approval for a crop variety with natural genetic resistance to certain herbicides, the PNT approval process can be a lengthy, less than transparent, and trials required to finalize a decision may be quite costly. Thus, the cost of attaining regulatory approval, under any system, may become a deciding factor that mediates the rate of advancement in agricultural biotechnological science.

Regulatory laws recently passed by the European Union Parliament reinforce constraints on soybean imports posed by the EU's 5-year moratorium on GE crops. Recognizing that the moratorium was not legally sustainable, the European Council of Ministers enacted legislation that establishes a comprehensive mandatory labeling and traceability regime for GE products (Europa, 2003). Under this legislation, all products from sources with 0.9% or more GE material must be labeled, regardless of the ability to detect

DNA or protein in the product. In addition, exporters of GE products (except for cheese and wine) will be liable for documenting the origin and composition of agricultural commodities at each step of the production cycle. This information may give consumers the option to choose among alternative agricultural food and feed products. However, it also may be regarded as a barrier to fair trade, because market competition may be impeded by the added cost and logistics necessary for compliance. American producers also argue that there is no scientific basis to suppose that the GE food products on the market today are more risky or substantially different from other food and feed products. Indeed, the safety of GE soybeans has been tested by time and volume. On a cumulative basis from 1996 to 2003, empirical estimates indicate that GE soybeans accounted for about 48% of the soybean products consumed in the U.S. Conservatively, that equates to about 360 billion pounds [66 billion pounds (30 MMT) oil and 290 billion pounds (130 MMT) meal] of oil and meal crushed from GE soybeans produced in the U.S. (FAS, 2003).

Every nation has a sovereign right to regulate technology, protect its people and preserve the environment. By 2004, 35 countries covering half of the world's population are expected to require mandatory government safety assessments before GE products are allowed in their markets. The EU likely will defend its regulatory framework with the argument that standards set by the Codex Alimentarius Commission, the UN food standards agency, support strong regulations for GE foods (Codex, 2002). These standards provide detailed guidance for determining if GE food contains new toxins or allergens, is altered nutritionally, or exhibits unexpected effects. They also establish a baseline that GE products should be 'as safe as their conventional counterparts'; and endorse the use of 'product tracing' as a tool of risk management. For reference, the Codex Alimentarius is a joint effort of the World Health Organization (WHO) and the Food & Agriculture Organization (FAO). Standards adopted by the Codex Alimentarius are automatically considered to be based on science and immune from challenge at the World Trade Organization (WTO).

The question of whether the EU Framework constitutes a barrier to fair trade has been brought before the WTO. Pending that decision, it should be noted that the Codex Alimentarius standard for GE food represents only one of 15 international instruments that may be used to settle trade disputes (CBD, 2003). Seven of those regulatory laws are legally binding, including the recently enacted (11 Sept. 2003) 'Cartagena Protocol on Biosafety to the Convention on Biological Diversity' which provides a viable forum for prospective and member countries to jointly consider procedures that address safe transfer, handling and use of living GE materials.

Adaptive change in the future application of biotechnology

As international regulatory systems continue to develop, the main factors that probably will guide future development and use of GE soybeans will be Labeling, Legislation and Litigation. These constraints will significantly influence how GE technology and resources are invested in varietal enhancement. The trend toward higher costs of regulatory approval probably will restrict the commercial application of GE research on soybeans with 'next-generation' transgenic improvements. In view of this apparent impediment, innovative scientists already are finding alternative ways to apply biotechnology toward the resolution of critical problems. Such advances in genetic engineering include: development of selectable marker-free transgenic soybean lines, multiple gene delivery systems, transformation and regeneration of elite cultivars, and more precise genomic insertion of transgenes. However, use of transformation technology to discover gene function, determine genome structure, and develop sophisticated molecular markers for specific genes is becoming the major application of genetic engineering. Hence, the role genetic engineering plays in soybean genomics is opening wider avenues for solving critical problems that effect soybean production and application through more effective use of natural or induced genetic diversity in the soybean genome.

The consumer-driven trend to replace 'trans-fat' in food products is an example of a critical emerging problem that will negatively impact soybean oil consumption. Trans-isomers of unsaturated fatty acids, a product of hydrogenation in refined soybean oil, are equated with the possible health risks associated with dietary levels of saturated fat (Institute of Medicine, 2002). One remedy for this problem is to develop soybeans with genetic traits that lower total saturated fat, increase levels of oleic acid, maintain sufficient linoleic acid to preserve flavor quality, and significantly reduce levels of linolenic acid. Such an oil should not require hydrogenation, and will give manufacturers greater flexibility in producing 'low saturated, low-trans' food products.

In two different ways, genetic engineering is involved in the development of germplasm that will help the soybean industry comply with FDA guidelines for labeling the composition of food products. First, soybean transformation enabled the production of transgenic germplasm with high-oleic acid concentration, a trait that may help reduce trans-isomers in foods. Second, transformation technology has facilitated the discovery of mutations or polymorphisms, and molecular markers for those mutations, in genes at Quantitative Trait Loci (QTL) in the soybean genome. QTL markers are genomic tools that not only speed breeding progress, but also to direct the

assembly of DNA sequences in genome mapping. Currently, there are about 2000 molecular markers for QTL in the soybean genome (Chung, et al., 2003). Several of these markers are associated with gene mutations that govern the fatty acid composition of soybean oil.

The ability to locate, and the capacity to associate a gene sequence in QTL with a gene function and a phenotype has significantly moved the science of soybean enhancement forward at a much more rapid pace. For example, we now recognize and can distinguish several different isoforms of each gene that determines palmitic, oleic and linolenic acid concentration in soybean (Wilson, 2003). It is likely that seven separate genes, each with a recessive mutation, must be combined to achieve soybean oil with less than 7% total saturated fat, 55 to 65% oleic acid, enough linolenic acid to maintain acceptable flavor, and less than 3% linolenic acid. However, less than 0.01% of the progeny in a segregating F2 population may exhibit this phenotype, and have all seven homozygous recessive genes. The use of molecular markers for each isoform of the seven genes substantially increases the selection efficiency of progeny containing multiple or 'stacked' recessive genes because DNA screening helps increase the frequency of plants in the next generation population that may exhibit the desired phenotype. This approach will help expedite the development of non-transgenic low-saturated mid-oleic low-linolenic acid soybean varieties that are adapted to each maturity zone. In this manner, genetic engineering contributes to soybean enhancement and the solution of emerging problems in a way that probably will not require stringent regulatory approval, except perhaps in Canada.

Summary

In conclusion, the science of agricultural biotechnology will continue to evolve and adapt to solve emerging needs in the oilseeds industry. Development of the regulatory process will keep pace, with 'transparency' to maintain and build consumer confidence in the products of biotechnology. However, the fulcrum for risk/benefit decisions on commercial development of advanced technology likely will be the cost of regulatory approval. As a cost-effective response, more attention and resources will likely be directed toward innovative applications of genetic engineering in 'genomic' approaches to solve critical problems in an acceptable manner. Thus, in one way or another, agricultural biotechnology will continue to play a beneficial role in the evolution of world agriculture, and will help ensure a plentiful and safe supply of soybeans and soybean products.

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PLENARY

MARCH 4

Global environmental benefits of soil carbon management: soybean concerns

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Abstract

Agricultural carbon (C) sequestration may be one of the most cost-effective ways to slow processes of global warming. Numerous environmental benefits may result from agricultural activities that sequester soil C and contribute to environmental security. As part of no-regret strategies, practices that sequester soil C help reduce soil erosion and improve water quality and are consistent with more sustainable and less chemically dependent agriculture. While we learn more about soil C storage and its central role in direct environmental benefits, we must better understand soybean contributions to the secondary environmental benefits and what they mean to production agriculture. Increasing soil C storage can increase infiltration, increase fertility and nutrient cycling, decrease wind and water erosion, minimize compaction, enhance water quality, decrease C emissions, impede pesticide movement and generally enhance environmental quality. The sum of each individual benefit adds to a total package with major significance on a global scale. Incorporating C storage in conservation planning with soybean presents some challenges, but demonstrates concern for our global resources and presents a positive role for soil C that will have a major impact on our future quality of life.

Key Words: soil organic matter, soil quality, environmental quality, conservation tillage, zero tillage, direct seeding, carbon sequestration

Introduction

Conservation agriculture aims to conserve, improve and make more efficient use of natural resources through integrated management of available soil, water and biological resources combined with external inputs. Conservation agriculture contributes to global environmental conservation as well as to enhanced and sustained agricultural production and can play a central role in global agricultural policy. Food security and sustainability of soybean production are important for all citizens. Agriculture, the major industry for food and fiber production, is known to cause emission and storage of

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greenhouse gases. Intensification of agricultural production has been an important factor influencing greenhouse gas emission. Agricultural activities contribute to carbon dioxide (CO_2) emissions to the atmosphere through the combustion of fossil fuel, soil organic matter (SOM) decomposition and biomass burning. Improved conservation agricultural practices have great potential to increase soil carbon (C) sequestration and decrease net emissions of CO_2 and other greenhouse gases that contribute to global environmental security.

World soils are an important pool of active C and play a major role in the global C cycle and have contributed to changes in the concentration of greenhouse gases in the atmosphere. Agriculture is believed to cause some environmental problems, especially related to water contamination, soil erosion, and greenhouse effect (Houghton, Hackler & Lawrence, 1999; Schlesinger 1985; Davidson & Ackerman, 1993). The soil contains two to three times as much C as the atmosphere. In the last 120 years, intensive agriculture has caused a C loss between 30 and 50%. Recent results suggest scientific agriculture can also lessen environmental problems and mitigate the greenhouse effect. In fact, agricultural practices have the potential to enhance soil quality by storing more C in the soil than farming emits through land use change and fossil fuel combustion (Lal et al., 1998). Soil quality is largely governed by SOM content, which is dynamic and responds effectively to changes in soil management, primarily tillage and C input. This review will primarily address soil C and its associated environmental benefits while raising research concerns about intensive soybean production.

Key role of soil organic matter

Soil organic C represents a key indicator for soil quality, both for agricultural functions (production and economy) and for environmental functions (C sequestration and air quality). Soil organic matter is the main determinant of biological activity because it is the primary energy source. The amount, diversity and activity of soil fauna and microorganisms are directly related to SOM content and quality. Organic matter and the biological activity that it generates, have a major influence on the physical and chemical properties of the soils. Soil aggregation and stability of soil structure increases with increasing organic C. These factors in turn increase the infiltration rate and available water holding capacity of the soil as well as resistance against erosion by wind and water. Soil organic matter also improves the dynamics and bio-availability of main plant nutrient elements.

Soils contain relatively small amounts of C that could be considered analogous to a catalyst for biological activity where a small amount has a big impact. Farmers are the primary soil managers who each have a

tremendous responsibility to maintain SOM for environmental benefit of the global population. Thus, farmers who use conservation agriculture or direct seeding techniques are providing ecosystem services and helping to maintain environmental quality for all of society. Quality food production and economic and environmentally-friendly management practices that are socially acceptable will lead to sustainable production and be mutually beneficial to farmers and all of society. It is important, therefore, that C loss from the soil system through historical land use of farming practices be restored to its natural potential using direct seeding and conservation tillage methods for sustainable production.

A case for conservation agriculture and zero tillage

Tillage or soil preparation has been an integral part of traditional agricultural production. Tillage is also a principle agent resulting in soil perturbation and subsequent modification of the soil structure with soil degradation. Intensive tillage loosens soil, enhances the release of soil nutrients for crop growth, kills the weeds that compete with crop plants for water and nutrients and modifies the circulation of water and air within the soil. Intensive tillage can adversely affect soil structure and cause excessive break down of aggregates leading to potential soil movement via erosion. Intensive tillage causes soil degradation through C loss and tillage-induced greenhouse gas emissions that impact productive capacity and environmental quality.

Recent studies involving a dynamic chamber, various tillage methods and associated incorporation of residue in the field indicated major C losses immediately following intensive tillage (Reicosky & Lindstrom, 1993, 1995). The moldboard plow had the roughest soil surface, the highest initial CO_2 flux and maintained the highest flux throughout the 19-day study. High initial CO_2 fluxes were more closely related to the depth of soil disturbance that resulted in a rougher surface and larger voids than to residue incorporation. Lower CO_2 fluxes were caused by tillage associated with low soil disturbance and small voids with no-till having the least amount of CO_2 loss during 19 days. The large gaseous losses of soil C following moldboard plowing compared to relatively small losses with direct seeding (no-till) have shown why crop production systems using moldboard plowing have decreased SOM and why no-till or direct seeding crop production systems are stopping or reversing that trend. The short-term cumulative CO_2 loss was related to the soil volume disturbed by the tillage tools. This concept was explored when Reicosky (1998) determined the impact of strip tillage methods on CO_2 loss after five different strip tillage tools and no-till. The highest CO_2 fluxes were from the moldboard plow and subsoil shank tillage. Fluxes from both slowly declined as the soil dried. The least

CO_2 flux was measured from the no-till treatment. The other forms of strip tillage were intermediate with only a small amount of CO_2 detected immediately after the tillage operation. These results suggested that the CO_2 fluxes appeared to be directly and linearly related to the volume of soil disturbed. Intensive tillage fractured a larger depth and volume of soil and increased aggregate surface area available for gas exchange that contributed to the vertical gas flux. The narrower and shallower soil disturbance caused less CO_2 loss suggests that the volume of soil disturbed must be minimized to reduce C loss and impact on soil and air quality. The results suggest environmental benefits and C storage of strip tillage over broad area tillage that needs to be considered in soil management decisions.

Reicosky (1997) reported that average short-term C loss from four conservation tillage tools was 31% of the CO_2 from the moldboard plow. The moldboard plow lost 13.8 times more CO_2 as the soil not tilled while conservation tillage tools averaged about 4.3 times more CO_2 loss. The smaller CO_2 loss from conservation tillage tools was significant and suggests progress in equipment development for enhanced soil C management. Conservation tillage reduces the extent, frequency and magnitude of mechanical disturbance caused by the moldboard plow and reduces the large air-filled soil pores to slow the rate of gas exchange and C oxidation.

Environmental benefits of soil carbon

The main direct benefit of conservation agriculture or direct seeding is the immediate impact on SOM and soil C interactions. Soil organic matter is so valuable for what it does in soil; it can be referred to as "black gold" because of its vital role in physical, chemical and biological properties and processes within the soil system. Agricultural policies are needed to encourage farmers to improve soil quality by storing C that will also lead to enhanced air quality, water quality and increased productivity as well as to help mitigate the greenhouse effect. Soil C is one of our most valuable resources and may serve as a "second crop" if global C trading systems become a reality. While technical discussions related to C trading are continuing, there are several other secondary benefits of soil C impacting environmental quality that should be considered to maintain a balance between economic and environmental factors.

Soil C is so important that it can be compared to the central hub of a wagon wheel that represents a circle, which is a symbol of strength, unity and progress. The "spokes" of this wheel represent incremental links to soil C that lead to the environmental improvement that supports total soil resource sustainability. Many spokes make a stronger wheel. Each of the

secondary benefits that emanate from soil C contributes to environmental enhancement through improved soil C management. Soane (1990) discussed several practical aspects of soil C important in soil management. Some of the "spokes" of the environmental sustainability wheel are described in following paragraphs.

Increased SOM has a tremendous effect on soil water management because it increases infiltration and the water holding capacity. The primary role of SOM in reducing soil erodibility is by stabilizing the surface aggregates through reduced crust formation and surface sealing, which increases infiltration (Le Bissonnais, 1990). Enhanced soil water-holding capacity is a result of increased SOM that more readily absorbs water and releases it slowly over the season to minimize the impacts of short-term drought. In fact, certain types of SOM can hold up to 20 times its weight in water. Hudson (1994) showed that for each one % increase in SOM, the available water holding capacity in the soil increased by 3.7% of the soil volume. The extra SOM prevents drying and improves water retention properties of sandy soils. In all texture groups, as SOM content increased from 0.5 to 3%, available water capacity of the soil more than doubled. Other factors being equal, soils containing more organic matter can retain more water from each rainfall event and make more of it available to plants. This result plus the increased infiltration with higher organic matter and the decreased evaporation with crop residues on the soil surface all contribute to improve water use efficiency.

Ion adsorption or exchange is one of the most significant nutrient cycling functions of soils. Cation exchange capacity (CEC) is the amount of exchange sites that can absorb and release nutrient cations. Soil organic matter can increase CEC of the soil from 20 to 70% over that of the clay minerals and metal oxides present. In fact, Crovetto (1996) showed that the contribution of the organic matter to the cation exchange capacity exceeded that of the kaolinite clay mineral in the surface 5 cm of his soils. Robert (1996) showed a strong linear relationship between organic C and CEC of his experimental soil. The CEC increased four-fold with an organic C increase from 1 to 4%. The toxicity of other elements can be inhibited by SOM, which has the ability to adsorb soluble chemicals. The adsorption by clay minerals and SOM is an important means by which plant nutrients are retained in crop rooting zones.

Soils relatively high in C, particularly with crop residues on the soil surface, are very effective in increasing SOM and in reducing soil erosion loss. Reducing or eliminating runoff that carries sediment from fields to rivers and streams will enhance environmental quality. Under these situations,

the crop residue acts as tiny dams that slow down the water runoff from the field allowing the water more time to soak into the soil. Worm channels, macropores and plant root holes left intact increase infiltration (Edwards, Shipitalo & Norton, 1988). Water infiltration is two to ten times faster in soils with earthworms than in soils without earthworms (Lee, 1985). Soil organic matter contributes to soil particle aggregation that makes it easier for the water to move through the soil and enables the plants to use less energy to establish to root systems (Chaney & Swift, 1984). Intensive tillage breaks up soil aggregates and results in a dense soil making it more difficult for the plants to get nutrients and water required for their growth and production.

The reduction in soil erosion leads to enhanced surface and ground water quality, another secondary benefit of higher SOM. Crop residues on the surface help hold soil particles in place and keep associated nutrients and pesticides on the field. The surface layer of organic matter minimizes herbicide runoff, and with conservation tillage, herbicide leaching can be reduced as much as half (Braverman et al., 1990). The enhancements of surface and ground water quality are accrued through the use of conservation tillage and by increasing SOM. Increasing SOM and maintaining crop residues on the surface reduces wind erosion (Skidmore, Kumar & Larson, 1979). Depending on the amount of crop residues left on the soil surface, soil erosion can be reduced to nearly nothing as compared to the unprotected, intensively tilled field.

Another key factor is SOM that can decrease soil compaction (Angers & Simard 1986; Avnimelech & Cohen, 1988). Soane (1990) presented different mechanisms where soil "compactibility" can be decreased by increased SOM content: 1) improved internal and external binding of soil aggregates; 2) increased soil elasticity and rebounding capabilities; 3) dilution effect of reduced bulk density due to mixing organic residues with the soil matrix; 4) temporary or permanent existence of root networks; 5) localized change electrical charge of soil particles surfaces, 6) change in soil internal friction. While most soil compaction occurs during the first vehicle trip over the tilled field, reduced weight and horsepower requirements associated with forms of conservation tillage can also help minimize compaction. Additional field traffic required by intensive tillage compounds the problem by breaking down soil structure. The combined physical and biological benefits of SOM can minimize the affect of traffic compaction and result in improved soil tilth.

Maintenance of SOM contributes to the formation and stabilization of soil structure. Another spoke in the wheel of environmental quality is improved soil tilth, structure and aggregate stability that enhances the gas exchange

properties and aeration required for nutrient cycling (Chaney & Swift, 1984). Critical management of soil airflow with improved soil tilth and structure is required for optimum plant function and nutrient cycling. It is the combination of many little factors rather than one single factor that results in comprehensive environmental benefits from SOM management. The many attributes suggest new concepts on how we should manage the soil for the long-term aggregate stability and sustainability.

A secondary benefit of less tillage and increasing SOM is reduced air pollution. CO_2 is the final decomposition product of SOM and is released to the atmosphere. Research has shown that intensive tillage, particularly the moldboard plow, releases large amounts of CO_2 as a result of physical release and enhanced biological oxidation (Reicosky et al., 1995). With conservation tillage, crop residues are left more naturally on the surface to protect the soil and control the conversion of plant C to SOM and humus. Intensive tillage releases soil C to the atmosphere as CO_2 where it can combine with other gases to contribute to the greenhouse effect. Thus a combination of the economic benefits of conservation tillage through reduced labor requirements, time savings, reduced machinery costs and fuel savings, combined with the environmental benefits listed above has universal appeal. Indirect measures of social benefits as society enjoys a higher quality of life from environmental quality enhancement will be difficult to quantify. Conservation agriculture, using direct seeding techniques, can benefit society and can be viewed as both "feeding and greening the world" for global sustainability.

Environmental concerns of soybean production

Movement towards more conservation agriculture systems with intensive cropping sequences can contribute to soil C sequestration and environmental quality. Unfortunately, some soybean physical and chemical properties often limit the plant's ability to protect the soil and maintain the soil resource for long-term sustainability. Environmental concerns with intensive soybean production need to be addressed with respect to soil C sequestration and water quality issues. Soybean has long been considered a crop that accentuates soil erosion. Soil erosion in the United States has increased in the last 40 years principally because the increase in row crop area and partially due to the amount of soybean grown in the various rotations. During five years of research in Iowa, USA, Moldenhauer and Wischmeier (1969) found that soil loss after soybeans was greater than after corn. If other practices are the same, soil erosion using a rainfall simulator was frequently greater after soybean rather than after corn (Siemens and Oschwald, 1976; 1978; Oschwald and Siemens, 1976). They attributed the increased soil and water loss after soybean to a less stable soil surface

and smaller amounts of residue. Laflen and Moldenhauer (1985) found that soil losses were greater following soybean than corn in either a corn-soybean rotation or in continuous corn. Similar findings in Africa were reported by Lal (1985).

In South America, Cogo (1985) suggested several known effective erosion control practices. Conservation tillage systems can reduce soil losses substantially to maintain soil sustainability for the long term. Uses of mulches of crop residues and cover crops have also been beneficial showing tremendous reductions in soil loss from the physical protection of the cover crop and carbon input. Diverse crop rotations also show benefits in decreasing erosion and helping maintain a productive and sustainable system. There is continued need for research on erosion control including a better understanding of the rainfall characteristics, soil erosion vulnerability after soybeans, tillage methods used, management practices and soil losses that can be acceptable.

Soybeans leave little crop residue to protect the soil and the soybean residue has that has a low C: nitrogen (N) ratio decomposes rapidly and leaves the soil bare and vulnerable to erosion very quickly after soybean harvest. Thus, the amount of residue cover for protection of the soil surface from raindrop impact is small for soybean. There is definite need for research to establish a wider C:N ratio so that the soybean residue can maintain soil cover for a longer period of time. During tillage, the soil surface is often left more rough and cloddy following corn and cereals than soybean. In fine or medium-textured soils, the soil is often more mellow, less cloddy and contains small aggregates following soybean in the contrast to corn. The small aggregates maybe susceptible to dispersion and sealing of the soil surface. Fahad et al. (1982) found that the rank of order of geometric mean diameter of the soil aggregates for several soybean crop sequences was continuous soybean < soybean after corn < soybean after sorghum < soybean after fallow. The surface sealing enhances runoff and the dispersed soil material may be readily transported in water runoff. All these factors contribute to the usually observed greater runoff and erosion following soybean as contrasted to corn or cereals.

Layese et al. (2002) summarized a comprehensive study using natural abundance 13C to study the effect of tillage-residue management, N fertilization and crop change on soil organic C dynamics. They evaluated three tillage systems: no till, moldboard plow and chisel plow and two residue options: either returned or removed and two N rates. Corn was grown for 13 years followed by four years of soybean with no fertilizer added to soybean in the rotation. They found a 24% reduction in soil

organic C between year 13 and year 17 after soybean had been grown for four years. After four years of soybean, the percentage of soil organic C derived from soybean i.e., the current C content ranged from -5.8 to +26.6% of the current C with most of that C in the 0-5 cm depth of the no till treatment and the chisel plow treatment and at 25-30 cm depth under the moldboard plow treatment. These results suggest alternate strategies for growing soybeans that could include diversified rotations and cover crops that would replace the C that is depleted during soybean production.

The soybean rhizosphere effect was first identified in a study comparing four plant species (Fu and Cheng, 2002). Cheng et al. (2003) further studied the rhizosphere priming effects of soybean (Glycine max L. merr.) and spring wheat (Triticum aevestium L.) on soil organic matter distribution at four phenological stages under three levels of fertilization in greenhouse experiments using natural 13C tracers. They concluded that the soybean rhizosphere priming effect can substantially intensify residue decomposition. The residue priming effect was responsible for a major portion of the total soil C efflux from that system. Cumulative soil C loss caused by rhizosphere effects during the whole growing season equated to the amount of root biomass C for the soybean treatment and 71% of the root biomass C for the wheat treatment. The overall rhizosphere priming effect of soybean was significantly higher than for wheat and peaked at 380% above the no plant control during the late vegetative stage and remained at high levels onward. They also noted that NPK fertilization did not significantly modify the rhizosphere priming effect. The stronger rhizosphere effect of soybean may have been caused by the higher substrate quality of the soybean rhizo deposition because the N concentration of tissues is generally higher than tissues from non-legume plants. The N-rich compound usually produce stronger priming effects than compounds that are low or no N. This clearly demonstrates the role of plants as an important factor controlling soil organic matter decomposition through the rhizosphere processes and the important role of soybean in the pools and fluxes of C within agricultural ecosystems.

Research is needed on altering the shoot: root ratio of the soybean to put more of the root biomass below ground to enhance soil C sequestration. The enhanced rooting capability will increase the amount of bio-pores available for water and nutrient transfer in the soil. Carbon more deeply distributed with root systems will contribute to greater sequestration and improve soil properties that will lead to enhanced nutrient cycling and improved crop yields. All of these factors can contribute to soybean maintaining the soil resource and are very important in maintaining environmental quality.

Farmers using conservation techniques stand to gain from protecting the environment because it is in their fundamental economic interest to conserve natural resources for the future. It is in all our economic interests to have healthy and sustainable ecosystems to enhance our quality of life. The true economic benefits of soybean can only be determined when we assign monetary values to externalities of environmental quality. Conservation agriculture without intensive tillage in soybean production can play a major role in sequestering soil C and providing long-term global economic and environmental benefits. Conservation agriculture with enhanced soil C management is a win-win strategy. Agriculture wins with improved food and fiber production systems and sustainability. Society wins because of the enhanced environmental quality. The environment wins as improvements in soil, air and water quality are all enhanced with increased amounts of soil C. The win-win scenario will increase productivity, improve soil quality, and mitigate the greenhouse effect with major impact on our future quality of life.

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Soybean processing and products research: progress contributed by the U.S. Department of Agriculture

L. F. Flora[1]

Abstract

United States Department of Agriculture, and later Agricultural Research Service, researchers have contributed significantly to the food and non-food uses of soybeans. Most of the work was conducted at the four USDA/ARS regional (utilization) research laboratories in Peoria, IL, New Orleans, LA, Wyndmoor, PA, and Albany, CA. Early USDA contributions included improving the quality of soybean oil for food use and developing soyoil epoxide plasticizers and dimer resins. More recently, ARS researchers have developed printing inks, lubricants, composites, nutraceuticals, personal care products, glue, adsorbents, more stable and lower cost soy diesel, methods for improved oil stability without hydrogenation, and alternatives to hexane extraction of soybeans. Future efforts by ARS researchers, combined with collaborations with public and private sector interests, promise additional market opportunities for soybeans.

Introduction

Processed soybeans are the largest source of protein feed and vegetable oil in the world. The United States is the world's leading soybean producer and exporter. Farm value of U.S. soybean production in 2000/01 was $12.5 billion, the second-highest value among U.S.-produced crops, trailing only corn. Soybean and soybean product exports accounted for 43 percent of U.S. soybean production in 2000. Soybeans equal about 90 percent of U.S. total oilseed production, while other oilseeds – such as cottonseed, sunflowerseed, and peanuts – account for the remainder. (ERS, USDA, 2003)

Soybeans have grown from a minor forage crop little over 60 years ago to the second biggest row crop, the second biggest cash crop, and the biggest export crop for the United States. Soybeans are the number one source of vegetable oil and of oilseed meal for livestock and poultry. Many people and organizations have contributed to this success, including U.S. Department of Agriculture (USDA) researchers.

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The Agricultural Research Service (ARS) was created in 1953 as the primary in-house research agency of the USDA. Four regional research centers provide the major portion of ARS's capability for research and development of technology to increase the use of agricultural products and thereby enhance the economic viability and competitiveness of U.S. agriculture. These centers are: the National Center for Agricultural Utilization Research (NCAUR), located in Peoria, Illinois; the Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania; the Southern Regional Research Center (SRRC) in New Orleans, Louisiana; and the Western Regional Research Center (WRRC), in Albany, California. Authorization for the four USDA utilization centers was a part of the omnibus Agricultural Adjustment Act of 1938. In 1939, Congress appropriated $4 million to build the laboratories and by the end of 1940 or early 1941, the buildings had been completed and equipped, the first scientist employed, and research begun. The original purpose of the centers was to find new chemical and technical uses and markets for farm commodities, particularly those like wheat, cotton, milk, and potatoes, with "regular or seasonal surpluses." Although the centers have expanded their mission over the decades to address other important agricultural issues, they maintain core programs in agricultural utilization.

Food uses of soybean oil

Historical developments

In the 1940's, soybean oil made neither a good industrial paint nor a good edible oil. Shortages of cooking fats in World War II forced processors to add soybean oil to margarine, with an upper limit of 30%. The flavor of soybean oil in those days was variously described by consumers as "grassy" or "beany" or "fishy," and it tasted even worse after it had been stored for a while. USDA scientists at the Northern Regional Research Center (NRRC) (now the National Center for Agricultural Utilization Research [NCAUR]) in Peoria, IL, initiated the use of taste panels and had panelists rate the flavors of soybean oil with numerical values instead of with imprecise adjectives. These more objective methods for assessing flavor and odor meant that the ratings of panelists in one processing plant could be compared with those in another plant and that panel results could be reproduced elsewhere. While this early research seemed trivial, it turned out to be the first significant milestone in improving soybean oil.

Guided by judgments of taste panels, the Peoria researchers identified the source of many of the off-flavors in soybean oil as trace metals, particularly iron and copper. Even extremely small amounts of these contaminants sped oxidation of the oil, shortening its storage life and promoting undesirable flavors. Responding to these findings, industry removed brass valves in refineries and substituted stainless steel for the cold rolled steel in equipment that came in contact with soybean oil. These actions alone improved the flavor of the oil.

The discovery, at the Peoria lab after World War II, that citric acid would deactivate the trace metals in soybean oil soon led to finding many other chemicals that could do the job. Industry response was prompt and in the 1990's practically all soybean oil was protected during processing by citric acid.

Although metal contaminants could speed the development of flavors, linolenic acid was identified as the ultimate source of the off-flavors in soybean oil and the reason why soybean oil turned rancid on the shelf or when it was heated repeatedly in deep fryers. Its presence in soybean oil is about double the level in other vegetable oils, except for canola oil. Peoria investigators discovered a highly selective copper-chromium catalyst that would enable processors to remove much of the linolenic acid through hydrogenation. While some features of the NRRC process were adopted by the soybean industry, others were not due to high costs. But the NRRC discovery that it was linolenic acid that caused soybean oil to go bad spurred plant breeders in ARS and state research facilities to develop soybean lines with lower linolenic acid content or to develop lines that lack the enzyme responsible for oxidizing linolenic acid.

Another early research breakthrough came in the 1950's with discoveries about so-called hidden oxidation in soybean oil after it was deodorized. The cause was found to be oxidation compounds in the oils that decomposed during deodorization of the oil and formed new compounds that brought about undesirable changes in flavor and stability. Peoria researchers recommended certain alterations in processing the oil before deodorization that overcame the problem. From the 1950's on, production of soybeans and soybean oil – and exports – began to climb significantly.

In the mid-1980's, an NRRC team found that deterioration of the flavor of soybean oil exposed to light could be reduced by adding small amounts of beta-carotene. This discovery was also widely adopted (Kelley, 1993).

Recent developments

At the NCAUR, hydrogenation of both soybean oil and admixtures of soybean and high oleic soybean oil were carried out under higher pressures and lower temperatures than normally employed by the industry. This technology produced margarine/spread oils with 4-5% trans acids compared to the 10-11% found in commercial products. These findings may help the edible industry comply with impending FDA labeling regulations. Studies on the formulation of margarines/spreads from hi-stearic acid soybean oils have shown that products formulated from them have suitable physical and sensory properties, thus providing an outlet for these crops estimated to have a market value in excess of $130,000,000.

Also at the NCAUR, tocopherols, tocotrienols and phytosterols were found to have the greatest impact in inhibiting frying oil degradation; future studies will evaluate the role of these constituents in inhibiting frying oil and fried food degradation. In specific tests with gamma tocopherol, ferulic acid or oryzanol, researchers found that they each inhibited deterioration of both the fried food during storage and the frying oil. These results are of interest to oil processors and food manufacturers in determining the appropriate composition of oils such as soybean, sunflower, cottonseed, canola, and corn oils to maximize their quality for the 8 billion pound/year frying oil industry.

Edible oil research at the NCAUR on effects of fatty acid composition on oil quality has led to recommendations to plant breeders for new cultivars of soybeans. These oils that have improved stability to oxidation without hydrogenation include low linolenic acid soybean, high oleic soybean and the more recent mid-oleic soybean which has an ideal composition for frying.

More recently, ARS scientists in Wyndmoor, PA, developed the necessary technology to produce structured lipid products from commodity type fats and oils, in collaboration with a Cooperative Research and Development Agreement (CRADA) partner. These lipid products, which are formed by a combination of physical and enzymatic steps, have been shown in studies by the CRADA partner to have beneficial cholesterol-lowering effects when administered as dietary supplements to hypercholesterolemic subjects. A jointly owned patent application on this technology was prepared and filed by the collaborator.

Alternatives to hexane extraction

In the early 1980's, ARS scientists at the NRRC (now NCAUR) in Peoria, IL, began experimenting with supercritical carbon dioxide extraction of soybeans as a substitute for hexane extraction. Carbon dioxide is more environmentally friendly than hexane, less hazardous, and can be recycled. But costs of plant conversions and problems in processing large quantities of oilseeds have so far discouraged adoption of supercritical extraction in the vegetable oil industry. Current research at the NCAUR focuses on using liquid carbon dioxide to effectively remove solvents such as hexane from mixtures of hexane/soybean oil resulting from the solvent extraction of oilseeds. This separation process will be more energy efficient than current methods employing heat and vacuum separators and will reduce costs associated with oilseed extraction.

The U.S. Environmental Protection Agency (EPA) published a Maximum Achievable Control Technology (MACT) standards for the oilseed extraction industry in early 2001. The oilseed industry is expected to comply with the

hexane loss standards by 2005. This new regulation further prompts the industry to consider an alternative solvent to replace the commercial hexane used in their facility. Research at the SRRC in New Orleans, LA, suggested that commercial isohexane can be a good alternative solvent for hexane. Plant trials proved that isohexane can be used effectively and offers the opportunity to save energy, increase productivity and reduce the emission of n-hexane and have encouraged the oilseed extraction industry willingly to examine commercial isohexane in their oil mills or to convert to isohexane from hexane. As of 2003, it is estimated that more than 20% of the oilseed extraction capacity in the U.S. is already being converted from hexane to isohexane.

Industrial uses of soybean oil

Historical developments

During World War II, USDA scientists at the ERRC in Wyndmoor, PA, developed epoxidation technology for fats and oils, facilitating the manufacture of flexible vinyl plastics. Of the 50,000 tons per year of epoxidized ester plasticizers used annually for this purpose, 75-80% use soybean oil.

Research begun at the Peoria lab in the 1940's led to commercial production and use of polyamide resins prepared from dimer acids derived from soybean and other vegetable oils. A hard, transparent, thermoplastic resin used in lacquers and adhesives was produced during World War II using the USDA technology. Today, polyamide resins are used as hot-melt adhesives for shoe soles, book bindings, solders to close seams in cans, and packaging. The widely used 2-tube glues containing epoxy resins and polyamide curing agents also use polyamides developed from the USDA research. Other uses include moisture-proof coatings, paints for porous surfaces like concrete and cinder blocks, and special-purpose inks. U.S. production of dimer acids a decade ago was over 330,000 tons per year; over half of this is used for polyamides.

In the late 1960's, chemists in Peoria converted soybean or linseed oil derivatives into a versatile new raw material. Working under a USDA contract, a Minneapolis firm developed several ways to use the new material to produce a variety of industrial products. One was nylon 9, a tough plastic especially suited for use in electrical insulation and gears, bearings, cams, and similar parts.

ARS technology enabled the production of liquid branched-chain fatty acids such as "isostearic acid" from soy oil fatty acids. The branched products are used widely in cosmetic applications and in fine lubricants as substitutes for sperm whale oil.

Recent developments.

Technologies developed by ARS scientists at Peoria and currently undergoing commercial development include lithographic news ink, sheet-fed and heat-set inks consisting of pigments and 100% vegetable oil (soy oil)-based vehicles. A U.S. Patent has been awarded for news ink technology, and the potential domestic market is more than 500 million pounds per year for this technology. The inks degrade faster and more completely than either hybrid soy oil-based or petroleum-based inks. Also, comparison of de-inking properties and analysis of volatile organic chemicals (VOC) content showed the superiority of vegetable oil-based inks over petroleum resin based inks. For sheet-fed and heat-set ink, the market is more than 100 million pounds and 400 million pounds per year, respectively. Sheet-fed and heat-set web offset ink vehicles prepared in the absence of petroleum products were comparable to conventional mineral oil-based vehicles. A U.S. Patent has been awarded for sheet-fed and heat-set ink technologies. These ink technologies alone present an opportunity to triple the market. News ink technology has been licensed (non-exclusive) to an Oklahoma-based company and licensing and technology transfer activities are continuing (with great interest from domestic and international companies) for all three (news, sheet-fed, heat-set) ink technologies. A small company is in the process of completing the licensing application.

Epoxidized vegetable oils (largely epoxidized soybean oil, ESO) account for about 15% of the approximately one billion pounds of the current domestic plasticizer market. Recent proposed regulations would ban a dominant petrochemical-derived plasticizer, thereby elevating epoxidized oils to a potentially larger share of the plasticizer market. ARS scientists at the ERRC isolated peroxygenase enzyme from an inexpensive source, oat seeds (Avena sativa), and immobilized it onto synthetic membranes. The immobilized enzyme preparation can be used to epoxidize unsaturated fats and oils to produce high levels of epoxidized materials and can be reused. This new process provides an improved method for producing plasticizers from oils and fats. A patent was granted that covers the process used for producing these epoxy fatty acids and esters.

ARS researchers in Peoria prepared soybean oil-based composites by cross-linking epoxidized soybean oil with EPON resin and five different gelling agents and using Solid Free-forming Fabrication Method (SFF). SFF is a method of making shapes without molds. The combination of natural fibers with soybean oil will produce composites that will be cheaper than the alternatives available. In addition using SFF will open the door to a broad range of new applications, such as in automotive, construction, aircraft

and military industries. The U.S. Patent has been awarded and a CRADA with Advanced Ceramic Corporation has been established. ARS scientists at Peoria developed new biodegradable lubricants and hydraulic fluids made from soybean oil which have the industrial quality and performance characteristics of current petroleum-based lubricants and hydraulic fluids. These products are being performance tested with industrial partners. The U.S. Patent has been awarded. CRADA's with Caterpillar, Inc. and Alcoa have been established.

A biodegradable hydraulic fluid was sold commercially for the first time in 1997 by an industrial partner based on the estolide technology developed by ARS scientists at Peoria which can use several vegetable oils. An end-user has demonstrated that this material lowers emissions when used as an additive to vegetable oil-based lubricants.

A soy oil-based aircraft deicing agent has been formulated by Peoria researchers and preliminary test results are very promising. Further modification and testing is continuing. Scaled-up samples will be tested by Boeing Company for FAA certification in Seattle, WA.

ARS researchers at Peoria created a new "all-natural" sunscreen active ingredient, SoyScreen, derived from vegetable oil and other natural plant components (cinnamates). This patented product can be used to provide UVA and UVB protection from the sun. It can replace the synthetic chemicals currently used in sunscreen formulations as well as many other related cosmetic applications. This product represents a new and novel market for commodity vegetable oils and specialty plant-derived components. The work also highlights the great potential of unconventional solvents and enzymes to perform environmentally friendly chemistry. Further development of this product is being conducted with an industrial partner. A license application has been submitted.

Starch and vegetable oils (e.g., those derived from corn and soybeans) are biodegradable, non-toxic materials that are produced annually by American agriculture in billion pound quantities. Fantesk, a stable microemulsified composite of oil in a starch-water matrix, was developed and patented by ARS researchers in Peoria and has food and non-food applications. Its fat mimicking properties coupled with its inherent emulsifying and encapsulating properties make it ideal for many commercial applications. Foods such as ice cream, salad dressings and processed meats have been prepared successfully under license agreement. One licensee of this technology is now marketing a seed coating formulation to inhibit water uptake of seeds during cold weather planting, a CRADA partner is testing another formulation in oil well drilling muds, another is testing formulations in moisturizing

lotions and barrier creams, and yet another is testing it as an industrial adhesive and lubricant.

Using soy-based glue formulations developed by ARS researchers at Peoria, a full-scale mill trial was conducted at a plywood mill in Arkansas. Results indicate that the soy-based formulations gave satisfactory performance (based on mixing, processing, and adhesive properties). Successful plant trials bring the soy protein-based adhesive closer to commercialization, and will create a substantial new market for soybeans.

ybean hulls were modified with citric acid and a manufacturing process design and product cost estimate was developed in collaboration with scientists at the SRRC, New Orleans, LA and cost engineers at the ERRC, Wyndmoor, PA, in preparation for commercial production of this metal ion-adsorbing product. This technology was transferred to Peat Technologies Corporation (Peatec), Aitkin, MN and a CRADA established to assist in product scale-up. Peat Technologies has had numerous requests from companies to use the modified hulls as a replacement cation exchange resin for the more expensive synthetic, commercial resins. The citric acid-modified soybean hulls, called BioSorb 100 by PeaTec, were very effective in removing zinc, copper and lead at several small companies where removal of toxic metals from their waste effluent is a problem.

Soy oil-based fuels

Biodiesel is made by esterifying fatty acids from vegetable oils and animal fats. In Europe, most biodiesel is made from rapeseed oil, whereas soybean oil is the most common feedstock in the United States. More than 200 vehicle fleets in the U.S. use biodiesel. Cold flow properties and storage stability require additional work to make biodiesel more competitive with petro diesel. ARS researchers at Peoria, IL, have improved biodiesel's ability to start engines in cold weather. Fundamental chemical research also addresses biodiesel pre-ignition and ignition compounds which influence efficiency, emissions and economics. ARS researchers at Wyndmoor, PA, have applied for patents on methods to use a byproduct of soy oil refining (soapstock) and its derivative (acid oil) to lower biodiesel cost. About 100 million pounds of soapstock are produced in the United States each year. Their method could convert soapstock into more than 6 million gallons of soy diesel fuel or soy diesel fuel additives annually.

Recent developments

Researchers at Wyndmoor, PA, developed a method for biodiesel production from acid oil, a coproduct of edible oil refining that sells for approximately

half the price of refined oils. This work successfully achieved high efficiency conversion of acid oil to biodiesel. Acid oil is produced from soapstock. The developed process is superior to those previously developed under the predecessor project, which used intact soapstock.

Future directions

As part of a broader project aimed at genetically engineering oilseeds to produce a safe domestic source of ricinoleate (the key functional component in castor oil) and other industrially useful fatty acids, researchers at the WRRC are identifying enzymes and cloning the respective genes needed to engineer oilseeds, including soybeans, to produce oils with a high proportion of one fatty acid. One thrust of a project at the SRRC proposes to develop new synthetic chemical methodology for the conversion of oleic and linoleic acid, the most abundant fatty acids of many vegetable oils (including soy) to more valuable fatty acids such as branched-chain fatty acids, eleostearic acid, and ricinoleic acid. Researchers at the ERRC will employ biotechnological or biomimetric processes (chemical reactions that mimic enzyme reactions) to produce branched-chain fatty acids from common fats and oils. A related and complementary project at the ERRC will explore fermentation of fats and oils for the production of biopolymers, biosurfactants, biolubricants, thickening agents, bioemulsifiers, and biobased specialty chemicals. Another project at the SRRC proposes to develop a microbial conversion system for bioconversion of low-cost oils, including soy, into tung-like drying oils (high in eleostearic acid). Research at the NCAUR will use biocatalysis to produce hydroxy fatty acids with industrial applications from soybean oil. Research projects at the NCAUR and the ERRC will use complementary approaches to produce metalworking lubricants using soybean oil. A project at the NCAUR will employ non-aqueous enzymatic transformation of vegetable oils to high value products with novel functional groups and properties, such as feruloylated monoacyl- and diacylglycerides, for food and non-food applications. Yet another project at the NCAUR proposes to chemically modify vegetable oils to provide improved biolubricants, monomers, polymers, composites, and polymeric blends. Soy protein extenders will be evaluated in plywood glue formulations at the NCAUR.

Another project at the SRRC focuses on developing enhanced, thermostable phytases animal feed additives that could increase the use of soybean meal in such feeds. Yet another project at the SRRC aims to convert plant by-products, such as soybean hulls previously used to adsorb metal ions, to activated carbons, resulting in metal ion entrapment in the carbon structure, which in turn will attract various anions such as phosphates, arsenates, chromates, and selenates.

One project at the NCAUR on edible oils will focus on developing new technologies to reduce trans and saturated acid contents of edible fats and oils and on developing starch-lipid emulsifier systems. Another project will focus on the mechanisms by which minor oil constituents such as antioxidants react in edible oils and how they can be used to control degradative oil processes such as oxidation, polymerization and hydrolysis.

ARS scientists look for opportunities to leverage research capabilities and commercialize promising new technologies by cooperating and collaborating with other public and private sector interests, both in the U.S. and in other countries. It's likely that these efforts will maintain a strong focus on soybeans as a source of food and industrial products well into the future.

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VII WORLD SOYBEAN RESEARCH CONFERENCE

MARCH 1

Host plant resistance in IPM and the advances and restraints on Bt engineered soybean/crops

M. E. Baur and D. Boethel[1]

Soybean remains important in the U.S with almost 30 million hectares of soybean planted in 2002 worth nearly US$14 billion. The soybean crop makes up almost 30% of the total agricultural production of some states in the Mid West, and production in states in the Mid South constitutes about 10% of the annual agricultural crop production.

The broad acreage planted to soybean and the mandate to incorporate integrated pest management (IPM) in most agricultural systems necessitates the use of IPM in soybean. The IPM approach to insect control involves multiple tactics that includes host plant resistance (HPR). Pest-resistant plants can reduce pest population growth, the number of pesticide applications and environmental impact of pesticides.

The potential of HPR in soybean has been recognized since the 1960s with discovery of resistance to multiple insect pests in three plant introductions (PIs), PI229358 ("Sodendaizu"), PI171451 ("Kosamame"), and PI227687 ("Miyako White") (Boethel et al. 1999). These PIs became the model for research on inheritance of insect resistance, mechanisms of resistance, compatibility of plant resistance with other management tactics, and genotype by environment interactions. They have been frequently used as donor parents in public breeding programs in the U.S. to develop insect-resistant germplasm and cultivars. The USDA Soybean Germplasm contains more than 16,000 soybean accessions from South Korea, Japan, Russia, and China, thus providing adequate resources to conduct research and development of resistant cultivars in the U.S.

Host plant resistance against insect pests of soybean has been extensively studied (reviewed by Boethel et al. 1999). Resistance to Mexican bean beetle (*Epilachna varivestis*), bean leaf beetle (*Cerotoma trifurcate*), and striped blister beetle (*Epicauta vittata*) has been reported in PIs 171451, 227687, and 229358. Resistance to banded cucumber beetle (*Diabrotica balteata*) was reported in PIs 171444, and 417061 in addition to those mentioned previously. Resistance to lepidopteran defoliators including velvetbean caterpillar (*Anticarsia gemmatalis*), soybean looper (*Pseudoplusia includens*), corn earworm (*Heliocoverpa zea*), beet armyworm (*Spodoptera*

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exigua), and tobacco budworm (*Heliothis virescens*) has been reported in PIs 171451, 227687, and 229358. Stinkbug resistance occurs in the PIs 227687, 274453, and 274454 has been reported in the cultivar IAC 100. Research in the U.S. (Baur and Boethel, unpublished, McPherson et al., unpublished) has identified advanced breeding lines with resistance to stinkbugs, where the genetic background for stinkbug resistance was PIs 227687, 229358, and IAC100.

Resistance in soybean is attributable to both constitutive and induced factors. Allelochemicals associated with feeding deterency and antibiosis in soybean are isoflavones (phaseol, afrormosin, coumestrol, diadzein, and glyceollin), phenolic acids, and phytoalexins. Primary metabolites also have been implicated in resistance mechanisms. However, PIs 227687 and 229358 do not accumulate nitrogen as rapidly as other susceptible cultivars (reviewed by Boethel 1999), and lack of nitrogen has profound effects on the growth and development of soybean looper. Resistance to stinkbugs has been attributed to reduced time spent by the plant in reproductive stages susceptible to stinkbug injury and reduced seed size.

Cultivar development in the public sector by government agencies (either state or federal) has played an important role in establishing soybean as a major crop in the U.S. Progress has been made toward development of acceptable insect-resistant cultivars, but success has not been achieved primarily because yield has been the primary focus of breeding efforts. Breeding strategies use germplasm to obtain genes for quantitative traits. Then recombinant phenotypes are identified from segregating progenies that possess the most favorable genes contributed by the PI. However, herbicide-tolerant soybean has become widely adopted and producers in the U.S. planted more than 75% of their acreage to this crop in 2003. This technology has been widely adopted because of lowered production costs, reduced crop injury, and simplicity and flexibility in weed management. This move to herbicide-tolerant soybean has meant soybean cultivars currently grown in the U.S. are primarily being produced by the private sector.

What is the future of host plant resistance in soybean? At present, our laboratory is exploring three lines of investigation. Transgenic technology allows for the rapid development of insect-resistant plants that also have good agronomic characteristics. Along these lines we have been evaluating Bt soybean (soybean plants expressing the Bt toxin Cry1Ac). Increased understanding of signaling pathways involved in induced responses has led to the discovery of natural and synthetic chemicals, called elicitors that induce chemical responses similar or even identical to those responses

induced by natural herbivory. Elicitors that have been tested with soybean include jasmonic acid and BTH [Benzo(1,2,3) thiadiazole-7-carbothioic acid (S) methyl ester]. Genetic techniques to identify the precise nature of the resistance, such as the Quantitative Trait Loci (QTL), will aid with breeding programs designed to incorporate host plant resistance (Terry et al. 2000, Narvel et al. 2001). We will be exploring ways to use this technology to determine mechanisms of HPR in soybean.

How does Bt soybean fit into the IPM paradigm? The IPM approach is based on the use of economic injury levels (EILs), where the EIL is the pest population level at which the loss in yield caused by pest damage to the crop is equal to the cost of controlling that pest using chemical intervention. Pest populations are maintained at a level below the economic injury level by monitoring and the as-needed use of insecticides. By employing economic injury levels, the producer and environmental costs are reduced by eliminating unnecessary insecticide applications. Use of economic thresholds also limits the exposure of the pest population to control measures, thereby reducing the possibility of pesticide resistance. However, in recent years, researchers involved in developing IPM strategies have studied preventive treatments. This strategy differs from the strategy involving economic thresholds and as-needed applications of insecticides, but still can be viewed as falling within the IPM approach. Preventive treatments can be especially useful in geographic areas that have historical, annual problems with certain insect pests. The transgenic technology in which proteins with insecticidal properties are engineered into plants might be viewed as a preventive measure. As with other preventive treatments, this technology could be useful in areas that historically experience high populations of target pests.

The target insects for Bt soybeans in the U.S. would be velvetbean caterpillar, soybean looper and corn earworm. These pests feed on the leaves or pods (in the case of corn earworm) of the soybean plant and can severely limit yield by reducing light interception of the soybean canopy. Velvetbean caterpillar populations can reach damaging levels very rapidly. Many producers in areas where velvetbean caterpillar is a significant problem apply a preventive treatment of Dimilin (diflubenzuron) when plants are in full bloom. In areas where soybean looper is a significant problem, such as those where soybeans are grown close to cotton, few preventive measures are accessible for the control of this pest because Dimilin is ineffective against soybean looper (Willrich et al. 2002). Furthermore, this insect has been difficult to control using many of the historically recommended insecticides, such as pyrethroids and carbamates. The corn earworm has proven to be difficult to control in Bt cotton and is especially damaging to soybean because of its pod feeding behavior. Usually the second generation

builds to very high population densities that have the potential to limit yield. These lepidopteran pests can be managed using early-maturing soybean varieties planted early in the season, but this may not always be an option.

In 1998, we began conducting field trials to evaluate the effectiveness of Bt soybean lines developed by Monsanto Company that contained the insecticidal protein from *Bacillus thuringenesis* (Bt). That year, and in subsequent years, we have seen exceptional control in the field and the laboratory of velvetbean caterpillar and soybean looper using these Bt soybean lines. In addition, there was no direct impact on insect predators often found in these fields. Trials in recent years have continued to examine the effectiveness against insects but have begun to focus on the agronomic properties of experimental lines.

There are several concerns about the effect of Bt crop plants that need to be addressed before widespread adoption. These concerns include: 1) the ability of Bt plants to rapidly select for resistance in the target insect because the toxin is constitutively expressed, 2) variation in control of the target insect due to variation in levels of toxin in different plant parts and during different times of the year, 3) the effect of Bt plants on natural enemies, and 4) toxin residues in the soil.

Control of soybean looper in the U.S. using foliar applications of Bt is variable despite apparent susceptibility in the laboratory. Across the geographic range of soybean looper (including North, Central and South America and the Caribbean Basin), susceptibility varies widely and some populations from the Caribbean Basin are very tolerant of Bt toxins. Population genetic analysis of neutral genetic markers of soybean looper populations suggests links among geographically distant populations (Baur and Boethel, unpublished). Insect-resistant Bt soybean has the potential to provide significant control of soybean insects. Long-term effect will be sustained if effective insect resistance management strategies are adopted to facilitate the durability of the resistance. These strategies must be aimed at preventing the mating of the resistant insects with nonresistant insects and insects resistant to other forms of Cry proteins. Use of resistance management strategies used in other Bt crops such as tissue- and time-specific promoters, gene pyramiding, and refuges can decrease the rate of insect adaptation in Bt soybean.

Although we have not directly tested different Bt soybean cultivars for their effectiveness against target pests, students in this laboratory have examined the effect of different Bt cotton cultivars on growth and

development of soybean looper (Clemens 2000). For three Bt cotton cultivars tested (PayMaster 1220 RR/BG, NuCotn 33B, and Coker 312/531), the intrinsic rate of natural increase (rm) varied from 0.211 to 0.043, and doubling time for the populations varied from 3.4 d to 5.8 d. Furthermore, expression of Bt toxin in Bt cotton changes with the age of the entire plant as well as the plant parts (Greenplate, 1999).

van Emden (1991) warned that the use of Bt-crops expressing high levels of toxin may affect parasitoids by reducing the growth rate of the host and thereby decreasing parasitoid size and fecundity. Baur and Boethel (2002) conducted experiments with two hymenopteran parasitoids, *Cotesia marginiventris* and *Copidosoma floridanum*. These experiments demonstrated that cotton expressing the Bt toxin (NuCotn 33B) increased development time of larval *C. marginiventris*, decreased number of ovarioles carried by females and reduced adult longevity. *C. floridanum* residing in soybean loopers fed Bt cotton developed more slowly and fewer adults emerged from these hosts.

The long term effects of Bt in the soil profile can be better understood when research efforts evaluate such issues as secretion of Cry proteins through roots, length of persistence and accumulation of Cry proteins in soil under field conditions, and horizontal and vertical movement of Cry proteins to ground and surface waters. Furthermore, the effects of Cry proteins on soil organisms should be considered in a risk-benefit context by comparing the effects of Bt soybean with the effects of other insecticides used to control the target pest.

Soybean roots secrete proteins and other chemicals all the time. Cry proteins, similar to other proteins, adsorb to clay particles in the soil. Adsorbed proteins are not biologically active. The amount of protein a given soil can adsorb depends on the cation exchange capacity of the soil. Thus, the adsorption varies between soil types. A significant concentration of Cry proteins was reported to decline rapidly in about 14 days (Palm et al. 1996).

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Role of technology transfer for maximizing soybean production in India

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The soybean a "Wonder crop" of 20th Century being an excellent source of both proteins (40-44%) and oil (18-20%). Soybean protein is rich in valuable amino acid, lysine (5%) which is deficient in most of the cereals. In addition, it contains a good amount of minerals, salts and vitamins like thiamin and riboflavin. Its milk is comparable to cow's milk. Soybean is used for preparation of milk, curd, cheese etc. and can be affordable by poor people. Soybean today can surely help feed the people better and could do much to reduce nutritional deficiencies in India. Nutritive value of soybean is incomparable to any other supplement in supplying protein, fat, mineral salts like calcium, sodium, manganese, phosphorus and to some extent vitamins A, D and B complex too. The presence of enough phosphorus and protein in soybean is made use of in curing nervous disorders, rickets, pulmonary diseases and anemia. Since soybean contains less carbohydrate and more proteins, it forms one of the best foods for diabetic patients. Soybeans are alkaline in nature, use in diet reduces the acidity in blood. Soybean fat is free from cholesterol and causes no worry to heart patients. Soya meal is now used for manufacture of nutritional products for food. The cake flour is extensively used as animal feed. The Indian soy meal contains 48% protein compared to 44 per cent of soya meal of other countries, hence it is preferred in the world market. Being a leguminous crop, it helps in increasing the fertility of soil through symbiotic nitrogen fixation. It is thus, a multipurpose crop with many advantages. Current world soybean area is about 71 million hectare (m ha) USA occupies the largest area followed by Brazil, China, Argentina and India.

Changes in soya area, production and productivity in India

Soybean, ranking second among oilseed crops of India, has exhibited unparalleled growth in area in the past three decades. Soybean is established as major monsoon season crop in India, particularly in central part of the country. The area under crop is now stabilizing and occupies about 6.3 million-hectare area with 6.9 million tonnes of production (1100 Kg/ha). The estimate for 1991-92 was 3.2 million hectare with 2.49 million tonnes (782 Kg/ha). The scenario would indeed be satisfying particularly to those,

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who are associated with the soybean crop from the early years of development. Agricultural feasibility and economic viability of soybean as sole as well as inter-crop during rainy season and also in crop sequences have been established. It has resulted economic revolution in rural India and has dramatically improved living conditions of farmers, particularly women and children, who were the worst sufferers in villages of Central India. During these years the pace of growth in productivity did not commensurate with its horizontal expansion. India's average soybean yield is about one tonne per hectare which is not even half of the world's average (2.2 tonnes/ha). The productivity potential of soybean varieties developed in recent past is about 4 tonnes/ha. Under real farm conditions many farmers are harvesting about 3 tonnes/ ha. The productivity of soybean can be easily augmented to two tonnes per hectare. It could be attained through genetic improvement and adoption of appropriate production technology. The low productivity is attributed to sizable addition of new areas in last decade with inexperienced farmers every year and the technology gap and lack of awareness on production technology. Besides, occurrence of several biotic and abiotic stresses at various stages of crop growth is an important factor responsible for poor yield.

Since early seventies when the crop was first introduced in about few thousand hectares, the area increase in the decade of nineties has become phenomenal. The soybean area was 30,000 hectare in the year 1970-71 and increased spectacularly to 64,20,000 hectare in the year 2000-01. The change in soybean area on decadal basis since 1970 is given in table-1.

Table 1. Decadal change in soybean area, production and productivity in India.

Year	Area ('000 La)	Production ('000 tonnes)	Yield (kg/ha)
1970-71	30	10	426
1980-81	610	440	728
1990-91	2564	2602	1015
2000-01	6420	5270	822

Madhya Pradesh has its major share in area and production of soybean in India (nearly 70 per cent) and called as 'Soya State' followed by Maharashtra, Rajasthan, Chhattisgarh, Uttar Pradesh and Karnataka.

The productivity was hovering around 700 kg/ha till mid-eighties gradually increased to 1138 kg/ha in 1999-2000. It is very low as compared to world average productivity of about 2000 kg/ha plus. Low yield is our greatest concern in popularizing and expanding the area of soybean specially, last three years were bad because of drought in the entire soybean growing tract.

Potential soybean varieties

Grain yield in soybean is the most intricate polygenic character. Apart from yield, other following plant characters in soybean are crucial for genetic enhancement and harnessing of yield.

- Pod shattering resistance
- Resistance to biotic stresses
- Resistance to insect-pests
- Resistance to diseases
- Resistance to abiotic stresses
- Efficient nodulation
- Suitability for mechanical harvesting
- Earliness and determinate plant type

About 65 soybean varieties have been released in India. At present the following varieties have been proved as potential ones to different states.

S.No.	State	Varieties
1	Madhya Pradesh	JS-335, JS 80-21, IS-9, JS 71-05, Ahilya-1, Ahilya-2, Ahilya-3, Ahilya-4, JS-90-41 and JS-93-05
2	Maharashtra	JS-335, MACS-124, MACS-450, Pratikar, Preatishtha, Samrudhi
3	Rajasthan	JS-335, JS 80-21, IS-9, JS 71-05,Ahilya-4 and JS-93-05
4	Chhattisgarh	JS-335, JS 80-21, IS-9, JS 71-05,MACS-124, PK 472 and JS-93-05
5	Uttar Pradesh	PK 1024, PK-1029, VL Soya 21, PK 472, Pusa-20 and PK 564
6	Karnataka	Sheha, Pooja, PK 1029, MACS 124

The following soybean varieties are showing resistance/ tolerance to the biotic and abiotic stresses listed against

S.No.	Stress	Suitable Efficient Varieties
1	YMV	PK 416, PK 262, PK 472
2	Bacterial pustule	PK 416, PK 472, PK 564
3	Pod blight	PK 416, PK 262, PK 472
4	Seed longevity	JS 80-21, Ahilya-2, Pusa-16, JS-335, Pusa-20
5	Pod Shattering	Bragg, PK 416, HIMSO1520, VLS-1
6	Higher insertion point for the first pod	PK 416, MACS 58, JS 80-21, IS 9
7	Rust	IS 9, JS 80-21, PK 1029, PK 1024
8	High yield	JS 335, Bragg, PK 472, Pusa 16, JS 80-21, IS 9
9	Early maturity	Monetta, PK 327, MACS 330, JS 335
10	Drought	Ahilya-3, PK-327, PK-564, Hardee, JS-71-05
11	Photoperiod	JS 71-05, Ahilya-3, JS-335, Ahilya-2
12	P (native)	PK 472 and PK 416
13	P (applied)	JS 80-21, JS 335
14	Oil Quality	PK 416
15	Nodulation	JS 335

Technology transfer and its impact

Yet there exists a vast unexploited yield reservoir in soybean with the currently available technologies. The productivity potential of the currently adaptable improved soybean production technologies which could be exploited under real farm situations are considerably higher than the corresponding state or district average. A sound and viable technology transfer system was, perhaps, a missing link in bridging this gap.

Results of thousands of Frontline Demonstrations conducted on farmers' fields in last decade under real farm situations through Government financed scheme clearly established that adoption of research emanated production technology is capable of increasing the productivity by 30-40 per cent. Against the yields of 1.2 to 1.4 t/ha obtained under farmer's practices, the yields under improved technology varied from 1.7 to 2.0 t/ha. For improving the production and productivity of oilseeds crops in general and soybean crop in particular, Government of India has launched an ambitious project, called "Technology mission on oilseeds" since 1987. Through this programme there is good amount of support to the farmers in some critical areas of technology transfer and inputs like seed, S-fertilization etc.

Use of soya meal based products in India at present is limited to only 8 percent. It is high time to popularize soya products to Indian masses, particularly pregnant women, lactating mothers and children in villages where protein deficiency food is consumed.

Indians have become habituated to traditional pulses but not for the flavour of soybean. As taste is an acquired habit, with proper education and change of attitude of the people, soybean can become a pulse in demand like any other pulses, accustomed. The change has to be brought about gradually. Therefore, soybean, and its *dal* (pulses) and flour should be used in smaller quantities by mixing with wheat flour and then gradually could be replaced completely wherever acceptable.

Besides increasing demand for soybean by creating awareness about its benefits for food and feed in our country, remunerative and stable price of soybean should be ensured. Increase in yield by improved efficiency of soybean farming is necessary for its sustainability in India. There is a need to strengthen further to popularise available technologies to attain established yield potential (2 t/ha) in varied agro-ecological situations by reducing the technology gap. The constraint analysis done by several researchers at different places in India has indicated the following as the major yield constraints:

- Poor plant population due to poor germinabelity of seed, improper field preparation and improper sowing methods
- Poor management of biotic and abiotic stresses eg. diseases, pests and weeds.
- Inefficient harvesting, delay in harvesting, natural vagaries at the time of maturity and post harvesting management resulting yield erosion.
- Almost entire Soya crop is raised under rainfed condition; specially, in drought years the productivity loss is very high.

The above constraints are the consequence of poor exposure of farmers to latest techniques of soybean cultivation and farm management as well as meagre mechanization in soybean farming.

Supply of good quality seeds of desired varieties, establishment of soy-clinics equipped with modern information technology, development and updation of web-sites for farmers and remunerative prices of soybean, would go a long way in augmenting soy yield and ensuring soybean sustainability in India.

Technology transfer tools for agricultural research

P. B. Johnsen[1]

Introduction

The United States Department of Agriculture (USDA) was established in 1862 to assist the public through the collection, generation and dissemination of information. Scientific research has been an important component of the Department's mission since the beginning. The results of agricultural research can be classified into three general types: 1) basic knowledge that is used by other scientists to resolve other research problems; 2) best practices that can be adopted by many in the producer and/or processing industries when this new knowledge is freely substituted for prior practices and 3) inventions that require transfer through formal legal mechanisms or processes for successful implementation. This paper will generally focus on this third category of research results, inventions, and detail the development and use of some of these formal legal mechanisms for technology transfer. Examples from the National Center for Agricultural Utilization Research (NCAUR) will illustrate their use and, based on recent experiences, a new approach for technology development and transfer is described.

Early technology transfer

Soon after Congress authorized establishing USDA, a system of land grant universities was commissioned to provide additional mechanisms to develop new information through basic and applied research. A few years later the state agricultural experiment stations were authorized as public – private partnerships to establish and demonstrate best practices for scientific farming. To disseminate this accumulated knowledge, the Extension Service was established in 1914.

In 1938 the Congress authorized USDA to construct and operate four laboratories to find new uses for surplus agricultural commodities. The charter for these laboratories specifically included the authority to work directly with industry. Known as the regional utilization laboratories and now operated by the Agricultural Research Service (ARS), the National

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Center for Agricultural Utilization Research (NCAUR) located in Peoria, Illinois, is the largest of the four. NCAUR has an outstanding record of basic research that has been transferred to the scientific community through almost 9,000 technical publications. However, NCAUR is best known to the public for its many inventions and their transfer to the private sector. Notable inventions include: the methods for the mass production of penicillin, dextrans, xanthan gum, soybean oil processing technologies, enzyme systems for the production of high fructose corn syrup and starch graft co-polymers which function as super absorbents found in such products as fuel filters, baby diapers and surgical dressings.

While each of these inventions was patented and transferred from the laboratory to the marketplace, laws existing at the time prevented other useful discoveries from being commercialized successfully. Before 1980, patents held by the U.S. Government were in the public domain and freely available to anyone. This did not provide incentive and protection to industry to make further investments necessary to develop and commercialize public inventions. Other companies could follow behind benefiting from lessons learned and thus erase any competitive advantage the pioneer company may have established. Therefore, inventions, while useful and commercially attractive, often remained trapped in what has been called "Death Valley" between the laboratory and the marketplace. Industry simply passed up promising technology.

Patent licensing and cooperative research

To provide the necessary protection to companies making significant additional investments in time and money to commercialize government inventions, the Bayh-Dole Act (Public Law 96-517) was passed in 1980. This law and other regulations provided federal laboratories with the authority to grant exclusive licenses on federal technologies. Thus, market-ready technologies could be commercialized by the private sector. However, some inventions, not yet ready for commercialization, often remained trapped in Death Valley between the laboratory and its application in the marketplace. Death Valley could be bridged with direct interaction between government scientists and the private sector R&D scientists but there were no legal mechanisms for these scientists to work together to successfully implement government inventions. The exchange of confidential and proprietary information necessary for collaborative efforts was not possible under existing U.S. law.

The Federal Technology Transfer Act of 1986 (Public Law 99-502) provided the means for cooperation between federal laboratories and the private sector. This act established the legal mechanisms for cooperative research

and development agreements (CRADA) allowing government scientists to work as closely as necessary with private firms to commercialize technology based on the scientists' research. The law specifically allows such collaborative efforts to cover the full range of research, from fundamental to applied, developmental and even through to commercialization. A unique feature of the CRADA is that it grants special rights to the company for exclusive licenses on patents obtained during the course of the collaborative research.

Thus, by 1986 several laws and an Executive Order (12591) provided the legal authority for enhanced relationships between government labs and the private sector to benefit the economy and the well being of the nation. USDA and its major laboratories have been leaders and innovators in utilizing these tools to commercialize scientific discoveries that are in the public interest.

Risk sharing in technology development

The National Center for Agricultural Utilization Research has successfully commercialized technologies such as the innovative food ingredients, Oatrim and Fluffy Cellulose, as well as, pest control devices, fermentation technologies and other inventions through the use of exclusive patent licenses and CRADA agreements. However, as experience was gained, the need for another means to bridge the Death Valley was identified. In some circumstances, successful commercialization was hindered by the inability to produce sufficient quantities of the new material to demonstrate its application suitability, the commercial viability of the production method or the potential market demand. This is particularly the case for technologies licensed by small companies that do not have the financial and physical resources to produce test market quantities.

What was needed was the capability to produce pilot plant quantities and to test market the product. However, Federal law did not allow public facilities to be used in this manner. It was not possible for material produced in government pilot plants to be sold into commerce. This often produced a classic chicken or egg problem. Companies could not produce sufficient quantities of the product to prove its commercial viability, and with out this proof, they were unable to secure sufficient funding to produce the test materials.

The legal remedy for this problem was established in the Agricultural Research, Extension and Education Reform of 1998 (Public Law 105-185) through the efforts of NCAUR technology transfer specialists. Title IV Section

404 (c) permits cooperative agreements with private entities under which the facilities and technical expertise of the Agricultural Research Service may be made available to operate pilot plants for the purpose of bringing technologies to the point of practical application. Under Section 404 (g) the private partner may sell the product for the purpose of determining the market potential of the new product. Through this law the government and the private partner can share risk in the commercial development of new technologies.

Recently, NCAUR has used the full suite of technology transfer tools in the commercialization of a new food ingredient. Azure Waves, a small minority owned business, licensed Fantesk™ , a novel starch-lipid composition, for coating fried seafood such as shrimp. NCAUR scientists worked with the company under a CRADA to develop a usable coating recipe containing spices and the Fantesk™ material. For Azure Waves to secure commitments from food store retailers, successful test marketing was required. Because the company did not yet own production facilities for this unproven product, NCAUR made use of its pilot plant authority to produce 1000 gallons of batter mix for the test market samples. These were then sold in mid-western Kroger Foods stores. Consumer responses and purchases were very positive and the company has secured a commitment from the retail chain. At this time the company is now searching for commercial facilities to go into full-scale production.

Technology development *vs* product marketing

In the previous example, and in all other technologies licensed by NCAUR, there is a product in hand that is ready to be produced and sold by the licensee. While minor development work may be required to bring the product to market, the business plan required by the government in its licensing procedure must detail how the company will go into production and sell product to customers. This means that government technologies that may be technically innovative but not yet ready for specific market applications are difficult to license because it is not possible to define a business plan. To advance these innovations in early stage development, yet another technology transfer approach is needed.

Currently, typical government patent licensees can be classified as marketing companies. But in some instances what is required is a development company. This concept is based on the biotech pharmaceutical industry where small companies begin technical development of a new unproven product. As risk is reduced through development work, the new product or the company itself is often acquired by a major company. NCAUR has

recently established a relationship with such a development company that may demonstrate how USDA can utilize this additional approach to technology transfer.

The National Center for Agricultural Utilization Research has established a CRADA with zuChem, a startup biotechnology company interested in developing glycochemical and related bioprocesses for the fine and specialty chemicals industry. The project involves development of mannitol a specialty chemical used in food, drug and agricultural applications. NCAUR has developed and applied for a patent on a bioprocess for the production of mannitol by fermentation. This process, with additional research and development, would allow improved production economics compared to currently used chemical processes. As an R&D development startup company, zuChem has raised venture capital funding and research partnership funding as it moves to develop and prove the NCAUR technology. The most likely business model in this instance is for zuChem to sublicense the proven technology to customers or even sell out the whole business to a major customer of the technology. Thus, zuChem functions as a technology development company rather than a chemical marketing company. By this arrangement the unproven NCAUR technology is advanced to the point where marketing companies find the technology attractive in terms of cost and risk.

NEXT generation technology transfer

This first experience of NCAUR with zuChem illustrates the potential of similar arrangements being developed under a unique collaboration and partnership in Peoria, Illinois known as Peoria NEXT. This entity is a not-for-profit company that is formed by the research institutions NCAUR, Bradley University, The University of Illinois College of Medicine at Peoria, Illinois Central College and Caterpillar Inc. In addition, commercial partners include First National Bank, the Biotechnology Research and Development Corporation and the Heartland Economic Development Council. Peoria NEXT functions as a catalyst to promote commercialization of promising technologies that will aid in economic development within the community. Peoria NEXT has partnered with Arch Development Partners, an early stage venture capital fund to identify and promote technologies with commercial potential requiriing development company assistance.

Under this plan, the partners would assess the business potential of technologies developed by NCAUR or the other research partners. A company would be formed with an experienced entrepreneur CEO with funding from the venture capital community. This newly formed business

would then license the technology, enter into a CRADA and work with NCAUR to develop the technology to the point it is commercially viable. Benefits of this partnership include the experience of the CEO, the technical expertise of NCAUR scientists, outside funding and the possibility to seek additional research and development partners. Other funding sources such as the Small Business Administration and Small Business Innovation Research grants would be available to this startup company. The benefit to the local community is that these development companies would provide high value employment and economic growth in the region.

Summary

Since its creation, USDA has developed innovative mechanisms to transfer the knowledge and technology it creates. These have included dedicated laboratories charged with the mission of technology development and transfer. It has used its legal authority, gained over time, to grant exclusive patent licenses, to enter into cooperative research and development agreements and to operate ARS pilot plant facilities to share the risk in technology commercialization. However, all of these approaches are designed to commercialize market-ready technologies with the assistance of a company prepared to market the product. Some technologies are not yet at this stage of development and require a different approach. Partnerships with technology development companies rather than product marketing companies are needed. These companies would develop government innovations to a stage where the technology or the company itself can be marketed. While this is a new approach to conventional technology transfer for Federal laboratories, USDA continues to develop mechanisms to meet its original mission of transferring new knowledge to the public for the benefit of the Nation.

University partnership in the development and transfer of technology

D. J. Myers[1]

There is an increased emphasis to find new value-added uses for farm commodities. The impetus for this research is the oversupply of some of these commodities and the need to find new ways to increase value of these commodities. Funds provided by the government and commodity boards, researchers have permitted researchers to investigate potential opportunities in a variety of areas including food, feed and nonfood products. The result of many of these efforts have been the development of technologies that has produced protein-, carbohydrate- and oil-based feed and food ingredients and/or chemicals for nonfood products that may be able to replace traditional chemicals used in these applications. In order for these efforts to be successful however, the technology developed in these laboratories will have to be transferred to a company or group of companies that has an interest in using this technology in their current operations or develop a totally new company or group of products. This presentation will discuss the efforts of the Biocomposites Research Team at Iowa State University in the process of technology transfer and commercialization of soy protein-based adhesives technology. The presentation will focus on the technology developed, the efforts to locate companies interested in the technology, and the current technology transfer and commercialization efforts that are underway. This presentation will be of interest to researchers who are interested in transferring technology that they have developed to industry and industry representatives who are interested in finding ways to work better with university researchers in finding new technologies that may benefit their companies.

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Strategies of technology transfer in soybean

P. R. Galerani[1]; J. F. F. de Toledo[1] and L. A. Domit[2]

Introduction

The strategies for technology transfer (TT) for soybean research results at Embrapa have been adapted over the time. Initially, the process of TT was based exclusively on activities such as training courses, meetings with extension agents and farmers. Field demonstrations, field days and technical visits have also been extensively applied in TT programs.

The development of a more competitive agricultural sector in Brazil due to globalization of the economy demanded from agricultural research institutions greater integration with other components of the productive chain and a better knowledge of the agribusiness.

In soybean specifically, the competitiveness of the sector goes beyond the boundaries of the country. Production of high quality soybean, efficient management by the farmers, lower production costs are important points, which makes Brazilian soybean more competitive in the world market.

The National Soybean Research Center of Embrapa has contributed to the high competitiveness of Brazilian soybean in the world. Release of new varieties with higher yields, and resistant to diseases, have contributed to more stable and higher yields. It is important to mention also that technologies related with improved soil management, such as no tillage, crop rotation, soil fertility have contributed to a more sustainable production systems well adapted to the new production areas in the country.

The Brazilian model of TT

In Brazil, the official agriculture research system is under responsibility of Embrapa, the Brazilian Corporation for Agricultural Research, linked to the Minister of Agriculture. At state level, institutions linked to the state government conduct agricultural research and extension. Some agriculture universities are also involved in agricultural research. Frequently these institutions develop joint projects in several areas of research. The budgets of these institutions are mostly funded from government resources.

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After the approval of the Varieties Protection Law, the private sector have started some research activities, mainly on the development of soybean varieties, attracted by a promising seed production market.

The extension and technical assistance to farmers are under responsibility of official extension institutions, private farmer cooperatives and, at some extent, chemical and fertilizer commercial companies.

The Embrapa system

Embrapa was created in 1973 with the main objective of developing and offering applied technology for farmers. An aggressive training program at MSc and PhD level was one of the priorities of the organization. With the increase in the expertise of the researchers, basic technology became part of the program. Often joint projects with state research institutions and universities were established.

Embrapa has currently 40 research centers distributed all over the Country. The National Soybean Research Center, the Embrapa Soybean, which was created in 1975, is one of the branches of Embrapa. It is located in Londrina, Parana State, with national mandate to produce technologies for soybean production. Research projects in several areas of knowledge and programs of TT have been developed by Embrapa Soybean in many opportunities through integrated activities with other Embrapa research centers, universities and extension institutions and farmers cooperatives. This cooperation among the public and private sector institutions was a natural procedure to integrate activities.

The diversity of climate and soils in Brazil and the extension of the territory have been a challenge to Embrapa research centers, especially Embrapa Soybean. Soybean is planted in more than 18 million hectares distributed in an extremely diverse climatic and soil condition. The soybean production in such large area has to be supported through appropriated technologies in order to guarantee the economical return to farmers, quality of the product and environmental sustainability.

Differentiated programs of TT are also required to face such challenge. Embrapa Soybean has applied an advanced partnership system involving also other actors of the productive chain and agribusiness. Another TT program applied by Embrapa Soybean is an adaptation of the Training and Visit (T&V) methodology of TT.

Private and public sectors: partnership for breeding programs

The partnership with private sector has been done basically with Associations of Seed Producers in most of the soybean producer States. Production and release of new varieties of soybean adapted to several soils and climatic condition in the Country has been the objective of these cooperation. Seed producers have organized themselves in Research Foundations to support Embrapa Soybean on the development of new varieties. This partnership put together the scientific expertise of Embrapa Soybean and the financial support and the dynamic behavior of the private sector.

The advantages of this cooperation are basically, (a) economical support for R&D; (b) more dynamic TT process facilitating adoption by farmers; (c) increase interaction between researchers and agribusiness; (d) decentralization of teams of breeders in strategic regions; (e) definition of research priorities.

Together with the release of new improved varieties all over the production area other practices and technologies are also offered to the farmers. At the same time that the adapted varieties have been obtained and released, information on soil management, IPM (insects and diseases control), plant nutrition among others, has been taken to the farmers.

Historical establishment of partnerships

Embrapa soybean started an aggressive program to put together private and public sectors with the objective of increasing capability to cover the national territory with competitive soybean varieties. This cooperation is ruled through an official contract and Annual Work Plans are discussed between the parties to plan and implement the activities in the following season. In general, the public sector contributes with the scientists and the germplasm bank and the private sector provides economical support and infrastructure for the activities.

The first partnership, in 1993, was between Embrapa and Mato Grosso Foundation (FMT). Initially, the official State research institution participated in the partnership, but they decide to withdraw and only follow the activities in the State. The FMT was created among the soybean seed producers of Mato Grosso State, representing the private sector in the cooperation. Although this partnership resulted in the obtention of varieties covering up to 83% of the State, the agreement was broken in 2001, due to lack of interest of the private sector to agree with new Embrapa regulations related with the Protection of Cultivars Law.

In 1994, a partnership was established in Goias State with the objective to create soybean varieties for Central Region of Brazil. The seed producers organized the Technological Center of Agricultural Research – CTPA, representing the private sector, which became responsible for economical and infrastructure support to this initiative. The official State institution of research and extension, AgenciaRural GO, participate actively of this agreement.

In Minas Gerais State, the agreement was between Embrapa, the official State research institution (EPAMIG) and the Triangulo Foundation, representing the private sector. This Foudation was created through the initiative of seed producers of the State and this agreement started in 1996.

The more recent partnerships were established mostly with the objective of development of new varieties adapted to agro ecological regions. These partners of Embrapa are:

1- Fundação Pró-Sementes de Apoio a Pesquisa (Pro seeds Foundation for Research Support), with the objective of developing soybean and wheat cultivars for Rio Grande do Sul State.
2- Meridional Foundation for Agricultural Research – It is located in Paraná State and its activities covers the States of Paraná, Santa Catarina and part of São Paulo.
3- Vegetal Foundation – It covers the activities in Mato Gorsso do Sul.
4- Central-West Foundation for Research and Development – The partnership witth this Foundation supports the activities of soybean breeding in the States of Mato Grosso and Rondonia.
5- Foundation for Supporting the Research and Development in the West of Bahia State.
6- Foundation for supporting to research in the Maranhão State. It covers the North part of Brazil, which is an important soybean expansion region.
7- State Foundation for Agricultural Research (FEPAGRO) – this partnership was established to develop soybean varieties specifically in the State of Rio Grande do Sul.

Results of the partnerships

The Table 1 shows the evolution of soybean yield every five years. A considerable increase in yield in the 90's shows an increased competitiveness of soybean production through release of new and improved varieties and dissemination of the other production technologies.

The established partnerships increased also the efficiency of researchers to face important problems during these years, such as the disease stem

canker and soybean cyst nematode. In both examples, new resistant varieties were quickly produced and tested through the research network formed by the partnership.

Table 1. Soybean production in Brazil average of 5 years from 1975 to 2000 and average of three years from 2001 to 2003.

Years	Area	Production	Yield
1975 to 1980	7.35	11.43	1,555
1981 to 1885	8.88	15.25	1,717
1986 to 1990	10.56	18.38	1,740
1991 to 1995	10.59	21.69	2,048
1996 to 2000	12.34	28.76	2,330
2001 to 2003	15.98	42.92	2,684

Source: Conab – Brazilian Company of Supply

Training & Visit methodology for TT

The Brazilian Agricultural Research System (SNPA) and the extension and technical assistance institutions are responsible for the development, validation and diffusion of technologies to increase the living conditions of the Brazilian farmers.

The constant development, complexity and improvement of the agricultural technologies require a more organized and efficient process of TT in Brazil. The T&V system was therefore applied as an improved TT program to provide, in a continuous and organized format, technical and management training programs to integrate the researchers, extension agents and farmers and to establish a permanent forum of technical debates and discussions. The system aims also at monitoring and evaluating the results of the technology in the field, at farmer level.

This system works basically through the formation and training of groups of specialists from the extension and technical assistance organizations who are in constant contact with the researchers involved in the program. Each of these specialists is responsible to provide technical assistance and organize groups of extension agents and technicians who will be the monitors in the field. Each of these monitors will transfer the technical information to organized groups of farmers. This procedure integrates the researchers,

extension agents and farmers and facilitates a constant feedback and evaluation of results.

The T&V has been applied for more than five years by Embrapa, IAPAR (research institution of Parana State) and EMATER PR, (Extension agency) in Paraná State. Farmer cooperatives and other private companies are also participating in the initiative. The objectives of the program are:

- o Continuous training of extension agents on technologies for soybean, wheat and corn production;
- o Organized and controlled transference of technologies and agricultural practices to farmers;
- o Improve the technical relationship among the participants (researchers, extension agents and farmers);
- o Increase the management capacity of farmers.

The following steps are needed for application of this methodology.

1- Definition of the general coordinator of the process;
2- Motivational activities in the group, planning and preliminary structure organization of the participants;
3- Creation of the technical committee of researchers, and invited specialists and technical monitors (T M I) from the extension agencies.
4- Formation of the group of monitors II (T M II). Every TM I should organize a group of TM II and transfer technologies discussed in the Committee of Specialists, follow up and evaluate the results obtained by the group.
5- Formation of a group of farmers (F). Every T M II should organize a group of farmers, transfer technologies defined in the group, follow up and evaluate the results obtained in the group of farmers.
6- The participants of the Technical Committee will periodically follow the activities of the T M II and farmers (F).
7- The evaluation is done after harvesting each crop, at every level, (TM I, TMII, and F) and closing with an overall evaluation. The adoption of the technologies is estimated, and the economic and agronomic results are calculated. A model of report is defined to compare and analyze the results of the groups.

Conclusion

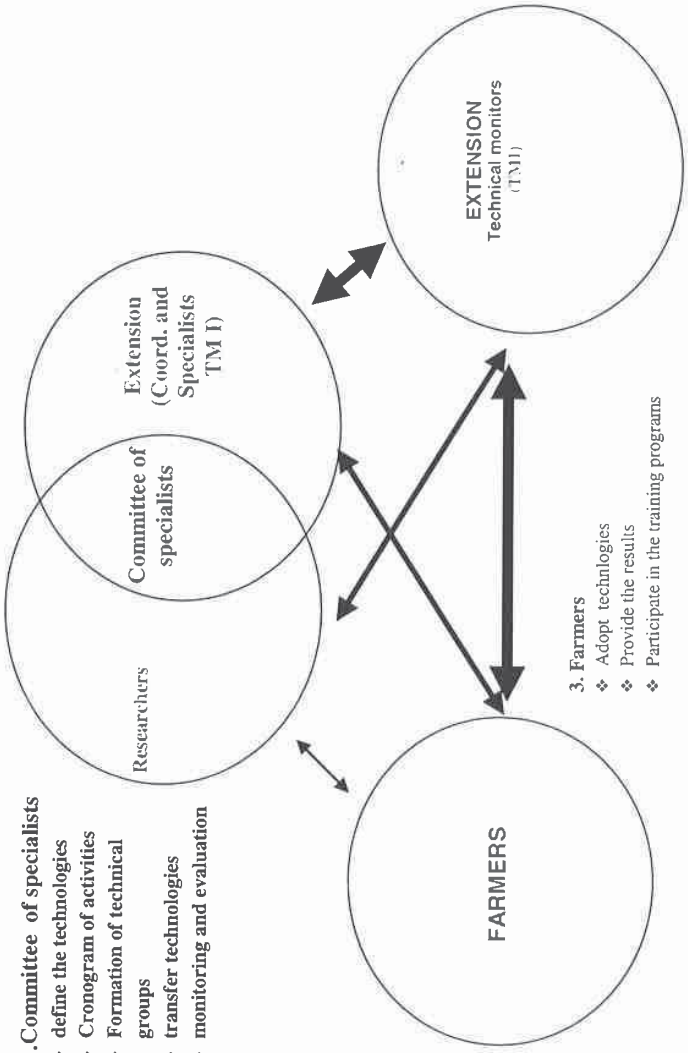
The development of agricultural technologies to support the modern agricultural production requires more efficient and special programs of TT for improvement and stabilization of sustainable yields of the crops.

The involvement of the components of the productive chain in the process is also important to support the scientific production and to speed up the process of adoption of research results, mainly new varieties adapted to different production regions.

Anexo

Operacional diagram

TRANING & VISIT



Embrapa Soybean has applied two different processes of TT: a) The partnership program to develop adapted and improved varieties; and b) the T&V methodology to organize and control a training program of extension and technical assistance agents. The high level of integration reached among researchers, extension agents and farmers, the constant feedback and the evaluation of the activities are important results of the T&V program.

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Strategies for technology transfer in Europe: the dissemination of research results to private sector, extension personnel and farmers

A. Messéan[1]

In the last fifty years, innovation has played an essential role for improving the competitiveness of European agriculture and of food industry: genetics through new varieties, crop protection through agrochemicals, equipment, new cropping systems, supply chain organization, etc. In order to achieve such a challenge, various strategies for technology transfer and a coherent R&D system have been put in place by a joint action of farmer organizations and public bodies.

In France, Chambers of Agriculture at the regional level and Technical Institutes skilled on specific production chains have been accompanying farmers in improving their competitiveness and meeting both regulation and market requirements. For the oilseed sector, the technical institute, CETIOM, was established in 1957 to assist in the development of oilseed production processes in France:

- improving production and conservation of domestic oilseeds (rapeseed, sunflower, soybean, linseed) and the use of products made from oilseeds;
- standardizing product quality and developing markets.

Funded by the 130,000 oilseed producers through a fee, CETIOM acts as an interface between research and field production. It focuses its activities in 4 major directions: analyzing contexts to steer research and development projects, participating in producing innovative products, contributing to environmental protection as well as improving product quality and diversifying markets. Downstream from public research and universities, CETIOM's research aims at co-producing references and innovations that can be used by all stakeholders: farmers, elevators, food/feed processors, national and regional regulation bodies, end users. Special attention is paid to local informational activities: field trials visits, focus groups, informational meetings, training workshops, publication of specialized reports in each region (Oléotech), advisory letters to farmers (Info-CETIOM).

Although the European demand with respect to agriculture is evolving, with new requirements in terms of multi-fonctionnality, it can be stated that the innovation process will remain one of the major factors for achieving

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the new objectives assigned to agriculture: reduce the negative impacts to the environment, ensure the food safety and contribute to a sustainable development. However, as the GMO example illustrates, there has been an increasing gap between innovation, and science to some extent, and society, together with a lack of confidence in public decision-making. It thus appears necessary to take into account the mid- and long-term impacts of innovation with respect to the environment and the agriculture.

Addressing these objectives is a real challenge for science and regulation as well as for technology transfer activities. On the one hand, new scientific approaches, methods and tools are required for such a systemic evaluation of the cost-benefit of GM crops. On the other hand, a new organisational framework for evaluation and monitoring of technologies has to be set up in order to address these new concerns.

VII WORLD SOYBEAN RESEARCH CONFERENCE

MARCH 2

Current status of soybean integrated pest management in Brazil

C.B. Hoffmann-Campo; F. Moscardi; B.S. Correa-Ferreira; L.J. Oliveira; D.L. Gazzoni; I.C. Corso; D.R. Sosa-Gomez and A.R. Panizzi[1]

The first activities related to soybean integrated pest management (IPM) in Brazil began during the 1974-1975 season. Prior to the implementation of the soybean IPM program, insect pests were controlled exclusively by chemical insecticides, often on a preventive or calendar basis. On average, five insecticide applications were performed during each cropping season, varying from three to ten (Gazzoni, 1994). Broad-spectrum insecticides such as endrin, DDT, toxaphene, methyl parathion were commonly used alone or mixed. As a result of a successful IPM campaign, the accumulated production costs of soybean were reduced by ca. $3 billion in 25 years and the number of insecticide applications dropped to less than two per season (Gazzoni, 1994).

The soybean IPM program is continuosly updated as new technologies are developed to meet consumer demands. Updated publications with recommendations for pest management and general information about IPM in Brazil are released as soon as new information becomes available.

The fauna associated with soybean also has increased from 10 species at the end of the 1960s to 60 species in 1997 (Panizzi and Corrêa-Ferreira, 1997). Currently, 37 species of insects and other organisms are considered primary, secondary or sporadic soybean pests (Table 1). However, only a few of them can actually be considered major pests of soybean as the velvetbean caterpillar (*Anticarsia gemmatalis*) and the stink bug complex (*Euschistus heros*, *Nezara viridula* and *Piezodorus guildinii*). As soybean is expanding to new areas and due to cultural practices changes, in certain regions, new outbreaks of different insect species have been registered. Some of them, after certain time, become less important but some have reached status of primary pests.

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Table 1. Pests of soybean, part of the plant attacked and its relative importance (Hoffmann-Campo *et al.*, 2000)

Pest	Part of plant attacked	Importance
Anticarsia gemmatalis	Le[1]	Main pest
Epinotia aporema	Le, Lb, Po	Secondary, importance in restricted areas
Omiodes indicatus	Le	Secondary, usually occurring at maturity, when defoliation is no long important
Pseudoplusia includens	Le	Secondary
Rachiplusia nu	Le	Secondary
Cerotoma sp.	Le(A)[2], No(L)	Secondary, in soybean areas, preceded by beans
Diabrotica speciosa	Le(A), No(L)	Secondary, in soybean areas, preceded by autumn season maize
Aracanthus mourei	Le, Pe	Secondary, occurring in the beginning of soybean development
Maecolaspis calcarifera	Le	Secondary
Megascelis sp.	Le	Secondary
Chalcodermus sp.	Le	Secondary, regionally important pest
Bemisia argentifolii	Le	Secondary, with high potential damage
Grasshoppers	Le	Sporadic
Thrips	Yl	Secondary, important in restricted areas as vectors of the virus that causes bud blight disease
Nezara viridula	Po, Se	Main pest
Piezodorus guildinii	Po, Se	Main pest
Euschistus heros	Po, Se	Main pest
Dichelops spp.	Po, Se	Secondary
Edessa meditabunda	Po, Se	Secondary
Thyanta perditor	Po, Se	Secondary
Acrosternum sp.	Po	Secondary
Ethiella zinckenella	Po	Secondary, with importance in restricted areas
Spodoptera latifascia	Po	Sporadic
Spodoptera eridania	Po	Sporadic
Maruca testulalis	Po	Sporadic
Sternechus subsignatus	St	Regionally important pest
Elasmopalpus lignosellus	St	Sporadic, usually important in seasons with extended dry period, in the initial phase of the crop
Myochrous armatus	Ha	Sporadic
Blapstinus sp.	Sd, Ha	Sporadic
Miriapods	Sd, Sp	Secondary, important in no-till areas
Snails and slugs	Sd, Co, Yl	Secondary, important in no-till areas
White grubs	Ro	Regionally important pest
Scaptocoris castanea	Ro	Regionally important pest
Scales	Ro	Secondary, important in no-till areas

[1] Lb = leaf bud; Co = cotyledons; Yl = young leaves; Le = leaves; St = stems; No = nodules; Pe = petioles; Sd = seedlings; Sp = smal plant; Ro = roots; Se = seeds; Po = pods.

[2] A = adult, L = larvae.

Strategies of IPM

Sampling: The recommended sampling method for caterpillars, stinkbugs and beetles is the shake-cloth (Boyer and Dumas, 1969). For assessment of other insects such as the shoot and axil borer (*Epinotia aporema*), pod borers, leaf rollers and the curculionid gall maker stem borer (*Sternechus subsignatus*), the examination of randomly selected plants or group of plants is the standard method. Soil samples are recommended to estimate the population of root insects such as white grubs (*Phyllophaga cuyabana*,

Plectris sp., *Liogenys* sp.) and burrowing bugs (*Scaptocoris castanea* and *Atarsocoris brachiariae*). Soil sampling is also used to estimate the next season potential population of *S. subsignatus*, helping farmers to decide on the appropriate crop rotation and other pest control practices.

Action threshold: In the initial phase of the program, action thresholds for lepidopterous defoliators and stinkbugs were the same as practiced in the United States (Kogan *et al.*, 1977). Studies conducted in Brazil confirmed and/or refined the action thresholds for these pests. Thresholds were also established for *E. aporema* and *S. subsignatus*.

Biological control: Natural enemies are important components of soybean IPM. Seven species of predators (carabids, geocorids and nabids), eight species of parasitoids (microhymenopterans and tachinids) and six species of entomopathogens (virus and fungi) are the most common natural enemies of above-ground soybean pests (Hoffmann-Campo *et al.*, 2000). The entomopathogen *Nomuraea rileyi* is responsible, for maintaining *A. gemmatalis* and Plusiinae populations under control, especially in the rainy season, and virtually eliminate the requirement of insecticide to control these pests. Other natural enemies are produced nowdays for release in the field, such as the nuclear polyhedrosis virus (NPV) of *A. gemmatalis* (Moscardi, 1999) and the parasitoid of stinkbugs eggs, *Trissolcus basalis* (Corrêa-Ferreira and Moscardi, 1995).

Cultural practices: Cultural changes in cropping systems affect the dynamics of soybean pests. Some cultural practices, such as manipulating planting dates, different tillage systems, crop rotation and mulch management can be used to reduce pest populations. Early-maturing soybean cultivars can escape stinkbug damage and are preferred by growers in southern Brazil, reducing the need for insecticide application. However, after harvesting short-cycle cultivars, stinkbugs usually migrate to late-maturing soybean, quickly and easily building up high populations, requiring frequent insecticide applications. Crop rotation has the disadvantage of the difficulty to persuade growers of its agronomic advantages, especially when soybean prices are higher. The low cost of some insecticides also limits the use of cultural practices; sometimes growers prefer to wait for insect pest populations to build up in order to apply low cost insecticides.

E. heros overwinters as diapausing adults under leaf litter (Panizzi, 1997). A cultural practice that reduces the seasonal population of this insect is incorporation of this leaf litter, or application of insecticides on the overwintering sites (Corrêa-Ferreira and Panizzi, 1999).

Chemical control: Chemical control is an important tool in IPM systems. Following the implementation of soybean IPM in Brazil, the number of

insecticide applications decreased, being the next step to seek for more selective products. In 1988, selectivity of insecticides became a major criterion for recommendation by regional entomology committees. These committees are responsible for annual revision and recommendation of insecticides for soybean IPM. One major result of this action was a significant reduction in the number of active ingredients recommended for velvetbean caterpillar control.

Empirical field observations had suggested that stinkbugs were attracted by sweat (Corso and Gazzoni, 1998). Sodium chloride was the most likely sweat composing substance to exert such effect. Adding a small amount of salt to the insecticide allows a reduction in dosage without loss of its effectiveness. Some insecticides are currently recommended at half of their former rates when applied together with a 0.5% sodium chloride solution.

Control of velvetbean caterpillar (*Anticarsia gemmatalis*) with NPV

*Ag*NPV is a naturally occurring control agent of *A. gemmatalis*. In spite of its widespread range, the natural incidence of *Ag*NPV is generally low (less than 10%). Basic studies at Embrapa Soybean focused on the development of a microbial insecticide to control *A. gemmatalis* leading to the world's most successful biological program, reaching an area of 2.0 million ha/yr.

Production of AgNPV: In the past, NPV was produced by Embrapa Soybean and the extension agents collected and distributed frozen NPV-killed larvae to growers. Afterwards, other institutions and growers' cooperatives began mass production of NPV. In 1991, the formulated *Ag*NPV started to be produced and commercialized by five private Brazilian companies under contract with Embrapa Soybean. The commercial production of virus is mainly carried out in the highly infested field with the hosts (*A. gemmatalis)* providing a ready source of inoculum for mass production. The cost of formulated *Ag*NPV is currently US $0.40/ha, at a retail cost of US $0.70 to US $1.00, lower than chemical insecticides.

Limitations of AgNPV use: Despite the advantages of using NPV to control *A. gemmatalis*, some factors may limit its use by growers, as: (i) demand for NPV is potentially higher than the available supply. According to informal surveys, approximately 30% of soybean growers are willing to use *Ag*NPV treatment. To meet such a demand, production would have to be increased threefold without significantly increasing the cost of the final product (Moscardi, 1999). Large-scale laboratory production of *Ag*NPV is currently being implemented. Recent results indicate that the cost of laboratory-produced virus is competitive with the cost of insecticides compatible with IPM criteria. (ii) Growers must know how to use the virus correctly, and

understand its delayed mode of action. Lack of information leds to partial misuses; most growers are umprepared for a careful monitoring of *A. gemmatalis* phenology necessary to ensure effective application of NPV (Moscardi, 1989; Moscardi and Sosa-Gomez, 1996). Preventive application mixed with seeds, herbicides, etc are also primary causes of unsuccessful *Ag*NPV utilization. In 24 years of virus usage, grower education by research and extension services has been the single most important factor for increasing *Ag*NPV use in Brazil. In regions where the extension program is weak, use of the virus is negligible (Moscardi, 1999). (iii) On average, the time-to-death by *Ag*NPV infection is 7 days, at least the double time of most chemical insecticides. In regions with lower mean temperature, the time-to-death of NPV-treated larvae can be even longer, leading growers to apply insecticide after 7-8 days. Formulations of virus with enhanced viral activity and speed of action are being investigated (Moscardi, 1999). The addition of a very low concentration of substances, such as boric acid and optical brighteners of the stilbene group, have being tested. Results are showing that they can increase viral activity and speed larval death (Morales *et al.*, 1997 and 2001, Shapiro 1995).

Potential for resistance to AgNPV: The possibility of resistance developing to *Ag*NPV has been raised as a potential cause of virus failure in some regions. However, *A. gemmatalis* populations collected in several regions of the country, submitted to 3 to 8 years of continuous *Ag*NPV application persist showing high susceptibility (Abot *et al.*, 1996). But, under some laboratory conditions with high selection pressure, *A. gemmatalis* demonstrated the ability to develop resistance to *Ag*NPV (Abot, *et al.*, 1996). Such resistance can be rapidly lost (in 4 generations) when resistant insects are crossed with susceptible wild-types, suggesting that immigration of susceptible insects to virus-treated areas may act as a mechanism to decrease the risk of resistance development by field populations. In addition, stilbenes can, in some cases, prevent the resistance of *A. gemmatalis* to its baculovirus (Fuxa and Richter, 1998; Morales *et al.*, 2001). Also, the utilization of resistant cultivars with high rutin concentration probably may also help to break such resistance (unpublished data).

Reducing insecticide dosage with AgNPV: Most insecticides can be mixed with *Ag*NPV at rates up to 1/4 to 1/6 below the usual recommended dose (Moscardi, 1999). When a sublethal dosage of insecticide is used, the virus can attack the remaining insects and serve as source of inoculum for the next generation. This strategy can be effective when *A. gemmatalis* larvae have exceeded the threshold level used for *Ag*NPV application alone, although the mixture of insecticides, including biological insecticides are forbidden by Brazilian legislation.

Biological control of stink bugs with egg parasitoids

Thirty-two species of stinkbug pests are associated with soybean in Brazil (Panizzi and Slansky, 1985). *N. viridula, P. guildinii* and *E. heros* typically represent 98% of the total stinkbug population (Corrêa-Ferreira and Moscardi, 1996). Over 20 species of stinkbug egg parasitoids have been reported, and the most important is *Trissolcus. basalis*, that preferentially attacks *N. viridula* eggs and *Telenomus podisi*, that prefers eggs of *E. heros* and *P. guildinii*. However, these parasitoids tend to occur in high populations only after the stinkbugs have reached threshold levels. For this reason, a program for laboratory production and inoculative release has been developed at Embrapa Soybean.

To protect early-season population of *T. basalis*, only biological agents such as *Ag*NPV, *Bacillus thuringiensis* (*Bt*), or highly selective insecticides, as insect growth regulators (IGRs), should be used to control *A. gemmatalis*. When stinkbugs begin to colonize the field, or no later than the soybean growth phase R4, 5,000 parasitoid eggs per ha are released. Using this technique, the egg parasitoids can successfully lower and maintain the stinkbug population below the threshold level. This technology was further modified for implementation in micro river basins (Corrêa-Ferreira *et al.*, 2000).
Production of T. basalis: This parasitoid is multiplied in the laboratory on *N. viridula* eggs throughout the year at Embrapa Soybean. The limitations to the use of *T. basalis* include parasitoid availability and the amount of labor involved. Another constraint is the reluctance of many growers to adopt *T. basalis* in lieu of more conventional control methods.

Host plant resistance: Host plant resistance is a highly desirable IPM tool, as its adoption does not require users to make complex changes in their routine. Soybean cultivars resistant to stinkbugs have been developed in Brazil (Rosseto, 1989; Hoffmann-Campo *et al.*, 1996). The soybean cultivar 'IAC-100' released by the Instituto Agronômico de Campinas (São Paulo State) presents double resistance to stink bugs and defoliators, as well as good agronomic traits (Rosseto, 1989). At Embrapa Soybean, the breeding program has been incorporating resistant genes from resistant genotypes (PI 171451, PI 227687, PI 229358 and PI 274454) into advanced lines that has showed good agronomic traits and high yields.

Control of the gall maker stem borer and white grubs.

The gall maker stem borer, *S. subsignatus*, rose from a minor to important pest as soybean fields expanded to new areas and systems changed to no-tillage practices. The adult *S. subsignatus* typically inhabits the lower third of soybean plants, protected by leaves and out of reach of insecticide

sprayings (Silva *et al.*, 1998). Larvae develop inside a stem gall. Before the IPM program, more than five applications per month of broad spectrum insecticides were used against the gall maker stem borer. These applications were performed early in the season and often resulted in increased pest problems, due to the depletion of important biological control agents. Recently, the technology of seed treatment with systemic insecticides has been developed and successfully used, preserving natural enemies. A strip of 30 to 50 m width on the field margin, sown with treated seeds, can maintain the pest population below threshold levels. Currently, aerial insecticide applications have been reduced to only one per season.

Cultural control methods: Populations of *S. subsignatus* usually become apparent in November, reaching a peak after mid-December (Hoffmann-Campo *et al.*, 1999). Early planting of soybean (mid- to late October) can prevent the synchrony of peak population of *S. subsignatus* with the most vulnerable plant stages. Crop rotation is also an effective cultural control strategy, especially for oligophagous and long cycle insects such as *S. subsignatus*. Populations of *S. subsignatus* have been adequately controlled by rotation with non-legumes such as corn, sunflower, or cotton (Hoffmann-Campo *et al.*, 1999). During crop rotation, one third to one half of the previous soybean area is planted with another crop. This strategy is essential for *S. subsignatus* control, and particularly efficient when it is used along with treated soybean seed in the border strip.

White grubs are root feeders and are also difficult to control with insecticides. Cotton, *Crotalaria juncea* and *C. spectabilis* are options for crop rotation in areas infested with white grubs, especially *P. cuyabana* (Oliveira *et al.* 1997). The timing of tillage can also be used as a strategy to aid in white grub control (Oliveira, *et al.*, 2000). Proper tillage timing can be an effective component of IPM for some soil dwelling insects, although further studies are needed to recommend its widespread use for soil insects pest control.

Soybean IPM in micro river basins

Soybean IPM program was modified slightly for implementation in environmentally sensitive river basins. The pilot program took place in the Rio do Campo basin. Laboratory rearing facilities were established in the river basin by farmers' associations in cooperation with county and state organizations. Strategies used in the river basin IPM program included (i) weekly pest monitoring; (ii) use of action thresholds to dictate control decisions; (iii) use of baculovirus or other highly selective insecticide to control *A. gemmatalis*; (iv) releasing *T. basalis* throughout the basin for adequate dispersion and control of stink bugs; and (v) application of insecticides only in border rows or at a reduced rate, mixed with sodium chloride to control stink bugs (Corso and Gazzoni, 1998).

A dramatic change in pest control practices occurred in the Rio do Campo basin after four years of IPM adoption. The average number of insecticide applications in IPM-treated fields dropped by 56%, from 2.80 to 1.23 per season (Corrêa-Ferreira *et al.*, 2000). The use of microbial insecticides also increased. In the 1997-1998 season, 62% of the river basin was treated with *Ag*NPV, and large areas outside of such river basins started to be treated with *Ag*NPV as well (L. Morales, unpublished data).

A significant increase in the use of more selective insecticides was a successful hallmark of the river basin IPM program. Before the IPM program, broad-spectrum and highly toxic insecticides represented 97% of the sprayings. After four years of the river basin IPM program, microbial insecticides and IGRs accounted for about 75% of insecticide use.

Final comments

IPM programs in Brazil contemplate release of biological control agents and strongly emphasize the reduction of broad-spectrum insecticide use. Continuous improvement of these programs is necessary to achieve wider use of IPM tactics against major pests, as well as to develop appropriate control tactics against newly emerging pests. Considerable improvements are expected in the methods of production and release of indigenous entomopathogens, parasitoids, and predators. Organic farming is a growing sector in Brazil, with an increasing demand for pest control methods that can also be used in organic soybean production.

Farmers are increasingly using safer and more selective insecticides, such as biologicals, IGRs and nicotinoids. Most likely this trend will continue, as private companies release new products. Furthermore, the public is demanding more high quality food and fewer chemical pesticides used in food production. In addition, policies for registration and use of insecticides in the country have become more stringent.

There is no legislation prohibiting genetically modified crops use in Brazil. According to the Brazilian legislation, presently in force, the Biossafety Comission (Comissão Técnica Nacional de Biossegurança - CTNBio) has the authority to decide upon cultivation and commercialization of genetically modified crops. Meanwhile, a consortium of NGOs argued on a Brazilian Federal Court that the environmental impact data provided for the Roundup Ready Soybean was not sufficient and inconsistent to assure the absence of negative impact of the technology on the environment. Given that, the matter is still *sub judice*, no genetically modified plants can be cultivated (except for research purposes) or commercialized in Brazil. Exceptionally,

in the present season, growers that attained their own genetically modified seeds, obtained outside of the official Brazilian seed system, can grow and commercialize Roundup Ready soybean cultivars. Several aspects of genetically modified crops are being evaluated, especially their impact on natural enemies, on nontarget insects, and other arthropods that feed on these crops, as well as the possibility of pest resistance. Thus, if genetically modified crops are approved, it is likely that IPM programs will need to be reevaluated and modified to account for this change.

Although continuous development and improvement of IPM programs in Brazil is important, improved technology transfer and outreach to growers is fundamental. IPM tactics must be made widely available to farmers through research institutions, official and private (farmer's cooperatives) extension services, and private companies. It is only by educating farmers of the importance and benefits of using IPM tactics for pest control that IPM programs can have a broader impact on Brazilian agriculture.

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Aspects of soybean insect resistance breeding in China

S. Wang[1]

Abstract

Progress in soybean insect resistance breeding in China is briefly outlined, which includes species and distribution of soybean insect pests in China, identification of insect resistance and screening of resistance sources, resistance mechanism and inheritance of soybeans to insect pests, and soybean insect resistance breeding. Some suggestions on soybean insect resistance breeding are proposed.

Key words: Soybean, insect resistance, breeding.

The damage caused by insect pests is an important factor impairing soybean production. It was estimated that soybean loss caused by insect pests is about 10-15% each year in China. At present, comprehensive measures are usually adopted in the course of the prevention and control of insect pests, such as improved cultural management, chemical control and biological prevention. However, the development of cultivars with resistance to insect pests is one of the most effective and economical methods to prevent soybean pests. Researches on soybean insect resistance breeding in China began in the late 1950's. A number of results have been achieved. Aspects of soybean insect resistance breeding in China will be outlined as follows.

Species and distribution of soybean insect pests in China

There are about 233species of insect pests identified in China so far, and 162 species of them are distributed in the Northeast (25). The major insect pests include soybean aphid, soybean pod borer, limabean pod borer, soybean root-miner, bean fly, onion thrips, Northeastern dark chafer, smaller velvet chafer, two-striped leaf beetle, Mongolian gray weevil, beet webworm, cotton red spider, bean pyralid, cotton geometrid, cotton worm, three-spotted plusia, flax bud worm, bean tussock moth, greenish yellow-brown hawk moth, and so forth (see Table 1.).

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Table 1. Species and distribution of major soybean insect pests in Chinat

Common name	Scientific name	Distribution	Damaged stage
Soybean root-miner	*Ophiomyia shibatsuji*	Northeast	Seedling stage
Mongolian gray weevil	*Xylinophorus mongolicus*	Northeast	
Smaller velvety chafer	*Maladera orientalis*	Northeast, North, East	
Onion thrips	*Thrips tabaci*	Widely distributed	
Two-striped leaf beetle	*Monolepta nigrobilineata*	Widely distributed	
Northeastern dark chafer	*Holotrichia diomphala*	Northeast, Northwest	
Soybean aphid	*Aphis glycines*	Widely distributed	Vegetative stage
Cotton red spider	*Tetranychus urticae*	Widely distributed	
Greenish yellow-brown hawk moth	*Clanis bilineata*	Widely distributed	
Cotton worm	*Prodenia litura*	Widely distributed	
Three-spotted plusia	*Plusia litura*	Widely distributed	
Cotton geometrid	*Ascotis selenaria*	Widely distributed	
Flax bud worm	*Heliothis dispacea*	Northeast, North	
Bean tussock moth	*Cifuna locuples*	Widely distributed	
Beet webworm	*Loxostege sticticalis*	Northeast, North	
Bean pyralid	*Lamprosema indicata*	Northeast, South, East	
Bean fly	*Melanagromyza sojae*	North, South	
Soybean pod borer	*Leguminivora glycinivorella*	Northeast, North, East	Pod-setting stage
Limabean pod borer	*Etiella zinckenella*	North, South	

Identification of insect resistance and screening of resistance sources

According to the different species of insects, there are different identification methods of insect resistance. In general, the evaluation of insect resistance is usually conducted under natural and/or artificial infestation conditions. The bioassay technique is also adopted sometimes (20).

In a study involving soybean pod borer, Guo and Feng (10) proposed an evaluating method. The percentage of injured seed in the field or in the germplasm nursery was investigated for screening resistant materials. Then the selected materials were investigated in test plots for one or two years. Resistant plant sources were also evaluated under conditions of artificial infestation during the period when adult pod borers occurred abundantly in the field. To determine the stability and adaptation of resistance, fields test were conducted at different locations. Gai et al. (9) put forward the methods and criteria of identification of soybean resistance to leaf-feeding insects (LFI) in soybean breeding. A criterion of visual rating of defoliation on a scale of 0 ~ 4 was suggested for field screening for source of resistance of soybeans under natural insect population. Arithmatical mean, weighed mean (WA) and the first principle component were used as comprehensive scores over several times of observations. Among them, WA was considered the best one in most situations. A criterion of rating the comprehensive scores on a scale of five categories was suggested for classifying the

levels of resistance. For the insect resistance of soybeans, five types of resistance are usually classified as follows: high resistant (HR), resistant (R), middle type (M), susceptible (S) and high susceptible (HS). The immune types have not been found so far.

A number of resistant sources to soybean pod borer have been identified in Northeast China (11,24,26). Fan (6) screened some materials with better tolerance to soybean aphid. Yue et al. (1991) identified three accessions of wild soybean with high resistance to soybean aphid. Lu et al. (15) screened some soybean varieties and lines with high resistance to limabean pod borer. Gai et al. (7) identified some resistant sources to bean fly. Shao et al. (1997) screened some cultivars and lines with better tolerance to soybean root-miner. Twenty genotypes from soybean germplasm accessions were identified to be highly resistant to leaf-feeding insects in Nanjing (3,5)(see Table 2.).

Table 2. Identified soybean sources of resistance to insect pests in China

Insect name	Major sources of resistance
Soybean pod borer *Leguminivora glycinivorella*	Jilin no.16, Tiejiaqing 1942, Tiejiaqing 1285, Maojiadou, Miaoyandou, Guoyu 98-4, moshidou, Heijiadou, Tiejiazi, Dongnong8004, Gongjiao 85164-92-1, ERI0001, ERI0002, ERI0003.
Soybean aphid *Aphis glycines*	Luzhadou, Guoyu 100-4, Suwuxiaobaimei, Duludou, Qingpipingdingxiang, Xiaoyoudou, Tiefeng 8, Tiefeng 24, Shennong 25104, 85-39, 85-32, 85-1.
Limabean pod borer *Etiella zinckenella*	Guoyu 98-4, Bainong 2, Tiejiahuang, Youbiao 30, Ludou 4, Zaosheng, Hui 8024.
Bean fly *Melanagromyza sojae*	Zheng 76064-1, Yangchunqingdou, Zhou 7327-3, Xudou 4, Yuejing 3, Yudou 2, Lin88-10, Heqing 1, N4582, N3498, N2980, N3697, N3008, N1657.
Soybean root-miner *Ophiomyia shibatsuji*	Hu94-41, Hu94-131, Hongfeng 9, Fengshou22, Bei 93-313, Bei 93-454, Bei 93-95,Gongye 85096, Pingdingxiang, Ke7311.
Cotton worm *Prodenia litura* Cotton geometrid *Ascotis selenaria* Bean pyralid *Lamprosema indicata*	N2549-2, N1178-2-2, N 5454-3, N 3697, N 3039, N 4908.

Mechanism of insect resistance

The resistance mechanisms of soybeans to different insect pests are probably either same or different. Meanwhile, it is likely that there exist different patterns of resistance mechanism for the same pest. In many cases, the different patterns of resistance mechanism play a comprehensive role for the insect resistance of soybeans. The resistance mechanisms of soybeans to insect pests are usually classified into three patterns—antibiosis, antixenosis and tolerance.

According to Guo et al. (10), resistance to soybean pod borer was found to be related to the performance for ovi-position, soybean maturity, the structure of the pod cortex tissue and agronomic characters such as absence or presence of pubesence on the pods. Wang et al. (24) reported that there was a significant positive correlation between the percentage of injured seeds and sugar content, while a significant negative correlation between the percentage of injured seeds and cellulose content in pod cortex tissue. Zhao et al. (27) indicated that soybean varieties with less branches, dark-colored pods and lower protein content usually had lower percentage of damaged seeds and stronger resistance to soybean pod borer. Feng et al. (1991) found that the most of resistant materials to soybean aphid belong to the type of bare pods. Fan (6) concluded that the performance of the resistance to soybean aphid was mainly tolerant. Liu et al. (12) reported that there was an obvious decrease of the reproductive amount of aphid in the resistant lines obtained by introducing exogenous DNA than that of in the check variety. Shi et al. (1995) found that varieties with dark-colored pods, longer and denser pubesence usually had a better resistance to limabean pod borer. Liu et al. (14) reported that the number insects, single insect weight, and single insect length in stem, rather than those in petioles, were major traits of antibiosis of the hosts to the bean fly. Resistance to boring from petiole to stem and resistance to growth and development in stem showed to be two different antibiotic mechanisms. The antibiotic traits were significantly correlated with stem diameter. Yan et al. (1992) indicated that soybeans had tolerance to the bean fly. According to Miao et al. (1996), the preference for ovi-position of the adults was founded and the seedling stems of the resistant varieties were all purple. Gai et al. (8) and Sun et al. (23) reported that soybean had obvious antixenosis and antibiosis to leaf-feeders, but the resistance of soybeans to cotton worm was mainly antibiotic.

Inheritance of insect resistance

The inheritance of insect Resistance of soybeans is very complicated. In general, major gene resistance and polygene resistance were both reported. Since the different experimental material, resistance criteria, indicating traits of resistance and environments, the conclusions on the inheritance of insect resistance were also different sometimes. There are only a few reports relating to inheritance of insect resistance in China.

A preliminary test made by Zhang and Fu (1983) revealed that the resistance to soybean pod borer controlled by nuclear genes and the susceptibility to the pod borer was partial dominant. Sun et al. (7) indicated that the percentage of damaged seeds or pods in F2 generation were unsymmetrical distributed, and were not characterized by the centrally symmetrical distribution pattern which a quantitative in character usually possessed. The resistant genes from the male and female parents might be different. Sun et al. (18) indicated that the recessive genes might control the resistance to soybean aphid. Liu et al. (13) reported that the inheritance of resistance to the bean fly controlled by a single dominant gene and no cytoplasmic effect was observed. Sun et al. (22) studied the inheritance of resistance of soybeans to the natural population of leaf-feeding insects. One major gene plus polygene mixed inheritance model was detected and the resistance was dominant. Sun and Gai (21) investigated the inheritance of soybean resistance to cotton worm in the net room under the inoculation condition. The results showed that the inheritability values of major gene and polygene were different at various growing date. The inheritability value of major gene varied from 50% to 70%, but only one major gene was shown, and these of polygene varied from 10% to 30%.

Soybean insect resistance breeding

Researches on soybean resistance breeding to soybean pod borer have been done in JiLin Academy of Agricultural Science (JAAS). A few resistant cultivatars released in past years, such as Jilin No.1, Jilin No.3, Jilin No.13, Jilin No.16, Jilin No.34, Jilin No.36, Jilin No.50, and so forth. Hybridization breeding is still a main method. Sun et al. (17,19) suggested that selection for pest-resistance in F2 generation was effective. In a breeding program, it is advisable to evaluate insect resistance by grouping the testing materials into maturity classes with minimum maturity differences. The procedure could greatly lower the interferences from maturity and flowering date, and enhance the accuracy of resistant screening.

In recent years, the transgenic technique has been used in the development of soybean resistant varieties to insect pests. Su et al. (16) reported that

soybean transgenic plants with Bt gene had been regenerated. Wei et al. (2001) reported that four resistant strains to soybean pod borer had been obtained by transferring Bt gene into soybeans. According to Liu et al. (2002), one strain (KCD-2) with high resistance to soybean pod borer and two strains (94-593,94-181) with high tolerance to soybean aphid had been obtained by introducing exogenous NDA. Liu et al. (2003) obtained five strains with high tolerance to soybean aphid by transferring GNA gene into soybeans through the pollen tube pathway (PTP) approach.

Some elite strains (Nannong 87-23,88-29) with resistance to the bean fly were selected in Nanjing Agricultural University (NAU). NAU has been done much research work on soybean resistance breeding to leaf-feeding insects (2). Other soybean insect resistance breeding programs, such as limabean pod borer and soybean root-miner, have also done in some institutions.

Prospects and suggestions

Accompanying the application of biotechnology and molecular biology in crop breeding, soybean insect resistance breeding may have a broad prospect. According to the present situation of soybean insect resistance breeding in China, some suggestions relating to the resistant breeding should be considered. First, the screening and introduction of resistance sources to insect pests should be strengthened. Second, researches on the mechanism and inheritance of insect resistance should be deepened. Third, combining conventional breeding programs, the development of multi-resistant varieties to soybean pests should be enhanced. Fourth, new techniques, such as marker-assisted selection (MAS) and transgenic methods, should be applied to improve approaches of soybean insect resistance breeding.

An overview of current soybean IPM practices and limitations in India

A. N. Sharma[1]

Abstract

Chemical insecticides have always remained farmers' first preference for insect control. Having relied solely on chemical insecticides for about last 20 years, they are now facing problems of insecticide resistance in insects, minor insects becoming major insect-pests, insecticides losing their efficacy etc. To cope up with these problems, a new thrust has been given to insect management in soybean. Insect resistance/tolerance has been made mandatory in varietal development programmes. Several insect resistant sources have been identified through large-scale field screening followed by laboratory screening – DS 396, TGX 855-53D (for defoliators), MACS 124 (for stem fly – Melanagromyza sojae), L 129 (for girdle beetle – Oberiopsis bravis) etc. These insect resistant sources have been used in breeding programmes and the resulting generations are being tested in hot spots for their reaction against target insect and for yield attributes. Bio-insecticides based on Bacillus thuringiensis and Beauveria bassiana have already been tested and recommended to the farmers. With the Integrated Pest Management (IPM) technology demonstrated under real-farm conditions, the farmers could harvest about 500 kg/ha more yield than with their own practices and earned a net profit of over Rs. 2200 per ha. Emphasis is also being given to exploit the potential of naturally occurring bio-control agents like – parasites, predators, insect pathogens, insectivorous birds etc. Chitin inhibitors viz. Diflubenzuron and Lufenuron have been found to be highly effective against lepidopterous defoliators and safe to natural enemies. Among newer chemical insecticides thiamethoxam 70 WS, thiamethoxam 25 WG and ethofenprox 10 EC against white fly, Bemisia tabaci, methomyl 40 SP and Lambda cyhalothrin 5 EC against Helicoverpa armigera have shown great potential in suppressing damaging populations of major insect pests of soybean. Some of the major impediments in implementation of IPM are: inadequate pest-monitoring system, lack of awareness among farmers and the extension functionaries, limited availability of bio-control agents esp. microbial insecticides, and non-utilization of native, potent insect pathogens.

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Major insect-pests of soybean in India

There are about 20 insect species that damage soybean and cause economic losses in India (Table 1). Damage potential of major insect-pests has been worked out, which varies from 20 to 80 % depending on the insect and the crop stage (Sharma, 1999). By and large, if the insect-pest population is managed properly, an additional yield to the tune of 27 % can be realized (Sharma and Shukla, 1997). The insect-pest complex varies form region to region. Stem fly and semiloopers damage soybean in all the soybean growing areas. Blue beetle and girdle beetle are dominant in central part of the country that accounts for about 70 % of total area and production. Incidence of *Helicoverpa armigera* in this region has become a matter of great concern during last two seasons. White fly and the YMV disease transmitted by it are major problems in northern states. In southern parts, tobacco caterpillar, stem fly and pod borers damage the crop extensively. For identification and management of major insect-pests, an expert system – SOYPEST has been developed (Saini et. al, 1997).

Table 1. Major insect-pests of soybean in India

A. Foliage feeders:	Semiloopers	*Chrysodeixis acuta* (Wlk.)
		Diachrysia orichalcea (Fab.)
		Mocis undata Fab.
		Rivula sp.
		Gessonia gemma
	Tobacco caterpillar	*Spodoptera litura* (Fab.)
	Gram pod borer	*Helicoverpa armigera* (Hub.)
	Bihar hairy caterpillar	*Spilosoma obliqua* (Wlk.)
	Leaf folder	*Hedylepta indicata* (Fab.)
	Leaf miner	*Aproaerema modicella* Deventer
	Blue beetle	*Cneorane* sp.
B. Stem borers:	Stem fly	*Melanagromyza sojae* (Zehnt.)
	Girdle beetle	*Obereopsis brevis* (Swed.)
C. Pod borers:	Gram pod borer	*Helicoverpa armigera* (Hub.)
	Pink pod borer	*Cydia ptychora* Meyrick
D. Sap feeders:	White fly	*Bemisia tabaci* Genn.
	Jassids	*Empoasca terminalis* Dist.
		Apheliona maculosa Dist.
	Thrips	*Thrips tabaci* Lind.
		Ayyaria chaetophora Karny
stinkbug	*Nezara viridula* (Linn.)	

Component wise status of IPM practices

Cultural control practices

Deep summer ploughing is strongly recommended and followed by the farmers to minimize the incidence of soil inhabiting insects, such as girdle beetle, blue beetle etc. Awareness about spacing and plant population is gradually creeping in. Depending upon the growth habit and seed size of a variety, row-to-row spacing of 30-45 cm and seed rate of 60-85 kg/ha is used. This practice helps in reducing incidence of defoliators. Some seed-producing farmers even go for seed dibbling for attaining higher seed multiplication.

Bio-control practices

Despite great potential, bio-control of soybean insect-pests has not gained desired momentum. Several natural enemies, including parasites, predators and insect-pathogens have been found to operate in soybean based cropping system (Table 2). Most of them are highly active during the month of August. Under congenial weather conditions insect pathogen, *Beauveria bassiana* alone can wipe off the population of lepidopteran defoliators. *Bacillus thuringiensis* (Bt) based bio-insecticides have been widely tested. They have been found to be as effective as chemical insecticides. Moreover, they are compatible with certain chemical insecticides as well as with fungicides (Sharma and Ansari, 1999; Ansari and Sharma, 2000). A tank-mix combination of Bt + monocrotophos + carbendazim / thiophenate methyl / triademafon can control defoliating larvae, stem borers and fungal diseases with single application. It is unfortunate that the potential of these naturally occurring bio-control agents is often suppressed because of heavy dependence on chemical insecticides.

Taking cognizance of the need to extend bio-control technology to the field, the Department of Bio-technology (DBT), Govt. of India, initiated a 'Bio-control Network Programme with total budget out lay of INR 160 m (approx. USD 3.2 m) (Wahab, 2001). While supporting more than 60 R & D projects, DBT has also addressed to the need of entrepreneurship-development. During the period 1996-97 to 2000-2001 consumption of technical grade Bt and neem based insecticides increased from 219 MT to 683 MT. As a result of this, more than 20 companies are now manufacturing bio-control products in India. Although, at present the market share of bio-pesticides is less than 1 per cent, but the steady growth in consumption of bio-pesticides indicates bright future ahead, and it is estimated that during next decade the share would be eventually 3-5 per cent (Ramarethinam *et. al.*, 2003).

Table 2. Some important naturally occurring bio-control agents of soybean insects and their potential

Natural enemies	Host insect	Potential
Parasitoids (*Appanteles, Trichogramma, Brachymeria, Bracon, Sturmia* spp.)	*C. acuta, D. orichalcea, S. litura, H. armigera*	5 to 30 % larval mortality
Predators (*Cantheconidia furcellata, Chrysoperla carnea*)	*C. acuta, D. orichalcea, S. litura*	10 to 12 % larval predation
Insect Pathogens (*B. bassiana. N. rileyi, Bt* & NPV)	*C. acuta, D. orichalcea, S. litura, H. armigera*	15 to 100 % larval mortality

Host Plant Resistance

Development of high yielding varieties was the only emphasis given during the establishment phase of soybean in India. During 70s and 80s, number of insect-pests attacking soybean were less and the losses were also not so alarming. But with more than 5 per cent annual increase in area, problems of insect-pests and accruing losses also increased. During mid 90s, concerted efforts were made to identify insect resistant genotypes and to incorporate the trait in cultivated varieties. Large-scale field screening trials have been conducted. To make field screening more result oriented following criteria are employed: 1. resistance against one insect spp. at 3 or more locations in any of the last 3 years, or 2. multiple insect resistance at any location in any of the 3 years, or 3. resistance against one insect spp. in 2 or more years at any location. Accordingly, varieties PK 262, PK 416, PK 564 and Shivalik have been found to be highly tolerant to damage due to stem fly (Sharma et. al., 1994).

In order to account for yield and tolerance to prevailing insect-pest complex, the 'maximin-minimax method' (Odulaja and Nokoe, 1993), is being used that helps to categorize genotypes into four groups, viz. High Yielding-Resistant, High Yielding-Susceptible (i.e. tolerant), Low Yielding-Resistant and Low Yielding-Susceptible. Released varieties viz. NRC 7, NRC 12, NRC 37, JS 80-21, JS 93-05 and MACS 124 are highly tolerant to insect damage (Sharma, 1996). At NRC for Soybean, Indore, screening of genotypes for insect resistance is also taken up under controlled conditions. 'Multiple Choice Test' (MCT) and 'No Choice Test' (NCT) approaches are employed to know the type of resistance in the genotypes. Accordingly, some germplasm lines have been found to possess '*antixenosis*' (non-preference) and '*antibiosis*' type of resistance. These lines have been used in domestic breeding programmes. The advanced generations are further field tested at hot-spots and other coordinating centers for their reaction against different insect pests as well as for agronomic acceptability.

Chemical control

Based on research trials, several chemical insecticides have been recommended to the farmers for management of different insect-pests (Table

3). Dose and time of application are explicitly mentioned in all the recommendations. But farmers seldom stick to the recommended quantity of insecticide as well as of water to be used. Moreover the spray schedule does not coincide with insect incidence rendering it to be a wasteful exercise. Mixing of two or more insecticides without knowing their compatibility is yet another unwarranted practice. However, with the experience of inadequate control of *Helicoverpa armigera* through irrational use of chemical insecticides, farmers are now more vigilant.

Table 3. Chemical insecticides recommended for soybean insect-pests

Insect(s)	Insecticide & Dose	Application time
Stem fly	Phorate 10G at 10 kg/ha /	At sowing
	Thiamethoxam 70WS at 3g/kg seed /	Seed treatment
	Thiamethoxam 25WG at 100 g/ha	7-10 day old crop
Blue beetle	Quinalphos 25EC at 1.5 l/ha	7-10 day old crop
Girdle beetle	Phorate 10G at 10 kg/ha /	At sowing
	Trizophos 40EC at 0.8 l/ha /	0.8 l/ha
	Monocrotophos 36SL at 0.8 l/ha	0.8 l/ha
Semiloopers and tobacco caterpillar	Chlorpyrifos 20EC at 1.5 l/ha / Endosulfan 35EC at 1.5 l/ha / Quinalphos 25EC at 1.5 l/ha / Trizophos 40EC at 0.8 l/ha / Methomyl 40SP at 1.0 kg/ha	At ETL
Gram pod borer	Methomyl 40SP at 1.0 kg/ha / Lambda cyhalothrin 5EC at 300 ml/ha	Up to 2nd instar
Sucking insects	Monocrotophos 36SL at 0.8 l/ha / Ethofenprox 10EC at 1.0 l/ha	Need based

Besides above, insect growth regulators (IGRs) have also been evaluated and found highly effective against defoliating caterpillars. Diflubenzuron 25WP @ 300 g/ha and Lufenuron 5EC @ 400 ml/ha could suppress larval populations significantly, if sprayed up to 2nd instar.

IPM Package

On the basis of component wise recommendations emanating from various research trials, the Technology Mission on Oilseeds & Pulses (TMOP), Govt. of India, finalized IPM package for soybean (TMOP, 1998). NRC for Soybean, Indore, further refined this package and validated under 'real-farm' conditions. The package included: 1. deep summer ploughing, 2. use of insect tolerant varieties, 3. phorate application at sowing, 4. installation of Light-Trap, 5. use of bird-perches, 6. mechanical removal of infested plants / plant parts, 7. spray of *Bt* based bio-pesticide, and 8. need based use of chemical insecticide. Conducted for two years, the IPM demonstration trials (20 in numbers) gave about 500 kg/ha more grain yield on an average than farmers' practices, and a net return of over Rs. 2200 per ha.

IPM for *Helicoverpa armigera*: In southern parts of the country, *H. armigera* is a perpetual problem in soybean. The coordinating centers located in this region viz. Dharwad and Bangalore have developed a sound IPM package for this pest. Besides deep summer ploughing, intercropping with maize / onion / red gram / coriander, pheromone traps and bird perches, this package also includes spray of HaNPV (@ 250 LE/ha), neem seed kernel extract (@0.2%) and *N. rileyi* (@ 1.0 kg/ha).

Farmers' ingenuity: Based on the traditional knowledge, some farmers spray soybean with herbal preparation, which is essentially a decoction of neem, tobacco, *Ipomoea* and *Pongamia* leaves mixed with cow urine and fermented buttermilk. This preparation is sprayed frequently to deter the insects from feeding on foliage. There is, however, no consistency of the treatment and farmers' experiences.

Limitations in implementation of IPM in soybean

Lack of awareness: National Research Centre for Soybean, Indore has fully utilized its whatsoever limited resources to bring awareness among the farmers and extension personnel through various training programmes. But still a large proportion of farmers are ignorant of IPM practices. For extension personnel, who belong to the state department of agriculture, soybean is one of several other crops to look after. Hence, needed attention is lacking both with respect to acquiring technical knowledge and transmitting to the farmers. A strong and efficient pest monitoring and surveillance system has to be operative.

Easy access to chemical insecticides: Owing to quick knock down and visual effect, affordable prices and developed market, chemical insecticides dominate insect management practices. Farmers mostly depend on non-technical retailers for advice. Often, the farmers incur economic loss for using sub standard insecticides.

Inadequate production of bio-control agents: Most of the bio-control agents producing companies cater to the needs of cotton, sugarcane and paddy growers. Production and supply of bio-control agents in major soybean growing area is very meager because of poor market development efforts by the companies. No government subsidy is available on these products.

Farmers' economic status: Most of the bio-control agents especially the microbial insecticides are manufactured by the private sector with imported strains. Consequently, the production cost increases and the product becomes beyond the financial reach of a common farmer. In retail market

microbial insecticides are at least 3-4 times costlier than chemical insecticides. Potentiality of native strains of insect pathogens should be exploited to bring down the production cost.

Lack of incentives: There is no financial incentive either from government or from industry for soybean produced without using chemical insecticides. Even the minimum support price (MSP) announced by the government does not account for the actual cost of cultivation using costly microbial insecticides.

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Current status of soybean insect pest management in the USA

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Soybean pest management is challenged by the simultaneous occurrence of biotic (e.g., various insects, disease pathogens, nematodes, and weeds) and abiotic (e.g., drought, excess moisture) stresses. Along with the concerns from existing and newer pest problems are the impacts from changing production practices, including the adoption of conservation tillage practices throughout soybean growing regions and ESPS (early soybean production systems) in the southern states, and the production of value-added soybeans including food grade soybeans.

Over the past decade, soybean research on insects focused on the impact of many of the newer production practices as well as on specific insect problems that continued to occur. With the introduction of transgenic and other herbicide-resistant soybeans, significant research was conducted on the impact on insect pests and natural enemies. The general conclusion was that the adoption of herbicide tolerant soybean would probably not significantly affect the soybean arthropod community (Buckelew et al. 2001; McPherson 2000; others, unpublished data). Thus, it was concluded that no specific changes in IPM approaches are necessary concerning the adoption of these new production practices. If and when transgenic soybeans resistant to arthropods, i.e., Bt-soybeans, are developed and enter the market, the impact on arthropod communities including non-target species will need to be determined. In the southern states, there was much research conducted to determine the impact of ESPS practices on the population dynamics of pest and beneficial arthropods on soybean. Adoption of ESPS was expected to have both positive and negative effects on insect management. Research has defined which and when certain insect pests should be a problem, and hence the critical periods for monitoring their abundance. Numerous conclusions and findings have been made. The early planting dates and early maturing varieties associated with ESPS generally allow for escape from late season migrating lepidopterous pests, such as soybean looper, velvetbean caterpillar, and green cloverworm (Boyd et al. 1997). However, it was determined that ESPS soybean may be at greater risk to attack by the stinkbug pest guild commonly found in the southern growing regions of the United States (McPherson et al. 2001). The incidence

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of bean pod mottle virus was higher in ESPS than conventional soybean, undoubtedly associated with larger numbers of the vector, bean leaf beetle, being found in ESPS (Baur et al. 2000).

Recently, soybean growers in the USA have experienced increases in certain insect pest problems and the introduction of a new and potentially significant problem. Interestingly, these problems are occurring more in the northern regions of soybean production rather than in the southern states. There are two specific concerns of importance that are being addressed across numerous states. The first concern is the increase in population densities of the bean leaf beetle, *Cerotoma trifurcata*, and a corresponding rise in the incidence of bean pod mottle virus, a pathogen vectored by the beetle (Rice et al. 2000). This relationship between bean leaf beetle and bean pod mottle virus, previously more common in southern states, is a relative new occurrence in northern USA. The second concern is the recent introduction of the soybean aphid, *Aphis glycines* (Marking 2001). Soybean growers are now facing widespread use of insecticide over potentially millions of acres of soybean in the upper Midwest and given the native range of this insect, soybeans throughout the United States are at risk of being invaded. The occurrence of both of these problems have challenged soybean growers in ways never imagined and dramatically changed their approach to insect management.

Bean leaf beetle

The bean leaf beetle has caused concern to soybean growers throughout the USA over the past 20 years. It defoliates the plant during most of the summer, and later switches to pod feeding that can result in direct injury to the seed and also cause seed quality issues because of the introduction into the pod of secondary disease pathogens. Bean leaf beetle management was usually directed against the pod feeding population in later summer.

Over the past decade, bean leaf beetle populations have increased to routinely exceed economic thresholds (Rice et al. 2000). Increased soybean acres planted in the Midwest and earlier planting dates are most likely the major factors contributing to the general increase in beetle numbers. The spring colonizing population, which has in the past been a relatively minor concern, is regularly entering fields during seedling emergence and causing great concern to soybean growers. Concurrent with the population increase has been an increase in diseases transmitted by the beetle (particularly bean pod mottle virus) (unpublished data from numerous states). Bean leaf beetle populations, having increased dramatically over the past decade, is thought to be the reason for the increase in bean pod mottle virus. The

occurrence of virus-caused symptoms, most notable "green stem syndrome" and mottled seeds, has caused growers great problems. This concern has been especially true for growers of food grade soybeans where seed appearance and quality is of utmost importance and to those growers producing soybean for seed, an industry that also demands clean seed.

For the bean leaf beetle generation that feeds on the pods, the standard management tactic has been sampling the numbers of beetles and determining the level of pod injury, and then making a treatment decision based on accepted thresholds. A new approach using the population levels of the first-generation in mid-summer has been suggested. Recent research from Iowa has resulted in a management strategy based on sweep-net sampling the first-generation population, using the population densities to predict if the second generation population will reach economically damaging populations, and if necessary, treating this second generation population early in its population growth period. A prerequisite of this approach is the use of a longer-residual insecticide. This approach is currently being examined by other states.

The ability of the beetle to vector the virus and cause economic losses as well as management of both the virus and its vector is also being researched. There are two times when virus transmission is thought to occur. The first time is at the early growth stages following crop emergence when overwintering bean leaf beetles initially transmit the virus. The second time period is in July when the first generation continues to transmit and spread the virus across the field. A few states have begun examining the ability to control the overwintering population of bean leaf beetle to limit the occurrence of bean pod mottle virus. Early indications are that an insecticide spray following soybean emergence, plus an additional insecticide application in July against the first generation of the insect, offers promise (Krell and Pedigo, unpublished data). A number of states are beginning to examine this in field studies. Additionally, the possibility of using this approach but using a systemic seed treatment to replace the initial spray for the overwintered population is being researched. Newer seed treatments, recently labeled on corn, are being examined on soybeans. These new seed treatments (currently not labeled on soybeans), all commercially applied to the seed prior to planting, are clothianidin (Poncho) and thiamethoxam (Cruiser). Two Midwestern states have had emergency-use labels approved for their use for this purpose in 2003 and 2004. Most northern states are conducting further studies with these materials to determine their promise in reducing the incidence of bean pod mottle virus.

Soybean aphid

Most recently, the discovery of the soybean aphid, *Aphid glycines*, in the Upper Midwest presents a new challenge to soybean production in the USA (Marking 2001). The soybean aphid is a recent invader of North American soybean fields. The aphid's native range is from northern China to Indonesia. In 2000, aphids were found in nine Midwestern states and by 2001, it expanded into more eastern and western states and into Canada. Soybean aphids can damage plants by reducing photosynthesis and reducing pod set by as much as 33%. Yield reductions in excess of 16 bu/A (1,076 kg/ha) have been measured

Numerous management strategies for soybean aphid are being examined. Preliminary studies by some states indicate host plant resistance holds promise for managing soybean aphid. Illinois studies identified 18 cultivars that exhibited resistance to soybean aphid. Trials are continuing to be conducted to identify and characterize soybean aphid resistant cultivars. Preliminary studies also indicate planting date plays a role in aphid population build-up and resultant feeding injury; late-planted soybeans appear more susceptible to large aphid population build-up and injury.

There is a large effort beginning in the biocontrol of soybean aphid. Foreign exploration of natural enemies, including parasitoids and predators, began in the summer of 2001 with two expeditions, one to northeastern China and one to Japan. There is considerable effort to develop a set of protocols to guide decisions on natural enemy releases and evaluation of impact, collaborate with Asia scientists to define essential relationships among soybean aphid, its natural enemies and host plants, determine whether there are different strains of natural enemies adapted to conditions similar to U.S. soybean production areas, and monitor releases and evaluation of the success of natural enemy establishment and the impact on soybean aphid populations and damage. There is also work on generalist predators, e.g., Nabidae, Anthocoridae, Chrysopidae, and Coccinellidae, underway in many states, as well as a suite of pathogens that will infect soybean aphids. Indeed, many think that there may be cultural practices that will encourage earlier onset of epizootics of entomophthoraceous fungi. Of note, the occurrence of the soybean aphid has resulted in incredible large numbers of the multi-colored Asian lady beetle, *Harmonia axyridis*, a known predator of aphids, in many states. Because of the large number of acres infested with the soybean aphid, extremely large populations of this lady beetle are being produced and are causing economic concern to small fruit growers in the fall following soybean maturity, later followed by homeowner concern from large numbers of lady beetles seeking overwintering sites and entering homes in numbers reaching into the thousands.

Currently the primary management tactic against the soybean aphid is the use of insecticides. Many insecticides registered on soybeans in the USA now have soybean aphid on their labels. A significant amount of research is done to determine which insecticides are the best and most appropriate, both economically and environmentally, and how they can best be used. For example, there are studies examining ground versus aerial application, and how best to apply insecticides in narrow-row soybeans. Insecticide trials are being conducted in all states where the aphid is a problem.

As part of the general IPM approach is research to determine accurate and useable thresholds to assist in decision making. Although provisional thresholds or action levels are in use in all states, most states are conducting studies to develop more accurate thresholds that take into account aspects of grower production practices (e.g., planting dates, variety differences, etc.) and economics.

An additional concern with the soybean aphid, similar to the bean leaf, relates to aphid transmission of soybean mosaic virus (Boethel 2003). Transmission of this non-persistent virus is from aphids passing through and probing soybeans in search of a suitable host. Aphids that do not colonize soybeans have, in the past, transmitted this virus. Symptoms of soybean mosaic virus are similar to those associated with bean pod mottle virus. Plants can also be infected simultaneously with both viruses that result in severely stunted plants. Symptoms are similar, and thus, correct identification is crucial. With the recent discovery of the soybean aphid, which is the first aphid able to colonize soybeans in North America and one that can transmit soybean mosaic virus, the incidence and impact of this virus may increase substantially. Thus, research on the interactions of soybean aphid with the viruses it can transmit is ongoing.

Other areas of concern

Although these two insects are currently receiving much of the attention, other areas are continuing to be examined. Researchers are continuing to develop management strategies for insect pests of soybean under evolving cropping systems, including further work in conservation tillage. In the eastern Corn Belt, slugs continue to be a major impediment to no-till growers. Slugs have the ability to cause 100% stand reduction in soybean fields, and when and how to manage them continues to receive a strong effort. In southern states, there is still much ongoing work on ESPS soybeans. Studies are being conducted in numerous states to examine the effects of early season and mid-season defoliation on value-added soybean varieties including high sucrose and high protein varieties. The question is if economic

injury levels are different, and if so, should thresholds be modified for these diverse varieties. There is also special concern on how insects will impact food grade soybeans because of the special need for high quality beans.

Coordination of research efforts

Much of the efforts in the USA are coordinated through various regional research committees. These committees were established to aid in assisting collaborative efforts and as a way to share information. Although not specifically guiding any one person's research efforts, they do assist soybean workers in their work. Indeed, much of the efforts against the bean leaf beetle and the soybean aphid are now under the auspices of S1010, a southern regional committee. The previous regional project, S281, recently collaborated in the writing of the most up-to-date publication on soybean insects, the Handbook of Soybean Insect Pests (Higley and Boethel, 1994). There are also regional efforts towards biological control of the soybean aphid through NC-125, Biological Control of Arthropods and Weeds. Research directed toward soybean viruses including some aspects of their vectors, the bean leaf beetle and soybean aphid, are through the auspices of the regional project, NCR-200, Management Strategies to Control Major Soybean Diseases in the North Central Region. Regional committees such as these have been extremely successful in the development of economically and environmentally sound management practices against many of the arthropod pests that soybean growers encounter in the USA.

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Soybean integrated pest management implementation in Argentina

J. Aragon[1]

Abstract

Several insect and animal pests represent a serious thread to soybean production in the large geographical area where this crop is cultivated in Argentina from southern Buenos Aires to the northern subtropical provinces. Major soybean pest in the Central Provinces (Buenos Aires, Cordoba, Santa Fe and Entre Rios) where most of the soybean is cultivated (12 mill. Ha. in 2003) are several defoliators worms mainly *Rachiplusia nu* and *Anticarsia gemmatalis* (Lepidoptera: Noctuidae), a bud borer, *Epinotia aporema* (Lepidoptera: Tortricidae) and a stink bugs complex : the green stink bug, *Nezara viridula* and the alfalfa stink bug, *Piezodorus guildinii* (Hemiptera: Pentatomidae). In 1979 the National Institute of Agricultural Technology (INTA) implemented a Soybean Research and Extension Program in order to improve crop management. Working with universities and private organizations growers gradually adopted better insect management as action thresholds and replaced highly toxic fosfate insecticides by synthetic pyrethroids. Field evaluation studies showed a great diversity of efficient natural enemies of soybean pests. As a result of an improved crop management yields and quality gradually increase while insecticide use was reduced. No-till soybean, widely adopted since the early 1990´s., favored an increase of soil related damaging pests as cutworms, grasshoppers, crickets, pillbugs and slugs. In the northern provinces two introduced pest , the stem beetles, *Sternechus pinguis* and *Promecops carinicolis*, develop heavy infestation to unprotected soybean fields. In this subtropical region soybean have more risk of pests attacks than the central provinces and several insectcides treatments usually are needed. In the last years a significant adoption of early maturing soybean group and early planting in the central producing provinces permited growers to reduce insectide application. Average soybean insecticide cost estimate at national level (approx. 12 mill. ha in 2002) since 1999 ranged 2,20-3 U$A per hectare and treatment number in pilot soybean IPM fields in eastern Cordoba ranged 0.2-0.4 per field in the last 8 years. Favourable environment condition for crop development and an effective biological control reduce pest negative impact on soybean.

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Introduction

Soybean has been the most cultivated crop in the last 10 years with more than 12 millions of ha in 2002-2003. High soybean yields (2700kg/ha) indicate good crop management and suitable environment conditions. In the late 1970's and early 1980's most of the soybean (1-2 mill. ha) were located in Central Pampa Region (Northern Buenos Aires, Southern Santa Fe and Eastern Cordoba Provinces, between 32° and 35° S. L) and frecuent outbreaks of insect pests and even light infestation were controlled mainly by the use of highly toxic organofosfate insecticides. Major pests of soybean were defoliators worms (mainly Noctuidae); a bud borer and a stink bug complex. 2-3 insecticide application per field were common. This situation caused considerable negative effects on biological control agents, pollinators and wildlife. Also great number of growers were affected by the highly toxic chemical insecticides. At that time tillage operations help control of most soil pests.

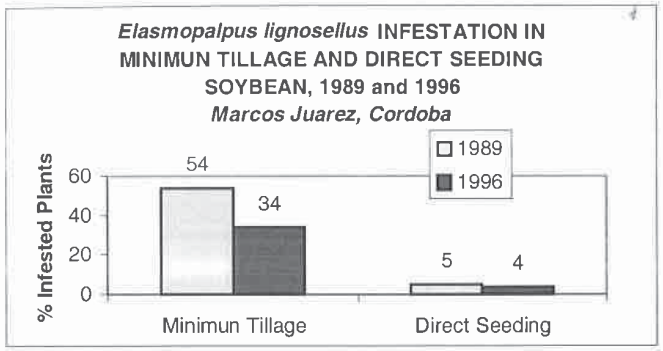
In 1979, the National Institute of Agricultural Technology (INTA) organized and provided funding for a Soybean Research and Extension Program. Working together with provincial goverments, universities, private organizations and commercial companies, several research projects provided to growers new cultivars and information about soil conservation with minimum tillage, crop rotation and weed, disease and insect management. All this work gradually improved agronomic and protection practices that increased yields. Early research works during the 1970's in INTA Experimental Stations as San Pedro Exp.St. (4), Parana Exp. St. (16) and Pergamino Exp. St.(9, 15) on stink bugs, defoliators and bud borer damage, economic thresholds and control techniques served as an important source of soybean-pest information for national distribution through Extension Service and private consultors. Sistematic sampling of soybean pest, use of action thresholds, a great diversity of efficient biological control agents (Table 1) and replacement of highly toxic insecticides mainly by synthetic pyrethroids were key factors that allowed a significant reduction of the number of insecticide application that were applied estimated in 0.5-1 application / field (3, 11). Similar results were obtained in pilots soybean fields in INTA Marcos Juarez Exp. St. (Figure 2). Synthetic pyrethroid insecticides introduced in 1978 in Argentina proved to be highly efficient weapons against the soybean defoliator lepidopterous larvae complex at extremely low dosages. In order to improve organic matter, water conservation and minimize soil erosion, soybean growers widelly adopted, since the late 1980's, the no-till production system with great success in Argentina major crops (15 mill. ha in 2002 , 50% of the cultivated land.). No-till planting have also another advantages as: decreasing fuel

consumption; reducing machinery investment cost and favor a rapid soybean planting. In the mid 1990's no-till soybean grew more after 1996, when new transgenic herbicide tolerant cultivars were marketed. This technology reduced dramatically weed control cost in no-till fields. However, the lack of soil disturbance and the residue cover provided a suitable habitad for several insect and related soil pests organism that increased their potential damage to soybean seeds and young plants. Among others emergent soybean pests, the more important at present time are cutworms, grasshoppers, a subterranean cricket, ants, slugs and pillbugs(2, 8). Despite these new limitations, effective and practical pest management alternatives for these pests were implemented and population dynamic preliminar studies indicate no significant population increase. New introduced soybean pests in the northern provinces as the stem beetle, *Sternechus pinguis* and the small stem beetle, *Promecops carinicolis* (Coleoptera:Curculionidae) cause serious damage to soybean (6). Fortunatelly, high rates of seed planting permit higher action thresholds for soybean soil pests as compared with corn and sunflower. Soybean cultivation in no-till fields expanded more after 1996, when new transgenic tolerant herbicide cultivars were marketed. This technology reduced dramatically weed control cost in no-till soybean. At the same time, the early planted (3-5 weeks before the conventional varieties planting date) and early-maturing soybean varieties (Groups III and IV) expanded as a new crop management estrategie to avoid summer drought stress and minimize late soybean deseases but also greatly reduced soybean pests incidence in the central provinces (12).

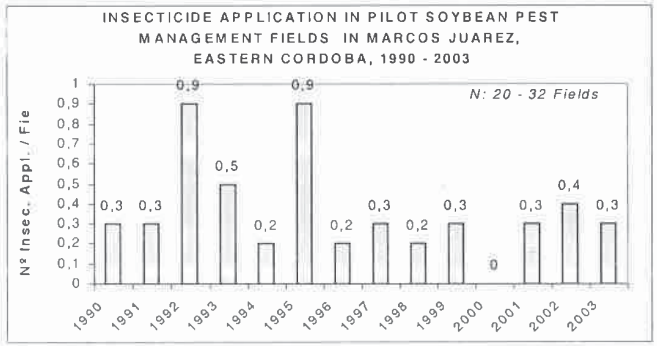
Table 1. Major soybean defoliators , bud borer and stink bugs natural enemies. SOURCE: INTA Exp. St. Oliveros, Pergamino, Marcos Juárez (3)

Pest	Parasitoids		Insects Diseases	
	Hymenoptera	Diptera	Fungus	Virus
Rachiplusia nu	-*Copidosoma floridanum* -*Campoletis grioti* -*Cotesia sp.* -*Euplectrus sp.*	-*Voria ruralis* -*Lespesia rufomaculata* -*Archytas sp.* -*Euphorocera sp.*	-*Erynia gammae* -*Nomuraea rileyi*	-*VPN*
Anticarsia gemmatalis	-*Pteromalus sp.* -*Trichogramma sp.* -*Microcharops bimaculata*	-*Europhocera bimaculata*	-*Nomuraea rileyi* -*Entomophthora sp.* -*Aspergillus flavus*	-VPN
Spodoptera frugiperda	-*Campoletis grioti* -*Ophion sp.*	-*Archytas incertus* -*Archytas marmoratus*	-*Nomuraea rileyi*	-VPN
Heliothis spp.	-*Campoletis grioti* -*Chelonus sp.*	-*Archytas incertus* -*Archytas sp.*	-*Nomuraea rileyi*	-VPN -GV
Epinotia aporema	-*Brassus sp.* -*Conura sp.*		-*Zoopthora radicans*	-GV
Nezara viridula	-*Trissolcus basalis*	-*Trichopoda giacomelli*	-*Beauveria sp.* -*Metarhizium sp.*	
Piezodorus guildinii	-*Telenomus mormideae*			

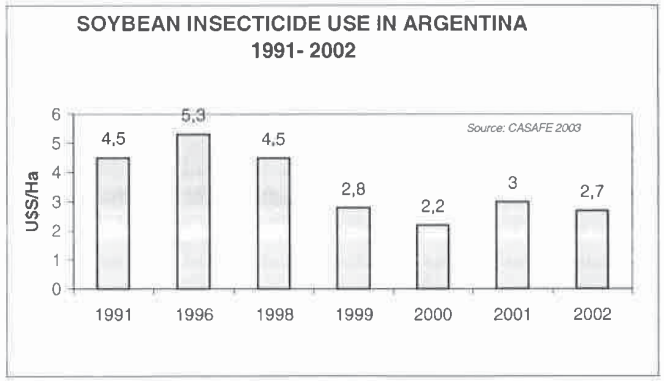
Graphic1



Graphic 2



Graphic 3



Defoliators pests

The most important soybean defoliator are several lepidopterous larvae, mainly Noctuidae. The plusiinae *Rachiplusia nu,* were the most frecuently detected species with outbreaks frecuently above the action threshold reaching 50-70 larvae/meter row in early summer but since the late 1990´s population outbreaks are sporadic. In the central provinces the soybean looper develops at least 4 generations per year. A large complex of natural enemies as the fungus *Erynia gammae*, parasitoids insects (Table 1) and predators as *Nabis spp., Geocoris spp., Chrysopa spp., Calosoma spp.*and a spiders complex contributes to maintain *R. nu* densities below action thresholds (3, 10). Another important soybean defoliator is the velvetbean caterpillar, *Anticarsia gemmatalis*, .mainly in the northern provinces where infestation start earlier than in the central provinces. Adult population dynamic studies with light traps in Eastern Cordoba indicate that from the subtropical provinces in the northern provinces moths migrates southward in February-March and laid eggs mostly in late maturing soybean. Outbreaks may occur despite predator and parasite that cannot keep them under control in soybean lots not treated with insecticides. Fortunatelly early fall rains are fairly common and trigger a naturally accurring fungus disease *(Nomuraea rileyi*) providing up to 90% of control. The fall armyworm, *Spodoptera frugiperda* and *Helicoverpa gelotopoeon* are also damaging Noctuidae species in soybean fields. *Spilosoma virginica* (Artiidae) was an sporadic late season pest of sunflower and soybean but since few years ago, it increased their population and frecuently reach defoliation action thresholds. Among the several registered insecticides for lepidopteroeus larvae control are 13 synthetic pyrethoids and their recommended rates are very low compared to the organophosphates insecticides (300-500 g.a.i./ha). Deltamethrin recommended rate for *A. gemmatalis* control is 4–7gr.a.i./Ha.; Cypermethrin: 30–50 gr.a.i./ha.; Beta-cyfluthrin: 1.7-2.5 gr.a.i./Ha.; Permethrin: 30 gr.a.i./Ha. and Lambda-cyalothrin: 6.7-8.5 gr.a.i./Ha. These recommended dosages had a limited and much less impact on predators and parasites than fosfate insecticides and allow a high level of biological control agents survival. Also are registered a *Bacillus thuringiensis* commercial formulation (*R. nu* and *A. gemmatalis*) and the new Naturalyte bio-insecticide class Spinosad (*R. nu*) (5).

Soybean bud borer, *Epinotia aporema* (Lepidoptera: Tortricidae) and lesser cornstalk borer, *Elasmopalpus lignosellus* (Lepidoptera: Pyralidae).

The soybean bud borer is a very important pest of late planting soybean varieties (Stante and Aragon, 1986) and economic infestations require high systemic organofosfate insecticides for their control. In the last years, as a result of a widwe adoption of early soybean maturity groups and early planting this insect have reduced their negative impact. In pilot soybean

IPM fields in INTA Marcos Juarez Exp. St. only 4.3% of soybean fields required chemical control in 2003 while in 1992 *E. aporema* was controlled in 55%, mainly in late planted soybean.

E. lignosellus severe seedling soybean infestation occurred in 1989 and 1996 associatted with severe drought and disturbed soil in plowed fields while no-till soybean fields showed a significant less infestation (Figure 1). The shift to no-till system preserve soil moisture reducing risk attacks by this pest (1) and only sporadic infestation were recently reported .

Stink bugs (Hemiptera: Pentatomidae)

The most important and damaging soybean pood-feeders are several stink bugs species as the green stink bug, *Nezara viridula*, the alfalfa stink bug, *Piezodorus guildinii, Dichelops furcatus* and E*dessa meditabunda*. Fields with high stink bug infestation have reduced yields and quality. These insects are widely distributed in most soybean-growing provinces. Stink bugs are considered a soybean key pest by their permanent thread to soybean crop. They also could transmit the causal organism of the yeast spot disease, *Nematospora coryli*. Early investigations in Argentina (16) determined that the most damaging species to soybean is *P. guildinii* following by *N. viridula* being both the most abundant species. Established stink bugs action thresholds are based on the number of stink bugs per linear meter of row from the pod-set (R3 and R4) and early pod-fill stages (R5) (4, 16). First *N. viridula* generation occur in alternate host as wheat, alfalfa and several weeds where preliminary studies show they have high mortality rates by natural enemies and environmental factors.

The scelionid egg parasitoid *Telenomus mormideae* are considered to be an efficient natural control of *P. guildinii* with up to 95% egg mortality rate in Cordoba Province. Another species, *Trissolcus basalis* was also identified as natural enemy of *N. viridula* eggs but are less efficient than *T. mormideae* (3). The extensive use of early planted and early maturity soybean cultivars favored to avoid most the 3rd stink bug generation, saving significant insecticide applications to farmers in a large number of hectares (12, 14). Several insecticides are registered for stink bug control on soybean.

Conclusions

Despite a large list of insect and other animal pests, envinronmental factors and biological agents permited soybean growers to produce high yields in 12 millions of hectares with an insecticide cost that in 2002 was estimated in U$A 2.70.- per Ha. (CASAFE , 2003) (Figure 3). Each year more soybean growers adopt IPM strategies and many of them have private monitoring

services that assure good crop management and sound control tactics for traditional and new soybean pest. Major research requirements for improve soybean IPM in all producing provinces are, among others, information about pest sampling methods, population dynamic and ecology studies in relation to cultural practices (weed control, crop rotation), establish new action thresholds for present soybean production systems. It is also important to promote available information for natural enemies protection , obtain and evaluate new insect resistant cultivars (17), determine new insecticide effectiveness and their impact on natural enemies. The EMBRAPA soybean biological control research projects in Brazil are excellent models to follow, mainly in the Argentina subtropical provinces (7, 13). Official and private extension services should encourage pests consultors training and promote alarm systems at regional level through modern electronic information systems.

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Soybean breeding program in Argentina

J. Ferrarotti[1]

It seems difficult to carry out a current analysis of genetic soybean breeding in Argentina without bearing in mind the regional frame provided by MERCOSUR. Several factors contribute to this view of the region which no longer limits the activity to some of the country members of MERCOSUR in particular.

In the first place, the position taken today by South America as the soybean complex ranks it at the highest position at world level.

On the other hand, the varieties developed in Argentina together with the Brazilian varieties are the ones that have contributed to develop soybean in the rest of the countries of the area. It would be fair to say that as far as developments of Argentine and Brazilian cultivars are concerned both play the role of generators whereas Paraguay, Bolivia and Uruguay are introducers of the new genetic materials.

However, research in the plant breeding field in the two main countries of the region come from histories that respond to markedly different realities. On one hand, Brazil has mounted its breeding genetic structure on a base of a political definition for over thirty years; where the Federal Government and the State Governments formed a very solid and effective public research network.

Although currently some achievements from the private sector can be sensed, it was the official infrastructure and the human resources generated by it the ones that gave way to the basis of the development of varieties in Brazil.

As for Argentina, the biggest efforts come from the risk taking from behalf of the private sector. Only by watching the panorama of cultivars it can be affirmed that almost 100% of the planted varieties come from the private sector.

When in the 80s INTA managed to introduce genetic materials to the argentine market, it was mostly done through private funds raised through agreements signed with private sectors of production. The same situation happens with EEAOC varieties, which come from the financial support carried out by local private companies.

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The particular situation of Argentina will be analyzed based on these foundational differences of the development of cultivars.

Production, industralization and exportation of soybean

Soybean area has shown a sustained increase since it incorporated to extensive agriculture in Argentina (Figure 1).

According to official numbers, during the 2002/03 season more than twelve point six million hectares were planted. Twelve point four million were harvested with a total production of almost 35 million of metric tons of grain. The unitary yields reached the historical record of 2.8 metric tons per hectare.

Approximately 70% of that production is transported to the region of Rosario, a city located southeast of Santa Fe Province (33° South Latitude).

The 200 kilometers around the city of Rosario focus the most important agro industrial export pole for soybean of the world, with modern infrastructure of crushing and a quick network of sixteen ports from where the grain and its elaborated sub products are exported to different buying destinations.

Today Argentina is the main export country of flour and soybean oil of the world. These exportations, together with the ones of grain *per se*, mean eight billion dollars for the country.

There is no better productive cost-benefit relation sector that the one corresponding to the soybean chain in Argentina.

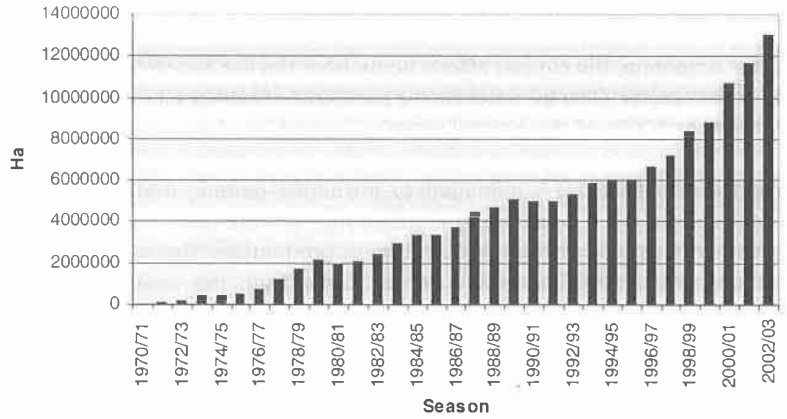


Figure 1. Evolution of the area planted with soybean. Source: SAGPyA.

Genetic breeding incidence in the soybean phenomena

The lines of work carried out by different breeding programs have played a fundamental role both in the horizontal expansion of the crop and the increase of unitary yields.

In a recent survey done on a segment of technologically more advanced producers of all the soybean regions, 90% gave more than seven points in a scale from one to ten when asked the value provided to the genetic breeding in the increase of productivity and 62% gave a value of over nine points.

Next, some of the main areas of interest where breeding has had a significant effect will be outlined.

Breeding related with adaptation characteristics

The expansion of the agriculture border that soybean gave to the argentine agriculture needed of enough vision and dedication to breed varieties adapted to the widest range of environmental conditions for the crop. As a result, many farmers today have the chance to plant varieties from a broad range of maturity groups (II to IX), with different types of growth (determined, undetermined and semi determined) and the inclusion of special traits for certain areas (for example long juvenile).

For such means, companies today count with wide networks of evaluation in the whole country, with numerous areas of experimental sites. Furthermore, certain companies which research infrastructures are located in the pampeana region have set experimental fields in the new areas of planting in order to offer high quality products from local development.

Breeding related with the increase of productivity

Just like the other extensive crops of Argentina, the unitary yields of soybean show a positive trend through time (Figure 2).

During the 1980/81 season Argentina showed unitary yields of 2 metric tons per hectare. The productivity increased to 2.15 metric tons per hectare in 1985/86, to reach the current 2.8 metric tons per hectare.

Such numbers come from an integral improvement of the different technical and productive areas related to soybean. The protection of the soil and the expansion of conservational systems of production, the advances in knowledge areas such as nitrogen biologic nutrition, mineral nutrition, irrigation and crop management have contributed to the yield increase.

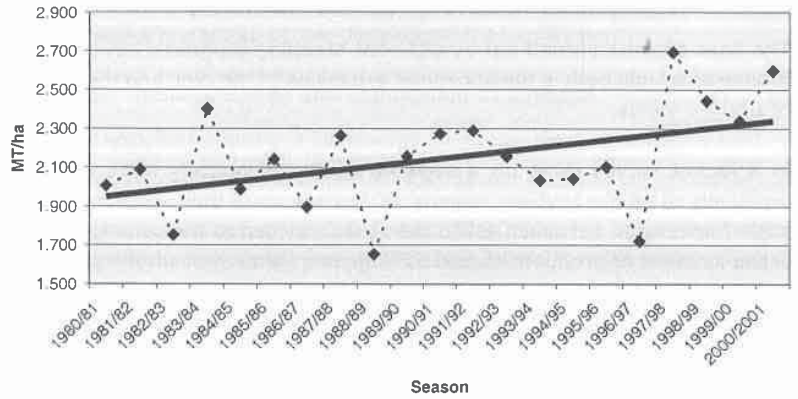


Figure 2. Soybean yield trend in the last twenty years. Source: SAGPyA

The genetic gain by selection cycle determines the degree of improvement of a breeding program for a certain trait. Like wise, such parameter applied to a certain area or region is an indicator of the joined efficiency in its improvement.

Several studies in Argentina where carried out in order to investigate the genetic advance for yield.

Some of those studies show a joined progress for all the maturity groups of 1% a year in the 80s. Further studies show a split gain for each maturity group. For the 1990-96 period the numbers are 0.7% for group IV, 1.3% for V, 1.6% for VI and 1.0% group VII. For the 1997-2002 period the numbers reported are 1.8% for group IV, 1% for V, 1.2% for VI and 2.0% for groups VII and VIII together.

Since the 2000-03 season, PROSOJA (the association that groups breeders and other soybean experts) is carrying out a broader study where several of its members participate in order to obtain updated information.

Table 1. Annual Genetic Gain for yield

Period	Maturity Group	Genetic Gain
1983-89	All groups	1,0%
1990-96	IV	0.70%
	V	1,3%
	VI	1,6%
	VII	1,0%
1997-02	IV	1,8%
	V	1,0%
	VI	1,2%
	VII y VIII	2,0%

Sources: Salado, Ferrarotti, Rossi

Breeding related with tolerance to biotic stress

Numerous biological agents such as fungus, bacteria, viruses and nematodes are natural enemies of soybean in Argentina. Being this the main crop of the country, many planted areas lack the necessary rotation with other planted species, such as corn or sorghum. This phenomenon has contributed to increase the disease pressure in said areas.

Another cause of the dynamic of pathogen agents and its increase is the great adoption of the no till planting system in Argentina (Figure 3). Such system has become a tool for soil conservation, better administration of operative time and reduction of cost for the farmer, increasing the efficiency and profitability of the agro business. In addition, according to numbers of the "Argentine Farmers Non Tillage Association" (AAPRESID), the surface with no till planting is in the order of 53% of the total planted area in Argentina.

On the other hand, the absence of the removal of the plowing layer benefits the increase of the population of some of these microorganisms which are soybean enemies. Fortunately different breeding programs in Argentina have warned ahead of time of the situation and today we can affirm that the losses caused by biotic effect are insignificant if they are to be compared with what is earned with the incorporation of resistance or tolerance in the varieties planted.

All efforts have been focused in the adversities caused by *Sclerotinia sclerotiorum, Phytophthora megasperma* var. *sojae, Diaporthe phaseolorum*

var. *meridionalis, Cercospora sojina, Fusarium solani, Colletotrichum dematium* var. *truncatum, Cercospora kikuchii, Phyalophora gregata, Meloidogyne incognita, Meloidogyne javanica, Heterodera glycine, Xanthomonas campestris, Pseudomonas syringae* and the Soybean Mosaic Virus among others. Currently different programs have been carrying out efforts in the monitoring of the variability in order to seek genetic solutions to decrease the possible future effects caused by *Phakopsora pachirrizi*.

Through the release to the market of improved varieties we could successfully overcome the possibility of epidemics caused by soybean stem canker and Soybean Cyst Nematode, or allow the planting of the crop in certain niches affected by Frogeye or Root Knot Nematode.

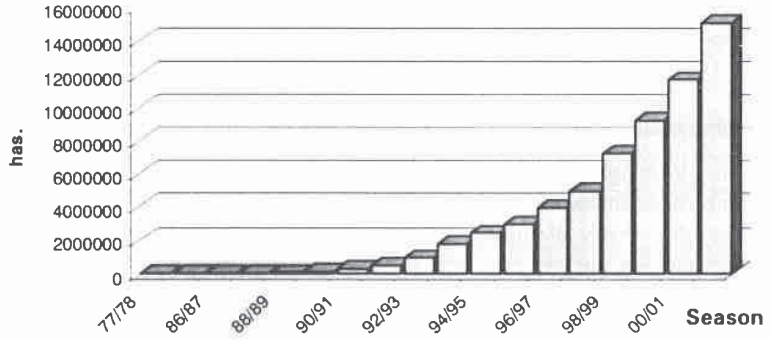


Figure 3. Evolution of the area with no till planting (Period 77/02). Source: AAPRESID

Breeding related with tolerance to non biotic stress

The colonization of new areas for agriculture made regional crops such as bean, cotton, rice, tobacco, peanut, sugar cane, etc. be replaced by soybean. Currently, soybean is planted beyond the pampeana region where environmental conditions are very different.

An effort in genetic breeding was necessary in order to release to the market varieties with capability to tolerate drought, heat, soil impedances caused by heavy soils or low water retention capability caused by sandy soils.

Several programs are still exposed to obtaining varieties with adaptation features to the new environments, being ones of the lines of interest the tolerance to salty soils and cold tolerance.

Breeding related to nutritive quality

Oil and protein are the energetic and nutritive sources of soybean, and at the same time its main sub products from the qualitative point of view.

The lack of internal laws regulating quality standards and bases for differentiated commercialization have discourage large investors to obtain varieties that carry the characteristics related with better quality in Argentina. Furthermore, the erratic overprices for better quality offered by the international market don't seem too encouraging for argentine producers.

Currently and probably linked to intense breeding in order to increase productivity, argentine soybean is not considered in the world for being the one with greater protein content. For this and other reasons, argentine breeders are watching the international perspective for the long run and keep developing programs with the purpose of increasing the percentage of protein for argentine soybean. Likewise, new transgenic events related not only with the concentration but also to the amino acid and fatty acids quality are in the process of deregulation by national authorities.

A special variety of the type called "edamamé", appropriate for human consumption in fresh has been recently developed by the Universidad Nacional de Rosario and an agreement with the private sector was signed for its production and commercialization. Most likely this recent fact may set grounds for future activities to which the public sector may have to dedicate.

Molecular breeding

Breeding by molecular methods is not detached to the argentine programs. The knowledge of the DNA molecular structure and the extension of lab techniques allow Argentineans to count on the finger printer of its varieties.

Assisted selection by molecular markers is used more and more to quickly introduce useful traits to soybean. In this sense, the selections for tolerance to different diseases and for characteristics related with the industrial quality are today matters of development in several companies.

Though the emergent developments in genetic engineering that reached the commercial standards come from licenses taken from third countries, public and private labs are working to develop their own methods. The office that regulates the process of release to the environment of transgenic materials is CONABIA. Eleven permits of release to the field were issued in 2002. Four of them are related to insect tolerance, two to herbicide tolerance, four to food quality and two to productivity growth. The training

of human resources, the return of investment and the political definitions of the National Government will be the main factors to encourage the better development of molecular breeding in Argentina in the future.

The generation of varieties tolerant to herbicides.

Soybean in Argentina has suffered dramatic changes since the release of varieties tolerant to glyphosate. Today we can assure that a different agriculture from the pre-transgenic era exists in Argentina. When soybean comes to our mind we no longer segregate the conventional varieties from the transgenic ones. The tolerance to glyphosate is for the Argentineans one more trait of the crop like cycle, height, or color of the flower.

The boarding of soybean to areas before marginal for agriculture, the prefect association of glyphosate with no till planting, the cost reduction of production and the better usage of operative time are among others, advantages from the utilization of modern transgenic varieties.

We only need to watch the increase of areas with RR™ together with the fall of the area with conventional varieties to realize the degree of adoption of this technology on the side of the farmers (Figure 4).

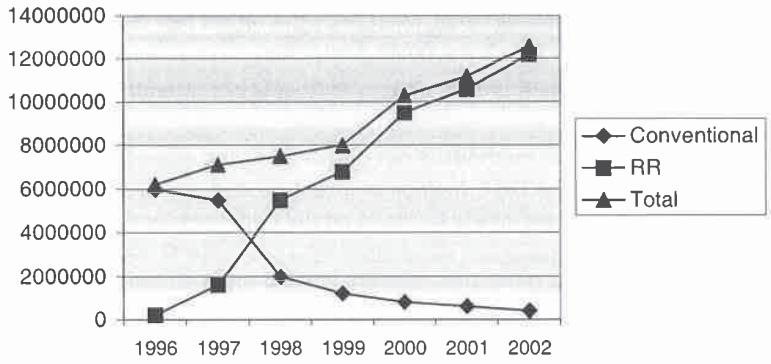
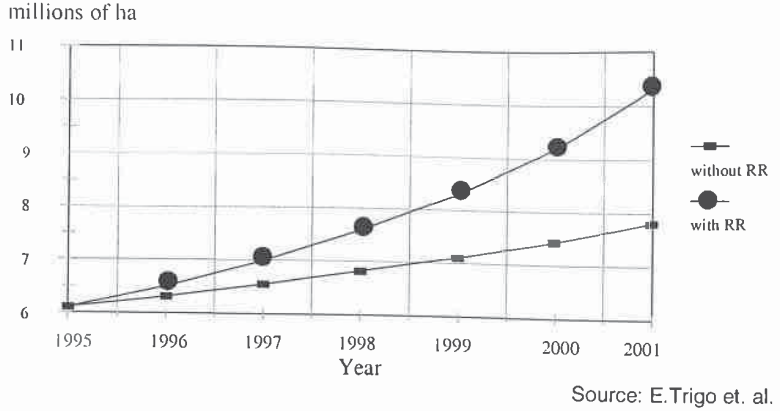


Figure 4. Hectares planted with conventional and RR™ varieties. Source: Ferrarotti, J.

An estimation reported by Trigo et. al. shows the evolution in millions of hectares for the crop bearing in mind the possible scenarios "with RR™ soybean and without it" (Figure 5).



Source: E.Trigo et. al.

Figure 5. Argentina: Evolution of the area with soybean (two alternative scenarios)

The same author figured the total profit accumulated between 1996 and 2001 and its distribution by sector of the economy. In Figure 6 we can clearly see the large portion of the profit that remains in hands of farmers.

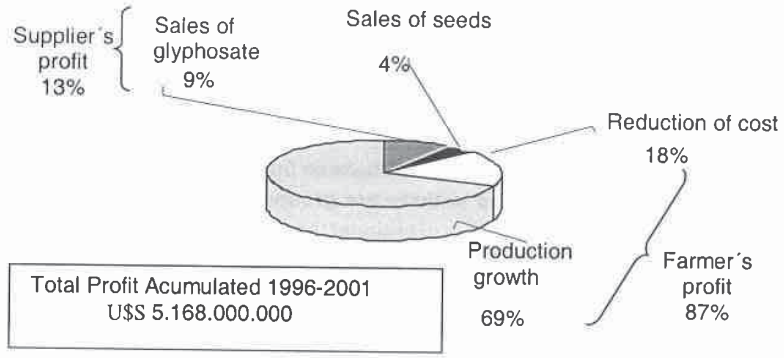


Figure 6. Transgenic soybean in Argentina. Distribution of the accumulated profits. Source: Trigo et. al.

The varieties tolerant to glyphosate are the perfect allied in the no till plant system. Figure 7 clearly shows the adoption rate of the planting system in the last years, together with the evolution of hectares planted with transgenic soybeans.

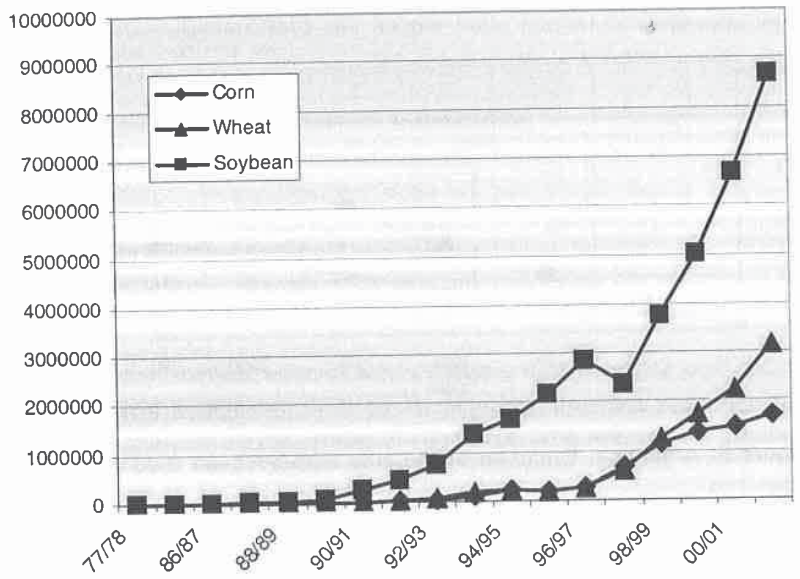


Figure 7. Evolution of area with no till planting of wheat, corn and soybean. Period 77-02. Source: AAPRESID

Today we can assure that the complex of transgenic soybean plus glyphosate plus no till planting has been a key element of the evolution of the argentine agriculture of the last years.

PROSOJA

A relevant fact that characterizes the soybean breeding in Argentina is that professionals devoted to this activity are grouped with other technicians and scientists of soybean in a professional Association called PROSOJA.

PROSOJA has been going on for 15 years. The professionals finance their activity with an annual fee. The objectives are centered in exchanging information and the integral improvement of soybean through soybean breeding, new crop management strategies and prevention towards sanitary risks among others. Besides, it is the association of soybean reference in Argentina where aspects related with economic practices or political measures are debated.

Final Considerations

Argentina has a vast history in genetic soybean breeding. This has been a decisive factor in the success of the crop. The grouping of technicians and scientists in an Association has turn out to be a catalyst of the process of genetic breeding with high efficiency in incredibly short terms. The future perspectives are linked to profitability of the seed companies and to political definitions in relation to a regulatory frame related to the intellectual property. This will be the main factor to mark the future of soybean breeding in Argentina.

Soybean breeding program in Australia

A.T.James[1] and I.A.Rose[2]

Abstract

In Australia soybean cropping extends from 16°S in the tropics to about 37°S in the temperate zone. Varietal improvement focuses on defining regions with broadly similar requirements for adaptation, overcoming disease or environmental limitations to the crop and defining specific grain quality traits necessary to permit greater functional quality of grain for culinary use.

Introduction

Australia is a net importer of soybean. Over time, human consumption or culinary markets have taken an increasing proportion of the domestic production and some high-value export markets have developed for culinary quality soybeans produced out of season to the main northern hemisphere producers (Colton 2000). The Australian breeding program must deliver varieties over the range of 16°S in the tropics to about 37°S in the temperate zone with limited resources. The program has increasingly sought to group regions of similar environment and potential for production of culinary quality together to simplify selection of new varieties, release and maintenance of seed stocks (see Figure 1). Generally these regions of production span a relatively wide range of latitude and planting dates. Increasingly, varieties must have quite broad adaptation to sowing date and to latitude but specific requirements for key culinary quality traits (James 2003). This paper will report a summary of the requirements for adaptation to each of the regions of soybean cropping in Australia and a discussion of research conducted to improve specific traits.

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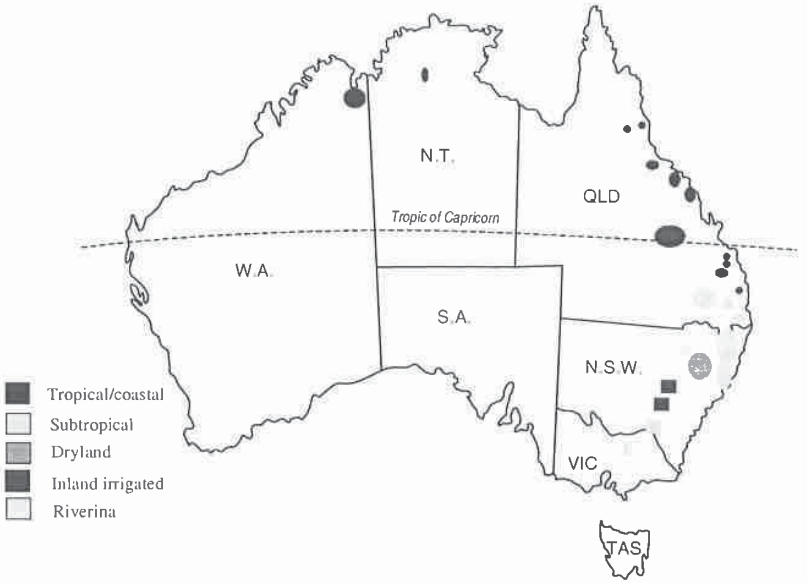


Figure 1. Regions of soybean cropping in Australia.

Tropical and coastal

Soybean is grown largely rain fed in rotation with sugarcane along the coast or irrigated in rotation with cotton or sorghum inland in this region. In the sugar cropping system, soybean is planted in rows up to 140 cm wide into 10 – 30 cm deep mulch left after harvest of sugarcane. Soybean crops may be cut and left on the surface of the soil as a green manure or managed to achieve insect control and harvested for grain. Yield of the subsequent sugar crop has been shown to increase by 10 to 30 percent. The increase is thought to be due to reduction in pest and disease pressures and in provision of slow-release nitrogen (Sparkes and Charleston 2003). Varietal improvement for this region must deliver varieties with tall growth habit, high vegetative vigour, adaptation to latitudes from 27°S to 16°S and tolerance to pre-harvest weathering of grain (James et al. 1997). More recently, growers have requested light hilum to improve their access to culinary markets and higher rust tolerance, however it is relatively difficult to reliably produce grain of quality suited to human consumption from this region (Christopher and James 1998).

Subtropical

In this region soybeans of crop duration around 120 to 140 days are grown rain fed on the north coast of NSW and irrigated in northern inland NSW and Qld. On the coast soybean is grown in rotation with sugar cane, in rotation with maize or winter cereals or as part of a farming system with cattle. Inland rotations almost exclusively involve cotton or winter cereals. Main improvement aims for this region have focussed on improving pre-harvest weathering tolerance and resistance to phytophthora root rot and other diseases (Ryley 2003). Other important characteristics include high yield under rainfed conditions, tolerance to periodically high levels of soil manganese, good standability, tolerance to *Sclerotinia sclerotiorum* and resistance to downy mildew. More recently, improving culinary quality of grain for the tofu, soymilk or natto markets has been given high priority.

Dryland

In northern inland NSW the approach has been to develop early maturing, hence drought-escaping, varieties. Among the early maturing types variation was observed in drought response when subjected to duplicate trials in fully irrigated and dryland conditions (Rose, McWhirter and Spurway, 1992). Valder, Intrepid, Valiant and Hale are varieties that have been developed at Narrabri by the NSW Agriculture breeding program specifically for dryland production. They are considered adapted to marginal moisture environments due to a combination of drought escape and drought tolerance. The levels of drought stress encountered have significant effects on seed quality through changes in seed size as well as changes in oil and protein content (Rose, 1988). This has generally had no deleterious effects on acceptance of dryland-produced beans for crushing markets however, this grain is not likely to be suitable for tofu and soymilk markets. Natto and soyflour types have therefore been targeted in selection of future dryland varieties.

Inland Irrigated. Irrigated production in central NSW river valleys has declined in recent years, this has occurred in part because of the development of a population of phytophthora races which quickly overcame new varieties as they were released, and in part because of higher profitability from other crops.

Riverina. Irrigated production occurs in this region in southern NSW and northern Victoria, in rotation with paddy rice or pasture systems. A proportion of the production from this region has gained acceptance as having high functional quality for tofu and soymilk manufacture. Generally, attempts to produce natto quality grain in this region have failed due to excessively large seed size. Maintaining functional quality for tofu manufacture, whilst changing to earlier maturity and enhancing phytophthora resistance are the major aims of the breeding program in this region.

Traits enhancing adaptation or quality

Weathering tolerance

Weathering of soybean seed prior to harvest can cause economic losses in humid environments such as the north coast of NSW and southern Queensland. Rainfall at or near maturity causes seed to take up moisture with weathering damage appearing as discolouration, swelling and shrinking, partial germination, loss of weight and invasion by Phomopsis and other fungi. Losses can be total where seed quality becomes unacceptable to markets. On average, where susceptible varieties are grown, substantial losses can be expected in 20-25% of seasons, with lesser, more sporadic losses in a further 25% of years.

Genotypic differences for weathering tolerance are measured in a rainfall simulator using field grown plants harvested at or after R7 and prior to any field weathering. They are placed upright under automated spray jets to ensure even coverage. Sprays operate continuously for 30 minutes and thence for 30 seconds every 20 minutes for five days. Temperature is maintained with 20-24°C max / 15-18°C min. Seed is harvested after plants have been dried and rated for weathering damage as a percentage of known control varieties. A seed is considered weathered if >50% of the seedcoat is damaged or discoloured by the weathering treatment.

Studies using the rainfall simulator have demonstrated that weathering tolerance is due to the pod wall excluding water from entering the seed for up to 48hrs (Rose and Desborough, 1997) and that hardseededness is relatively ineffectual as it does not develop until grain moisture content has fallen below 17 percent which is too late to prevent damage. The benefits of weathering tolerance were demonstrated in 1996 when heavy, prolonged rainfall caused extensive losses in susceptible varieties but greatly reduced losses in Dune and Manta (Table 1).

Table 1. Comparison of field weathering in two trials (1996 only) and rainfall simulator weathering mean ratings (1995-1998) for commercial soybean varieties with contrasting levels of tolerance.

	Rainfall simulator weathering relative to Dune (mean %)	Field weathering 1996, relative to Dune (%) Unweathered seed Experiment 1	Unweathered seed Experiment 2
Dune	100	100	100
Trochus	86	93	78
Manta	82	89	50
Soya 791	73	89	59
Warrigal	52	38	18
Manark	25	37	15
Poseidon	102	-	80
Zeus	108	-	103

Further enhancement of weathering tolerance. The weathering tolerant varieties developed in Australia have all been derived from common southern USA germplasm sources. New sources of weathering tolerance in diverse germplasm have been identified, particularly in landraces from tropical Asia. These are being crossed to Australian developed weathering tolerant varieties to test if it is possible to combine different sources of weathering tolerance and in so doing increase tolerance above existing levels.

Drought tolerance

In much of the potential Australian soybean areas summer rainfall is unreliable in amount and distribution. In many areas irrigation is essential to supplement rainfall. In others the development of cultivars better adapted to marginal rainfall areas is central to expansion of the crop. There are two approaches to enhancing the drought tolerance of soybean in the Australian environment. The most successful has been to develop early maturing, hence drought-escaping, varieties for inland NSW. The second strategy has been to develop longer duration drought-tolerating populations with potential adaptation to central Qld.

Early maturing strategy. Yields obtained from the early maturing strategy are clearly dependent on in crop rainfall. As an example, the expected rainfall at Narrabri for the 120 days from 1 December is 260 mm. This translates to a yield expectation of around 1.3 t/ha. In areas to the west of the dryland region (Figure 1) the corresponding rainfall expectation is less than 200 mm and yield expectations are below 1 t/ha. In the eastern part of the dryland region the growing season rainfall often exceeds 300mm and yields in excess of 2t/ha are frequently obtained. The yield potential of the dryland varieties that have been developed for the early maturing strategy is limited by their earliness, with full irrigation achieving around 4t/ha.

Longer duration drought-tolerating strategy. This strategy entails enhancing leaf survival in longer duration types so that the crop can survive intermittent severe water stress to go on to produce harvestable yield (James 1998). The key to improving leaf survival has been to select lines with extreme low epidermal conductance, moderate osmotic adjustment and low critical relative water content. Lines with appropriate combinations of traits have been shown to maintain leaf area for around 20 days longer than varieties selected in the early-maturing strategy.

Functional traits for tofu quality. A small-scale method for manufacture of silken tofu was developed after extensive modification of existing techniques (James and Bumstead 2002). The test measures soymilk yield, solid

solubility, milk solids, tofu yield, tofu texture and tofu colour on an 80 gram sample of grain. It was found that there is a large genotypic effect on functional traits, a moderate effect of environment and relatively little genotype by environment interaction. Significant correlations among functional traits and between functional traits and grain quality traits such as seed size, protein and oil content and seed germination also occurred.

It is possible to make significant changes to the tofu making potential of soybean through selection. For example there is a large genotypic effect on tofu texture even when using grain of similar protein content (Table 2). Manufacturers prefer cultivars with higher protein content and larger seed size than is usual for varieties selected for oil seed crushing, because this will give higher yields of tofu with firmer texture. However, it is important that protein content not be pushed too high because this will have a negative impact on grain yield, sugar content and flavour of the tofu.

Table 2. Protein content and tofu texture of soybean varieties averaged across seven trials conducted in subtropical Australia.

Variety	Grain protein content relative to the average of Jabiru and Manark (% DM)	Tofu texture (0-5 scale where highest gelling)
96130-2	0.1	3.5
A6785	0.1	1.9
Cawana	-0.1	2.3
Centaur	-0.1	2.8
Dragon	1.4	2.5
Jabiru	-0.3	2.5
Manark	0.3	2.8
Melrose	-1.1	2.3
Poseidon	0.9	2.5
Soy 791	-0.2	2.0
Warrigal	0.4	2.4
l.s.d. (0.05)	0.5	0.3

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Soybean genetic breeding in Brazil

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Abstract

The several participants of the soybean production chain continuously demand cultivars that guarantee sustainability and competitiveness to their activities. New cultivars bring opportunity for reducing the environment impact of soybean cropping and production costs while increasing crop yield, stability and quality. The contribution of the soybean genetic breeding programs to the development of Brazilian agribusiness has been very significant as it has developed higher yielding, higher quality and better adapted cultivars for the various ecological regions and cultivation systems. Stability of production is sought through the introduction of resistance or tolerance to disease and pests and through the introduction of special agronomic traits such as tolerance to acid soils. Other objectives are the development of cultivars with high physiological seed quality in tropical regions and with special traits for human consumption or industrial processing. The programs develop cultivars for all Brazilian regions, including the States of RS, SC, PR, SP, MS, MG, MT, GO, RO, BA, TO, MA, PI, PA, RR, AL and the DF. They are either isolated or multi-institutional programs carried out in partnership among public and private companies.

Key words: vegetable oil, soybean protein and yield, *Glycine max*, biotechnology

Introduction

The Brazilian soybean agro-industrial complex was worth US$ 60 billion in 2003. Total grain production was 52.0 million tons, of which 25.8 million tons were processed to produce 5.16 million tons of edible oil and 20.64 million tons of cake. The exports of grains, oil and cake totaled US$ 8.26 billion. The 52 million tons which were produced in 18.5 million ha corresponded to 26.5 percent of the world production and placed Brazil as a second major world producer in volume and the first in yield (2,816 kg/ha).

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The availability of technology for soybean production was vitally important for Brazil to reach this outstanding position. The results of the research developed by Embrapa Soybean and other national institutions contributed decisively to the achievement of the present levels of efficiency, competitiveness and sustainability. In this context of availability of P&D solutions for soybean cropping, the development of cultivars represented an important contribution. Cultivars that carry in their genome the genes capable of expressing high yield, wide adaptation and resistance or tolerance to biotic or non-biotic adverse factors represented perhaps the most significant contribution to the efficiency in the entire productive sector.

The genetic breeding programs carried out isolated or in partnership by public and private institutions throughout Brazil, resulted in efficient development of new cultivars that allowed yield genetic gains between 1.5 to 2% a year. They also brought about quality gains to the industry and increased production stability and reduced production costs to farmers.

Main recent issues

In Brazil, the soybean genetic breeding programs have contributed to change important national paradigms related to intellectual property, to the relationship between public and private institutions and to the funding of research activities.

Brazil changed significantly its legislation regarding intellectual property by signing the Convention of Biological Diversity (C.D.B.) in 1993 and ratifying the TRIPS in 1994. The adoption of these international legal instruments and the consequent perfecting of the Brazilian laws (variety protection law, seed law, etc.) opened space for private investment in the development of cultivars of the most economically important crops, such as soybean. This scenario also widened the perspective for business resulting from the introduction of genes by techniques of genetic transformation in the Brazilian crops.

The international companies working in the plant breeding area quickly responded to the new situation. Their action in the case of maize breeding was swift and with the acquisitions of Brazilian breeding companies rapidly controlled more than 90% of the Brazilian seed market. Regarding soybean breeding, however, the situation was different because of the institutional arrangement developed in the form of sound strategic partnerships between the public (Embrapa) and private sectors (seed production companies) which were already underway when Brazil adopted the current legislation. The soybean cultivars released by this institutional arrangement accounted for about 60% of the seed market in 2002/2003, which showed the partnership

efficiency in detecting the demand for cultivars by the production chain. The process of search for efficiency has been long but the excellent results of the last years showed that the strategy adopted and the solutions found have been adequate. Soybean production in Brazil increased by approximately 116.7% in the last 14 years, moving from 24 million tons to 52 million tons between the 1988/89 and 2002/03 growing seasons. This increase was essentially due to a significant increase in productivity which increase from 1,940 kg/ha in 1988/89 to 2,816 kg/ha in 2000/03 while the cultivation area expanded from 12.2 million to 18.5 million ha in the same period. Table 1 shows the yield increase along the years and the main cultivars developed.

Present main focuses of the Brazilian breeding programs

The significant advances of the biotechnology leave no room for doubts about its growing role in agriculture and, therefore about its effect on the breeding programs. Genes of interest will continue to arise and the breeding programs must be able to introduce them into the cultivars to make them available to the cropping system. There is an intricate legal system to be complied with, such as strict biosafety rules and environment and food legislation, which add work to the already well established testing procedures. Public and private companies that hold patents on those genes should negotiate to guarantee reasonable returns to their investments. A heated debate about the benefits transgenic soybean will bring to Brazil is ongoing.

Soybean rust caused by *Phakospora pachyrhyzi* Sidow is an important disease that has recently been found in Brazil. Resistant materials have been detected and four dominant genes Rpp1 to Rpp4 have been identified. These major gene resistances to the disease, however, have been broken in a single cropping season and, therefore, vertical resistance may not be a practical solution. Soybean lines or cultivars with partial resistance to rust have been identified and characterized based on the number of pustules and disease development rate. Several Brazilian inbred lines or cultivars show different levels of tolerance or susceptibility to the disease. Breeding for horizontal resistance to soybean rust is in progress in the country.

Table 1. Main released soybean cultivars.

Period	Yield (kg/ha)	Main cultivars
Until 1960	1,000	Amarela Comum, Abura, Pelicano and Mogiana.
1960 - 1968	1,060	Hill, Hood, Majos, Bienville and Hampton
1969 - 1974	1,394	Bragg, Davis, Hardee, Santa Rosa, Campos Gerais, Viçoja, Mineira and IAC-2
1975 - 1980	1,541	IAS-4, IAS-5, Paraná, Planalto, Pérola, BR 1, Bossier, IAC 4 and UFV-1
1981 - 1990	1,740	BR 4; Ivai; Cobb; Emgopa 301, 305 and 306; IAC-6; 7 and 8; Doko; Cristalina and Tropical
1991 - 1999	2,450	BR-16; FT Abiara, Iguaçu; Paiaguás; Embrapa 48, 59; BRS 133; Canário; Xingú; Tucano; Garimpo; Conquista; Emgopa 308, 313 and 314; Curió and
2000-2003	2,680	BRS 154, 184, 214, 231; BRSMT Pintado, Uirapuru; FT 106; MSOY 8914; BRS Celeste, Jiripoca, Sambaiba; BRSGO Santa Cruz, Jatay,

Heterodera glycine, the soybean cyst nematode (SCN), is the most damaging species to the crop in the United States. In Brazil, its presence was registered in February 1992 and currently SCN is one of the main problems in the soybean cropping areas. Large areas have been infested mainly by the intense movement of agricultural machines and implements. Race 3 predominates although races 1, 2, 4, 4+, 5, 6, 9, 10 and 14 have also been identified. Race 4+ was detected in Mato Grosso and is capable to parasite the Hartwig cultivar, which was previously accepted as resistant to all the SCN races. The use of resistant or tolerant cultivars is the most efficient, practical and economical control method and many of soybean cultivars with cist nematode resistance have been developed by the Brazilian breeding programs.

Gall nematodes are cosmopolitan and reduce the yield of several crops in various countries in the world. The *Meloydogyne* genus is present in almost all the natural systems were they survive on a wide range of host plants. The most important species associated with damage to soybean are *M. javanica*, *M. incognita*, *M. arenaria* and *M. hapla* and the first two are most important in the tropical areas like Brazil. They occur in the States of RS, PR, SP and MS. The use of resistant or tolerant cultivars is the most efficient, practical and economic method of control. Although several species may occur in a given field, usually only one species is dominant and accounts

for severe damage. The development of soybean breeding programs to obtain resistant cultivars to the different nematode species is essential for the sustainability of the crop.

Epidemiological studies carried out with Japanese men and women showed a diminished ratio of cancer hormone related deaths when a diet based on soybean is administered. The isoflavones are vegetable hormones that can act as anti-estrogens due to their similar structure. They link to the estrogen receptors altering their metabolism and diminishing the uncontrolled cell proliferation. The anti-cancer property of these soybean components has raised interest in introducing soybean in the human diet. The quantity of isoflavones is environmentally and genetically dependent. Genetic variability in isoflavone content has been observed in the Brazilian germplasm.

In principle, breeding genetically modified soybean follows the same steps as the non-modified soybean after the existence of a good event (the introduced gene working properly in the new host plant). The experimental process, however, must follow the strict regulations that exist in the country. Most effort is concentrated in developing cultivars tolerant to the RoundUp ready herbicide although several other interesting traits are being dealt with.

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An overview of current status of breeding programme in India

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Soybean is next only to groundnut in area and production among 9 conventional oilseed crops of India. Its share for national oilseeds area and production is 26 and 26.4% respectively. Between 1983-84 and 1998-99 compound growth rate of 14.2 18.3 and 3.6% was observed in soybean area, production and productivity respectively (Hegde 2001). The production has more than doubled since 1990-91 (2.60 m.t) and now stands at more than 6.0 m.t. The productivity of soybean is however hovering around 1 t/ha for last 13 years. Besides narrow genetic diversity among cultivars, water stress has been main reason for low productivity since soybean is cultivated as rain fed crop and productivity is subject to vagaries of weather conditions. Major soybean growing area (70%) is located in the central part of the country between 20 and 25 latitude where soybean is grown during Monsoon season (July –October). The average annual rainfall is between 500 –1000 mm, most of which is received during this period. The average temperature ranges from 30-20 C during soybean growing season.

Although commercial cultivation of soybean is of short history in India, scattered cultivation of black seeded soybean was in practice in hills from very early times. With the introduction of yellow soybeans in early sixties and establishment of first soybean processing factory in 1972, there was phenomenal increase in hetaerae. India now occupies V place on the world map of soybean.

Having been convinced of agricultural feasibility and economic viability of soybean cultivation in the country, Indian Council of Agriculture Research (ICAR) launched an interdisciplinary, multilocation All India Coordinated Research Project on Soybean in 1967. Under the aegis of this project systematic efforts to breed soybean varieties for Indian conditions were initiated at G.B.Pant University of Agriculture & Technology Pantnagar (U.P), J.N.Agriculture University, Jabalpur (M.P.), University of Agriculture Sciences, Bangalore (Karnataka) and Tami Nadu Agriculture University, Coimbatore (Tamil Nadu).

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Breeding Objectives

Breeding objectives were defined on the basis of problems encountered for its adaptation in subtropical climate in latitude ranging from 10 to 40. Following have been the prioritized goals.

- High yield potential (3.5-4.0 t/ha)
- Stability of yield under variable conditions of rain fed cultivation.
- Early to medium maturity (85-110 days) to escape moisture stress and to fit in the dual crop farming systems
- Good seed longevity and resistance to mechanical damage to seed
- Resistance to pod shattering
- Resistance to biotic and a biotic stress
- Photo insensitivity for wider adaptation of varieties

Genetic enhancement of yield and its stability under rain-fed conditions have been of utmost importance for soybean varieties. The ideal soybean plant for high yield should have determinate or semi determinate growth habit, (suited to short growing season), erect and non lodging. It should have long juvenile period and rapid seed filling duration for high yield in a duration of 100-105 days. The leaves should be broad for maximum light interception and checking weed growth. Most of the improved varieties have a yield potential of 2.0-3.0 t/ha with many varieties capable of yielding up to 4.0 t/ha. The important yield contributing characters are high number of pods per unit area and seeds/pod. Besides yield, the other important characters for harnessing yield potential soybean varieties in tropics are resistance to pod shattering and good seed longevity. The varieties should be able to withstand 10-15 days delay in harvesting after reaching harvest maturity and should maintain at least 70% germination after 8-9 months of storage at ambient temperature. While most of the improved varieties possess former, seed storability is poor particularly in bold seeded varieties.

The major biotic stresses are diseases like yellow mosaic virus , soybean rust (*Phakospora pachyrhizi*), rhizoctonia (*Rhizoctonia bataticola*), anthracnose (*Colletotrichum truncatum*) etc and insect pests like stem fly (*Melanagromyza sojae*), girdle beetle (*Obereopsis brevis*) and various defoliators cause extensive damage to the crop. Varieties resistant/tolerant to many of these stresses have been bred and the work is continuing. Among biotic stress drought is of common occurrence and tolerance/escape is must for better productivity. Early maturity is an important breeding objective, first to fit in the dual crop farming system and secondly, to escape moisture stress during pod filling stage of the crop. Farmers now have a choice of many varieties maturing within 100 days. Despite its rich nutritional profile, use of soybean in food has been limited because of its

beany flavor or convention bound detraction. Breeding for quality characters and development of food grade varieties is important future objective.

Breeding approaches

The breeding approaches in soybean have been those typically suited to highly self-fertilized species. Besides introduction and selection from existing variability, pedigree method has been widely used. Of late, single seed descent is being preferred. In this method the generations till F4 are advanced by taking a single seed form all F2 and F3 plants and selection starts only in F4 or F5. A modification of this method, single pod descent method is being largely practiced. This enables handling of large segregating material with better chances of obtaining favorable gene combinations. Mutation breeding approach is also practiced.

Evaluation of promising genotypes is carried out in 3 tier system under All India Coordinated Project on Soybean in multilocation trials in five agro climatic zones. The entries are first tested in Initial Varietal Trial (IVT) across the zones. The better promising entries in each zone are promoted to Advanced Varietal Trial -I (AVT-I) and then promoted to Advanced Varietal Trial –II (AVT-II) in the following year. Based on three years performance superior genotypes are identified for release.

Varieties

Resurrection of soybean began with the introduction of exotic varieties like Bragg, Lee, Hardy, Harsoy etc in 1963-64 at Pantnagar and Jabalpur. Subsequently, some of the cultivars were released for cultivation. Till 1980 most of the varieties were either introductions or selections from exotic material. These have been called as varieties of 'Selection cycle-1'. During 1981-90 exotic varieties were used as parents to generate new variability for selection. The varieties developed since 1990 have used breeding lines and cultivars as parents. The varieties belonging to these years have been grouped in 'Selection cycle-2'. The varieties in selection cycle-1 have produced 4 times yield than indigenous variety Kalitur by virtue of high number of pods per plant and seed weight, short duration and increased biomass. The varieties in selection cycle-2 showed 19% higher yield than selection cycle-1 varieties. This was due to improvement in harvest index and seed filling duration. The annual genetic gain in seed yield of soybean varieties in India between 1969 and1993 was approximately 22 kg per ha.(Karmakar&Bhatnagar 1996)

So far, 79 soybean varieties are released in India. Of these 8 are direct introductions, 21 selections, 5 mutants and 45 have been developed through

hybridizations. A majority of Indian varieties have been developed using exotic parents. Out of 45 varieties, 39 have at least one parent from 9 exotic introduced varieties. Variety 'Bragg' alone accounts for 14 varieties. The contribution of indigenous germplasm to varietal development has been limited as one of the parent in only 9 varieties is indigenous. The genetic base of Indian soybean varieties is rather narrow despite richness of germplasm (diversity analysis shows that most of the varieties fall in two less divergent clusters) and there is a need to use less exploited material as parents.

Future Perspective

The immediate concern of the breeders is to enhance the national productivity to 2t/ha. Although varieties with yield potential of 3.0 - 4.0 t/ha are available, there is need to prevent yield erosion due to stress factors through breeding for stress resistance. Efforts are required to broaden the genetic base of Indian soybean varieties by using diverse germplasm sources. The assessment of genetic diversity with the help of molecular markers is already in progress. The photosensitive nature of soybean leads to insufficient vegetative growth, early flowering and poor yields in tropics. The introduction of long juvenile trait from Brazilian accessions to prolong vegetative phase is being explored. The use of molecular techniques like marker assisted selection, mapping of QTLs, tagging of important genes and biotechnology / genetic engineering will receive increasing attention in soybean breeding. The development of cultivars for value added specialty market is another promising area where soybeans with altered fatty acid composition, low in anti nutritional factors like lipoxygenase or low in linoleic acid will command a premium in international markets. The use of soybean as vegetable is popular in many countries could be of importance in Indian context, too. The area, production and utilization of soybean are expected to increase steadily in India in years to come.

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Overview of recent genetic improvement in public and private breeding programs in the USA

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Abstract

Soybean breeding programs in the public and private sectors continue to make genetic improvement for yield and other important traits. Selection of parents is very important for success. Glyphosate tolerance is a major component of most soybean breeding programs in the U.S. Breeders have developed and refined breeding procedures and plot equipment in order to be able to evaluate more selections. Genetic marker information is being used for marker-assisted selection in public and private breeding programs. The use of conventional and genetic marker techniques should assume that continued genetic improvement occurs in the U.S.

Genetic improvement of soybean in public and private breeding programs continues to be made. Recent estimates indicate soybean yields are improving at a rate of about 23 kg/ha/yr due to improved genetics, better production practices, and the increasing atmospheric CO_2 levels (Specht et al., 1999). The genetic improvements in recent years have been accomplished mainly through the use of conventional breeding methods; however molecular-based plant breeding techniques are assuming an increasingly more important role in genetic improvement.

Conventional breeding strategies have been, and continue to be, very successful in improving soybean productivity and quality. Yield has been and remains the trait of greatest emphasis by breeders since most soybean producers are paid for the quantity of soybeans marketed. Wilcox (2001) estimated that public breeders in the northern soybean production areas have increased seed yield about 60% over the past sixty years. Estimates of progress in the public sector of a similar magnitude have been made in other areas of North America (Salado-Navarro et al., 1993; Specht et al., 1999; Voldeng et al., 1997). Similar progress has certainly been made by breeding efforts in the private sector (Specht et al., 1999), although major breeding efforts have occurred there only in the last 30 years or so. Besides yield, progress has also been made in selecting for resistance to pathogens, insects and nematodes, tolerance to other production hazards, improvement in seed protein, oil, and other quality traits, as well as other agronomic characteristics, such as standability.

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Genetic improvement in both public and private programs or the soybean industry as a whole can be considered a series of breeding cycles. Each cycle begins with the breeder making choices as to the parents to be used to create segregating populations. Those populations are then advanced toward homozygosity, with or without selection, to produce relatively homozygous lines that are then subject to yield and other trait evaluation. A given cycle ends when its best lines are released as improved pure-line cultivars. Since a cycle is initiated each year, genetic improvement is a continuous process.

Selection of parents is extremely important to the success in genetic improvement. In most private breeding programs elite lines from within a company, from other private companies or from the public sector are crossed to create segregating populations. This has also been the case in many public programs. However there has been some efforts to use exotic germplasm in the public sector. Although there are exceptions, research has suggested that elite parents of diverse origins are more likely to produce progeny superior to either parent than parents of similar ancestry (Burton, 1997). The parental selection method used depends on many factors, including the trait(s) of interest, the purpose of the cross, the relative importance of characters other than yield, the ancestry of the lines, and the resources and time available. Parents can be selected on the basis of comparative evaluation *per se*, by test cross evaluation, or experience of the breeder. In many cases, *per se* evaluation data are readily available from yield performance tests. However, if the objective is to identify parental germplasm with favorable alleles not present in existing cultivars, test-cross evaluations may be needed. A method for soybean was suggested by Kenworthy (1980). St. Martin et al. (1996) also developed a test cross procedure for identifying germplasm lines with the potential to contribute favorable alleles for improving pure-line cultivars. Panter and Allen (1995a) suggested the selection of parents for crosses to improve yield could be facilitated by using best linear unbiased predictions.

Selection of parents will continue to be very challenging but extremely important for success in genetic improvement. In many programs where time and resources are limited, use of existing comparative data using the best linear, unbiased predictions appears useful. If more resources and time are available, test cross evaluations can be used to identify parental germplasm with favorable alleles not present in current cultivars. As molecular and genomic data for potential parents become available, this information could further assist in the selection of parents. In some cases these kinds of data are currently being used.

Breeding populations for cultivar development are typically developed from 2- or 3-parent hybridizations involving cultivars, breeding lines, or other germplasm. The populations are then advanced through generations of selfed inbreeding. Selection can be practiced among plants during early generations before yield tests are initiated or later among lines during yield testing. The amount and effectiveness of selection depends on the heritability of the trait or character and the environment where the population or lines are grown. Nearly homozygous lines are then created from individually harvested inbred plants. These lines are then extensively evaluated to identify those with a performance superior to existing cultivars.

Many breeding procedures are available for advancing crosses toward homozygosity. They include pedigree, single seed descent, bulk, early generation testing and backcrossing. Longer term procedures like recurrent selection are aimed at population improvement. Where cultivar release is a primary goal most public and private programs use some form of single seed descent (or a modification there of) or backcrossing (or modifications including marker-assisted backcrossing) to develop lines for yield testing. A summery of some of the recent literature on comparing breeding methods was reported by Orf et al. (2004).

From a cultivar development perspective, the development of a glyphosate tolerant soybean line (Padgette et al., 1995) has had a major impact on the U.S. soybean industry since the late 1990's. Initially the glyphosate tolerance gene was transferred to cultivars via backcrossing, but more recently many programs have incorporated the gene into the majority of the breeding material in their programs. An example of the development of a backcross-derived glyphosate tolerant soybean cultivar using genetic markers to speed up the recovery of the recurrent parent is given in Orf et al. (2004).

As noted above, genetic improvement in soybean can be made using recurrent selection. Since recurrent selection is designed to improve the frequency of favorable alleles in a population for quantative traits, additional effort is needed to develop a finished cultivar from a recurrent selection population. Lewers and Palmer (1997) published an excellent review of recurrent selection in soybean.

The main challenge in using recurrent selection in soybean has been the difficulty of the intermating step. Accomplishing the number of random matings needed in a large-scale population improvement scheme can be a taxing effort if such matings must be made manually. An efficient method for producing hybrid soybean seed for recurrent selection was developed by Lewers and Palmer (1997) that takes advantage of the closely linked loci Ms_6 and W_1. Marker-

assisted recurrent selection can also aid in population improvement. Some recent recurrent selection studies are listed by Orf et al. (2004). A number of traits including yield have undergone recurrent selection. Even though recurrent selection is a longer term procedure it is evident from the literature that recurrent selection in soybean is effective.

Selection among lines that are near homozygosity is another step in the cultivar development process. A commonly used procedure is to select individual plants after several generations of selfing then grow out progeny rows and visually select desirable lines, and then conduct replicated yield tests. Recently, many programs have begun conducting yield tests on progeny rows that may or may not be replicated. Including appropriate checks *frequently* in the tests allows breeders to use these yield tests to advance the best lines and discard unpromising material as soon as possible. Since yield is the main criteria in most cultivar development programs this early assessment of yield also allows breeders to evaluate more crosses or more lines per cross. If additional traits are part of the selection process, testing becomes more complex. As the use of molecular markers becomes easier and less expensive, I would anticipate marker-assisted selection for yield to become more common.

Soybean breeders continue to develop breeding methods to improve the effectiveness and efficiency of their cultivar development programs. Specht and Graef (1990) described a breeding method called the male-sterile-facilitated cyclic breeding (MSFCB) method for cultivar development. This method provides a breeder the opportunity to annually introgress a set of elite lines into the breeding population as well as providing F_2 seed for generation advance using single seed descent. Several high-yielding cultivars have been released using the MSFCB method.

Hybrid soybeans are a continued topic of interest for soybean breeders and soybean growers. Palmer et al. (2001) published an excellent article regarding hybrid soybean. In a recent informal survey of public and private soybean breeders, most respondents feel hybrid soybeans are several years to a decade or more away from being commercially important in the U.S. (R.G. Palmer, personnel communication).

The development of better techniques and equipment for plot management, especially plots designed for yield evaluation continues on a regular basis. Major changes have occurred over time that have increased the efficiency of planting, managing, and harvesting plots. Significant changes have also occurred in the preparation of seed for planting, and the weighing and analysis of seed during or after harvest as well as data manipulation and

analysis. These changes have resulted in larger numbers of plots handled per person in a breeding program.

Most soybean breeding programs use commercial or internally developed computer programs to make entry and plot designations, randomize plots, prepare field plans and field books, print labels, and organize tests. In many cases seed packets or trays may be filled using seed counters while others are filled by hand. Continued improvements are being made on planters used for yield plot testing. Newly designed planters have better seed placement, more accurate plot length, and use air systems for more uniform placement of seed.

Harvesting of plots for yield is now performed using self-propelled combines. Specialized combines have been developed for the various plot sizes from hill plots to large demonstration plots. In fact, some combines have been developed that are separated down the middle, such that two different plots can be harvested at the same time. Most combines have electronic systems for determining the weight and moisture content of seed harvested from plots. Electronic systems for on-the-go measurements of seed quality (protein and oil) are currently being tested. Combines are also available that will automatically bag and label seed from plots, making the harvesting process a one-person operation. Data are generally collected on a combine computer and then downloaded to office or laboratory computers for analysis.

Soybean breeders have used genetic markers to map genes controlling a number of economically important traits. Molecular markers have been used to identify quantitative trait loci (QTL's) in soybean. Orf et al. (2004) summarize most of the recently reported QTL's in soybean. A greater challenge will be the development of methods for the widespread application of marker-based techniques in breeding programs. Markers are currently being used in some programs for some traits, however, markers will be widely applied by breeders only after the markers have been shown to be both effective and economical. Many breeders suggest that most of the time when markers will be applied in breeding, the markers will complement traditional breeding methods that are refined and proven successful. In a review of DNA markers in plant breeding, Lee (1995) states "The challenges loom large for marker-assisted selection (MAS); in many crops, conventional selection has had several decades to evolve into a very effective technology. Plant breeding programs in most crops conduct simultaneous selection for several (>10) traits, in many populations across diverse environments." The experience so far of incorporating MAS in soybean has been mixed. Currently, MAS might be best viewed as a supplement to conventional breeding methods.

Another use of genetic marker information in applied breeding programs that has some promise is aiding breeders in the selection of those parental combinations with the greatest potential in leading to new, high yielding cultivars. Cox et al. (1985) stated that optimal breeding populations should have high means for traits of interest and a high genetic diversity. It has been shown that the means of parents of soybean populations are predictive of the means of populations developed from crosses (Panter and Allen, 1995b). However, predicting genetic variance in populations is difficult and very time consuming in typical breeding programs where related, elite parents are crossed. According to quantitative genetic theory, the genetic variance of populations for traits, such as yield, will increase as the parents differ for more genes that affect the trait (Falconer, 1981). It is generally assumed that genetic diversity between parents is inversely related to coefficient of parentage (CP). It may be possible to estimate the number of genes differing between parents with either CP or genetic markers.

Backcrossing is a widely used method in soybean breeding. Soybean breeders have employed backcrossing to add new genes to existing cultivars or to quickly transfer a trait like glyphosate tolerance. The use of markers can speed the development of backcross lines through the selection for, or against, genetic regions during backcrossing. This includes selecting for one or more genetic regions from a donor parent that contains gene(s) of interest, selecting for the recurrent parent's genome to speed its recovery, or selecting against regions that are not of interest from the donor parent. The use of markers in backcrossing is especially applicable in transferring novel genes into elite breeding material that were obtained through genetic transformation or from exotic germplasm (Frisch et al., 1999; Ribaut and Hoisington, 1998).

In many cases the use of molecular markers in selecting for the gene being backcrossed can be especially helpful when the phenotype of the gene is not readily assayable in backcross F_1 individuals. Situations where this would occur include when the gene(s) being backcrossed are recessive, a destructive test is needed for phenotyping, the phenotype is only expressed after pollination, or the gene contributes to a quantitative trait that can only be evaluated in replicated tests in the greenhouse, growth chamber or field. In all four of these cases, if markers are not available, the time to reach a given generation of backcrossing would be much longer because progeny testing would be required during each backcross generation. Marker-assisted selection for the gene being backcrossed is especially useful in soybean, where manual pollinations are very tedious, many pollinations are unsuccessful, and successful pollinations rarely produce more than two seeds. This way the efforts of the plant breeder can be focused on the few plants that, based on marker analysis, are presumed to carry the gene(s) of interest.

Marker-assisted selection might also be used by breeders for selection among plants or experimental lines within segregating populations. Since traditional breeding approaches rely on phenotypic selection, MAS could potentially increase genetic gains by reducing costs, increasing the total number of plants or lines with the desired loci available for selection, increasing the effectiveness of selection, and reducing the time required for a breeding cycle. Although MAS seems promising, there are many challenges that need to be resolved before the full potential of MAS in breeding programs will be realized.

Soybean breeders will need to continue their efforts to increase the rate of genetic gain in seed yield, pest resistance, and soybean seed composition by the application of conventional and molecular breeding tools. The increased mechanization of field plot equipment, use of progeny row yield evaluations, new computer software programs to manage all aspects of a breeding program, along with the potential for development of hybrid cultivars should contribute to this progress. The successful incorporation of MAS for SCN and other disease resistance traits into the breeding program has already been accomplished in several private-sector breeding organizations and MAS has already been used to develop backcross derived cultivars. In the next decade we should see many additional applications of MAS in soybean breeding. The challenge for public and private soybean breeders will be to incorporate the new conventional and molecular technologies into their existing cultivar development programs and thus continue the genetic improvement of soybeans.

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Status and future direction of soybean genomics – genetic association analysis and linkage disequilibrium

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Abstract

Genetic association analysis is being used by human geneticists as the method of choice to identify regions of the genome that contain disease susceptibility loci. In particular, association analysis is suggested as a powerful approach for the mapping of quantitative trait loci (QTL) that underlie complex traits. This approach has a number of advantages over conventional linkage analysis for QTL discovery. The effectiveness of association analysis is dependent upon the level of linkage disequilibrium (LD) that is present in the population under study. Because essentially all empirical studies of LD have been done in outcrossing species, the autogamous nature of the soybean presents a new and challenging application of genetic association analysis. We are investigating the application of association analysis for the discovery of QTL associated with seed weight and seed protein concentration. The relatively large literature of conventional linkage analysis for discovery of QTL controlling seed weight and seed protein provides a number of candidate loci with which to proceed in the initial association studies. This will be followed by more general scans of the genome as more single nucleotide polymorphism (SNP) DNA markers become available.

Introduction

It is certain that the future of soybean genomics research will follow many and varied paths leading to detailed definitions of genome structure and gene function and interaction. This information will guide efforts to fashion new gene combinations, modify existing genes or create completely new genes and/or alterations in gene expression that will assist in the development of soybean cultivars with improved productivity, seed quality, and biotic and abiotic stress resistance. It is the intention of this paper to briefly outline one research path that we believe provides an approach to the discovery, cloning, and manipulation of genes and their allelic variants that underlie QTL.

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Genetic association analysis

The discovery of genes or QTL in plants has relied upon genetic linkage analysis. Linkage analysis involves determining the transmission of alleles or genes along with anonymous genetic markers and quantitative trait data within a population of individuals with a defined pedigree structure. The resulting phenotype and genotype data are used to calculate recombination distance, the order of loci on the genetic map, and the putative positions of genes or QTLs. Genetic association analysis provides an alternative approach for gene or QTL discovery. The advantages of this approach particularly for the discovery of QTL with complex inheritance were described by Risch and Merikangas (1996). They suggested that the important virtue of genetic association analysis in relation to linkage analysis is the superior ability to detect genes with modest effect on phenotype. In medical genetics, association analysis compares allele frequencies in patients and controls to dissect the genetic control of complex genetic diseases. In its simplest form, a population of patients is paired with an otherwise identical control population of individuals who do not exhibit the condition under study. Allele frequencies of marker loci from across the genomes of the two groups are compared by a Chi-squared test or non-linear regression. A significant allele frequency difference between patient and control groups is indicative of a gene or QTL in LD with the marker locus. Linkage disequilibrium is of critical importance to the discovery of QTL and is described in further detail below. In relation to linkage analysis, association analysis is reputed to provide more precise QTL positioning because it takes advantage of recombination occurring over many generations rather than over the few generations used to create mapping populations via artificial hybridization as commonly practiced by plant geneticists. Another advantage of association analysis is that all QTL in a diverse population are theoretically detectable, not just those segregating in a particular bi-parental cross. Similarly, because diverse populations are used in case-control genetic association studies every available genetic marker is useful, not just those markers that are polymorphic in the particular bi-parental cross under study. Various difficulties that can give rise to false negative or false positive associations have been discussed in a number of reviews including those of Risch (2000) and March (1999). One difficulty of particular importance arises as a result of population subdivisions which may result in a statistical association between a phenotype and a marker that is unlinked to the actual causal genetic locus. A phenotype that is very prevalent in one subpopulation will be associated with many alleles that are in high frequency in that subpopulation. Pritchard (2001) sites a good example of such a situation as reported by Knowler et al. (1988) in a study of type 2 diabetes in two Native American tribes that have an extremely high rate of diabetes.

Diabetic individuals in the case population were less likely than controls to have a particular haplotype at the immunoglobulin G locus. One possible conclusion was that this haplotype was protective against diabetes. However, many of the individuals in the study populations had some proportion of recent European ancestry. It was demonstrated that the controls had a higher proportion of European ancestry than the cases, and that the frequency of the haplotype was higher in Europeans than in Native Americans. When comparisons were made between cases and controls with similar amounts of European ancestry, the association disappeared. In order to detect population structure in case-control studies Pritchard et al. (2000) proposed a method to test for population structure and thereby avoid spurious associations between a phenotype and a genetic marker locus.

Linkage disequilibrium

Of particular importance to the successful use of genetic association analysis is the level of LD in the target species and most importantly in the specific population being analyzed. LD or allelic correlation is present when two alleles at different loci occur together in individuals within a population at a higher frequency than would be predicted by their allele frequencies. As a result of LD, the correlation of alleles at linked loci results in reduced genetic variability as a result of haplotype structure. The haplotype of an individual is the linear ordered arrangement of specific alleles on a chromosome or DNA fragment. LD is a function of the degree to which haplotype structure is present in a population. Single nucleotide polymorphisms (SNPs) defined as single DNA base differences between homologous DNA fragments plus small insertions and deletions (indels) are a useful tool to quantify LD. The analysis of SNP haplotype structure has been the focus of recent studies. Patil et al. (2001) analyzed SNP haplotype diversity across human chromosome 21 and found a mean of less than three common haplotypes (those with a frequency > 0.10) in 4135 blocks of physically consecutive SNPs based upon the analysis of 24,047 common SNPs (frequency > 0.10). Generally consistent results were reported by Stephens et al. (2001) who analyzed SNP haplotype diversity in 313 human genes in 82 individuals whose ancestors were from various geographical origins. They found a total of 4304 haplotypes (approximately 14 per gene). However, when based upon frequency of occurrence, Stephens et al. (2001) indicated that 80% of the global haplotype diversity detected was defined by three common haplotypes with mean frequencies of 0.5, 0.25 and 0.125, respectively. The important reason for determining the nature and extent of haplotype structure is to allow the identification of a sequence variant(s) (SNPs) that can define each haplotype block. In this manner a small number of carefully defined sequence variants can be used in whole genome scans.

In plants there are limited data relating to genome-wide haplotype diversity but haplotype structure can be inferred from analyses of the decline of LD. Tenaillon et al. (2001) reported the rapid decay of LD over only 500 bp suggesting limited haplotype structure in maize. Similar, but somewhat different conclusions were reached by Remington et al. (2001) who found highly variable rates of LD decline in different maize genes. A recent report in *Arabidopsis thaliana* (Nordborg et al. 2002), which like soybean is an autogamous species, indicated that LD decays within approximately 1 centiMorgan (cM) or 250 Kbp. Nordborg et al. (2002) concluded that "the combination of a relatively high level of polymorphism and extensive haplotype structure bodes well for developing a genome-wide LD map in *A. thaliana*".

Some data relating to haplotype structure of soybean are available. Zhu et al. (2003) analyzed a diverse set of 25 *G. max* genotypes 17 of which were reported to be direct introductions or selections from introductions from Asia. Fourteen of the 17 were selected to represent the germplasm base of N. American soybean cultivars based upon a pedigree analysis reported by Gizlice et al. (1994). The 14 genotypes were estimated to have contributed 80.5% of the allelic diversity present in N. American soybeans. Three additional genotypes that have been used as sources of resistance to the soybean cyst nematode (*Heterodera glycines*) were included. None of the 17 genotypes is derived from a program of hybridization and selection thus LD in this group of lines had not been dissipated as a result of artificial hybridization and subsequent recombination. Haplotype analysis of SNP-containing fragments revealed a severe deficiency of haplotypes versus the number that would be anticipated at linkage equilibrium. The DNA sequence of a total of 143 DNA fragments derived from 116 GenBank genes and cDNAs as well as random genomic sequence was analyzed in each of the 17 genotypes. In 49 fragments with three or more SNPs, five haplotypes were present in one fragment while four or less were present in the remaining 48. These fragments averaged less than 600 bp in length and their linkage relationships were not determined, thus an analysis similar to that reported by Nordborg et al. (2002) was not possible. However, data comparable to Nordborg et al. (2002) were reported by Zhu et al. (2003) for one 12 cM region of the genome. One commonly used measure of LD is r^2, the squared allele-frequency correlation (Weir 1996), which is a pair wise correlation coefficient for measuring the association between alleles at different loci. Zhu et al. (2003) reported two estimates of LD in soybean. The r^2 value was calculated as per Remington et al. (2001) based upon the haplotypes of the 17 soybean genotypes at seven loci in DNA fragments amplified from across a 12 cM region on soybean linkage group G. The significance of r^2 ($P < 0.05$) was determined via permutation analysis. The r^2 values were plotted against the known

genetic distances between loci to examine the relationship of genetic distance and LD. This analysis indicated a substantial decline in LD as measured across this 12 cM region as measured by r^2 (Figure 1) and suggests that association analysis may be a useful alternative for QTL discovery in soybean. At this time our laboratory at the USDA, ARS, Beltsville, MD USA is working in collaboration with other labs to define the extent and structure of LD in various soybean populations including the wild soybean (*Glycine soja*) and unimproved *G. max* genotypes from the Far East.

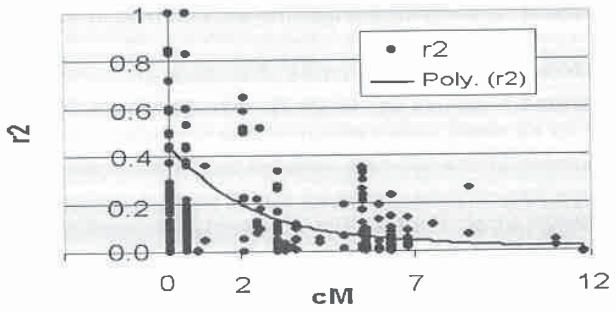


Figure 1. Linkage disequilibrium around the rgh'l locus on soybean linkage group G

Genetic association analysis for QTL discovery in soybean

Seed weight and seed protein concentration provide convenient quantitative traits to use in the examination of association analysis in soybean. Seed weight measured as g/100 seed can vary from 5 to 55 g (Hartwig 1973) and variation is continuous. Approximately 41% of the dry weight of the soybean seed is storage protein, however, soybean germplasm accessions are present in the USDA Soybean Germplasm Collection that have seed protein concentration greater than 50%. Soybean breeders have used these accessions and released high seed protein genotypes in which the protein component exceeds 50% of the seed dry weight (Hartwig 1990; Leffel 1992). Yaklich (2001) demonstrated that high protein seed lines released by breeders contained more glycinin and b-conglycinin than normal seed protein lines.

The use of SSR and RFLP markers have been used to map numerous seed weight QTL in cultivated soybean (Mansur et al. 1996; Mian et al. 1996; Orf et al. 1999; Csanadi et al. 2001; Lee et al. 2001; Specht et al. 2001; Hoeck et al. 2003) as well as QTL associated with seed protein concentration

(Lee et al. 1996; Mansur et al. 1996; Brummer et al. 1997; Orf et al. 1999; Qui et al. 1999; Csanadi et al. 2001; Specht et al. 2001). Seven seed weight QTL were reported in at least two different studies as were at least four seed protein concentration QTL. These "verified" QTL are more likely to be true genetic effects rather than statistical anomalies and provide candidate genome positions that can be initially examined in a genetic association analysis. Likewise, the glycinin and b-conglycinin genes reported by Yaklich (2001) provide candidate genes that may be associated with increased seed protein concentration.

Two case -control studies were developed. The first included one group of 86 germplasm lines with seed weights ranging from 4.2g to 10.0g/100 seeds and the second with weights ranging from 20.0g to 38.0g/100 seeds. The second study has one group of 159 germplasm lines with "normal" (40-42%) seed protein concentration vs. a second group of 159 lines with high (> 46%) seed protein. The germplasms were chosen based on balancing the case vs. control groups for maturity group, Asian origin, stem termination, and flower color. Initial DNA marker analyses of the germplasm lines employs SNP marker analyses and is focusing on regions of the genome where verified seed size or seed protein concentration QTL have been reported as described above. SNP DNA markers that map to other positions of the genome are also being assayed in an effort to determine if population structure exists in the germplasm lines being evaluated.

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Construction and use of microarrays of 30,000 soybean cDNAs to probe gene expression in soybean: examples from cotyledon and seed coat development and somatic embryogenesis

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DNA microarrays are powerful tools to analyze the expression patterns of thousands of genes simultaneously. As part of the NSF-sponsored "Soybean Functional Genomics Program", we have accumulated a set of unique genes from a larger collection of soybean 5' ESTs. The current "unigene" collection (or tentatively unique sequences) represents 36,000 low redundancy cDNA clones.

These include reracked libraries Gm-r1070 (a set of 9216 cDNA clones from various stages of immature cotyledons, flowers, pods, and seed coats); Gm-r1021 plus Gm-r1083 (a set of approximately 9216 cDNA clones from 8-day old seedling roots, seedling roots inoculated with B. japonicum, whole seedlings, and 2 month old roots); Gm-r1088 and Gm-r1089 (a collection of 9216 cDNA clones from a number of libraries made from cotyledons and hypocotyls of germinating seedlings and leaves and other plant parts subjected to various pathogens or environmental stress conditions). Functional assignments of clones were inferred by matching the BLASTX hits of the 5' and 3' sequences to the non-redundant databases. The inserts were amplified from each clone by PCR and were spotted onto glass slides for microarray analysis. Four arrays have been produced, each with approximately 9,216 cDNAs. We are currently using these arrays to study gene expression during soybean seed development and have used them to study reprogramming of cotyledon cells associated with induction of somatic embryos in soybean tissue culture (Thibaud-Nissen, et al., Plant Physiol, 132:118-136, 2003). In addition, we have analyzed isogenic lines containing mutations in the flavonoid pathway.

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Biotechnology and the challenge to increase crop yields

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Abstract

Genetic transformations of crops are considered for their potential for overcoming both the stagnation in yield potential and for limitations posed by abiotic stress. Limited flexibility in the resources required for plant growth and a diminishing impact of genetic transformations as one moves from the level of gene expression to harvestable yield result in a discouraging view that biotechnology will have major impacts on yield potential or abiotic stress resistance. Examples where basic physiological research has led to crop improvement are considered as a potential framework for ways that crop biotechnology may also be integrated successfully into research programs aimed at increasing crop yields.

Crop biotechnology has been offered as a solution to overcome the stagnation in crop yield potential, particularly in those areas of the world where yields are low and malnutrition and starvation are constant threats (Herrera-Estrella, 2000). Certainly, transgenic technology has altered several crop production systems, and this technology may provide developing countries assistance in battling biotic stresses of disease and insects. If solutions are found and implemented to overcome the biotic stresses limiting crop productivity in the developing world, then there is still an urgent need to: (1) increase the ability of crops to overcome the negative consequences imposed by abiotic stresses, and (2) increase the inherent yielding potential of crops. The purpose of this paper is to address the crucial points that we believe a plant biotechnological approach must consider in identifying traits and developing transgenic materials that will increase crop yield potential.

To begin this discussion, we examine the resources that plants require and which set physical limits on plant growth and yield. This analysis provides a framework for assessing how transgenic technology can, perhaps, make better use of these essential resources. Secondly, we consider a few rare examples of how the forerunner of crop biotechnology, crop physiology, has contributed to ameliorating the effect of specific abiotic stresses.

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Photosynthesis, plant mass accumulation, and the yield hierarchy

Under ideal conditions, when a crop is well supplied with adequate nutrients and water and in the absence of biotic stresses, crop mass accumulation is limited by the quantity of light intercepted (LI) by the crop (Sinclair, 1993; Cassman, 1999). Under these ideal conditions, crop mass accumulation for a given day (g m^{-2} d^{-1}) can be described as the product of LI and the radiation use efficiency (RUE, g MJ^{-1}), which is a long-term measure of crop (i.e., canopy) photosynthesis. Integrating the product of LI and RUE from emergence to maturity describes the total mass produced by a crop. Although plants using the C4 photosynthetic pathway have higher maximum RUE (~1.7 g MJ^{-1}) during vegetative growth than do plants using the C3 photosynthetic pathway (~1.4 g MJ^{-1}), there appears to be little variation in maximum RUE within the C3 or C4 pathways (Sinclair, 1993).

Because of the close association of photosynthesis and RUE, a seemingly logical target for biotechnology would be the modification of photosynthesis. For over forty years, photosynthesis was the target for crop improvement by plant and crop physiologists. From this research tremendous advances were made in understanding photosynthesis at scales ranging from the field to the gene. These research programs also identified genetic variation in photosynthesis among lines of soybean (Shibles, 1987), wheat (Austin et al., 1982), and maize (Moss and Musgrave, 1971) and that photosynthesis was inherited quantitatively (Wiebold et al., 1981). Despite these great academic achievements, there were no major increases in yield related to photosynthetic research.

One reason that photosynthetic research has failed to impact crop yields is that the effect of a trait when expressed at the level of the gene is diminished as one moves up the continuum to higher levels of complexity (Fig. 1; Sinclair et al. 2004). In Figure 1 is the response of soybean to a hypothetical 50% increase in mRNA levels for both subunits of Rubisco. The positive response at the transcriptional level decreases in magnitude as one moves up the 'yield hierarchy' to increased levels of complexity. This analysis predicts a modest (6%) yield increase given the scenario that adequate nitrogen is available for the added protein associated with Rubisco and predicts a decrease in yield if this added nitrogen is not available.

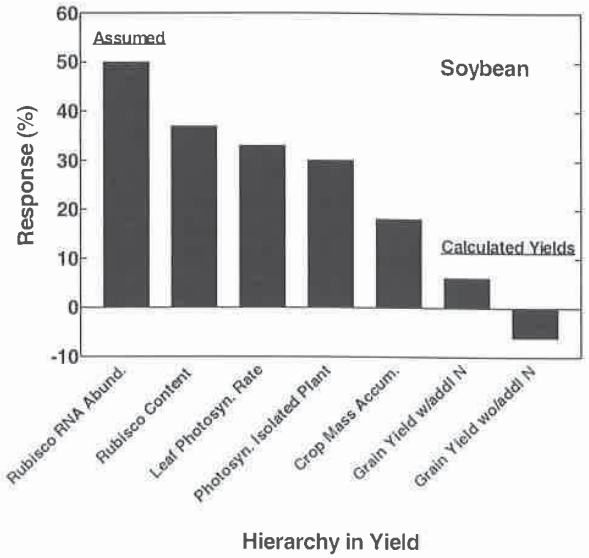


Figure 1. Yield hierarchy for soybean assuming an initial 50% increase in levels of Rubisco mRNA. For further details see Sinclair et al., 2004.

Plant biotechnology has also targeted photosynthesis as an important trait for increasing crop yields. Ku et al. (1999) isolated from maize PEP carboxylase, which is the initial enzyme in the C4 photosynthetic pathway. Further, they successfully transformed rice with the maize PEP carboxylase gene and achieved high levels of expression and PEP carboxylase activity. Despite these achievements, leaf photosynthetic activity of transformed plants had similar photosynthetic rates as untransformed plants. These results are similar to past studies using Panicum species that have C3, C4, and C3/C4 intermediate photosynthetic pathways (Brown, 1994). These studies documented that there was no advantage for C4 photosynthesis without the accompanying Kranz anatomy of C4 plants. If, indeed, C3 plants were transformed to have the necessary enzymes and the accompanying morphological changes that would double leaf photosynthetic rates, the accompanying yield increases would likely be modest due to the diminished response at higher levels (Fig. 1).

Harvest index, seed growth, and plant nitrogen

Yield may be expressed as the product of crop mass and harvest index (HI, ratio of seed mass to aboveground plant mass) (Sinclair, 1993; Cassman, 1999). A major advantage of wheat and rice cultivars that were introduced

in the 1960's was that they allocated a large proportion of their mass to grain. Past physiological research targeted at increasing allocation of dry matter to grain has included selecting for seed growth rates, seed growth duration, seed number, and decreased flower and ovule abortion (Egli, 1998). An important finding from these experiments was that a community of plants has an enormous plasticity for achieving a particular yield level. High-yielding cultivars may, therefore, achieve high HI values through various paths (Ball et al., 2000). Increases in HI above their current values (~0.50 to 0.55), however, appear unlikely (Sinclair, 1993; Cassman, 1999) because a minimum proportion of plant mass is required for supporting grain for mechanized harvests, for water and nutrient uptake, and for light interception.

Plant biotechnology has also targeted seed-growth characteristics as important traits for crop improvement. By decreasing the sensitivity of ADP-glucose pyrophosphorylase to phosphorus, Smidansky et al. (2002, 2003) increased seed growth of transformed rice and wheat plants when grown in isolation. Moreover, increased seed growth was accompanied by overall increases in individual plant mass, which resulted in no change in HI for rice and a slight increase for HI for wheat. The observed seed yield increase appeared to be simply a consequence of plant adjustments to changes in overall mass of individual plants. Substantial crop research has shown that increases in individual seed growth rates in a community of plants are compensated by shortened duration of seed growth so that little or no increase in yield results (Egli, 1998). To date there has been no published evaluation of the transformed rice and wheat lines when grown in a plant community. Under these conditions competition among plants and compensation among yield components would greatly affect any yield advantage associated with increased seed growth (Fig. 1).

In many conditions, light interception does not limit crop growth and yield. Rather, limiting nutrient and water resources are often restrictive, and keystones of the Green Revolution were crop cultivars that were responsive to nitrogen and investments in irrigation capacity. In less developed regions, yields are commonly limited by nitrogen availability and uptake by the crop. Yield improvements for these conditions will occur only if additional nitrogen is extracted from soil, or in the case of legumes, if symbiotic nitrogen fixation activity is increased. Pathways in the plant that lead to improvement in these traits would appear to be prime targets for crop biotechnology.

Water-deficits and crop growth

In addition to light and nutrients, water is often a limitation for crops reaching their full yield potential. With decreasing freshwater supplies and increasing

urbanization, development of drought tolerant crops is a critical issue facing world-wide agriculture. It is appealing to consider that specific traits may allow crops to obtain high yields with limited water supplies, but, unfortunately, the physics of water usage and mass accumulation by plants has limited flexibility (Tanner and Sinclair, 1983). This is because photosynthetic fixation of CO_2 is inseparably linked to transpiratory losses of water (Tanner and Sinclair, 1983; Purcell and Specht, 2004). Therefore, major increases in yield for water-limited conditions require increased access to soil water, and a prime target for crop biotechnology are traits that increase rooting depth (Purcell and Specht, 2004).

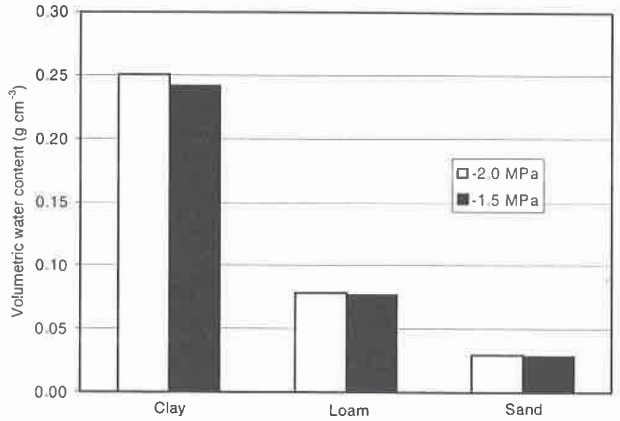


Figure 2. Volumetric water content for clay, loam, and sandy soils at water potentials of -1.5 and -2.0 MPa. The difference in water content between water potentials for a particular soil represents the increase in water content due to osmotic adjustment of 0.5 MPa.

Much of the molecular and transgenic research on water-deficit stress has focused on putative benefits of osmotic adjustment, which is the accumulation of solutes in response to stress above that due to cellular dehydration (Zhang et al., 1999). It is often assumed that osmotic adjustment will increase the water potential gradient between leaves and the soil and allow for greater water extraction as the soil dries. In principle this is correct, but if we consider the lower limits of water availability to be a soil-water potential of -1.5 MPa, the actual quantity of water that may be made available by osmotic adjustment is quite small.

If we consider that most plants can extract water to a soil water potential of -1.5 MPa, then crops selected or engineered to adjust osmotically by 0.5 MPa, could then extract water to a soil-water potential of -2.0 MPa.

Figure 2 shows the minimum volumetric water content that would be available from clay, loam, and sandy soils at water potentials of -1.5 and -2.0 MPa. The difference between the height of these bars represents the quantity of water available due to osmotic adjustment, which ranges from 0.009 g cm^{-3} for a clay soil to 0.0009 g cm^{-3} for a sandy soil. If we assume that the rooting depth was 1000 mm this would be an additional 9, 1.4, and 0.9 mm of water for plants grown on the clay, loam, and sandy soils, respectively. For comparative purposes, the water losses from a fully-canopied crop on a hot (37 C) day is approximately 7 to 8 mm.

Characteristics of successful physiological research

Although crop biotechnology is in its infancy, the expectations of this technology are high. The past 40 years of physiological research has laid the foundations for crop biotechnology but there are relatively few examples where crop physiology has led crop improvement. In the following paragraphs are three examples where crop physiology played a pivotal role in problem and trait identification, germplasm screening, and cultivar development.

The poor yield of cowpea under high temperatures led A.E. Hall and colleagues to evaluate the effects of high temperatures. They determined that high night temperatures resulted in pollen indehiscence and low viability (Warrag and Hall, 1983). Germplasm screening for pod set at a location with high night temperatures identified three potential lines with heat tolerance. A breeding program was established which successfully introgressed heat tolerance into commercial cultivars.

Recurring water-deficit stress in Australia's wheat region led G. Farquhar, R. Richards, and A. Condon and colleagues (Rebetzke et al., 2002; Richards et al., 2002) to search for wheat lines with high water use efficiency (WUE). Although there are physical limits as to how much WUE can be improved (Tanner and Sinclair, 1983), even modest increases in WUE will have a positive impact on yield in drought-prone regions. Their research first required them to develop tools to screen large numbers of lines, and theoretical considerations of WUE led to the development of carbon isotope discrimination ()) that could be used as an indirect measure of WUE. This methodology was successful in greenhouse experiments but initial field results with) gave the opposite of the anticipated response based upon the known WUE of the selected wheat lines. It was concluded that the anticipated relationship of WUE and) was masked because of interactions with other physiological differences among lines. The next step was an ambitious backcross program to move high WUE (low)) into selected cultivars. Those lines selected for increased WUE produced significantly

greater yields in eight of the nine field environments and the greatest relative yield increase being nearly 11 % (approximately 120 kg ha^{-1}) in the driest environment.

The last example was undertaken by us and our colleagues to ameliorate the sensitivity of N_2 fixation in soybean to drought (Serraj et al., 1999). Initial experiments identified the cultivar Jackson as having substantial drought tolerant N_2 fixation and it was used in crosses with drought-sensitive cultivars. Progeny lines developed from this cross have been evaluated in multiple rainfed locations and had yield 5% greater than commercial checks. Physiological evaluation of germplasm discovered that the inhibition of N_2 fixation to drought was associated with an inability to catabolize ureides (Purcell et al., 2000), which are the products of N_2 fixation in soybean. Progeny lines with high yield under drought also tend to be able to catabolize ureides rapidly (Sinclair et al., 2004).

Each of these successful physiological projects had commonalties that were crucial for their success. These included (1) a fully integrated team of physiologists, agronomists, and plant breeders working together from inception through cultivar development; (2) beneficial identification of traits near the top of the yield hierarchy and evaluation of traits in the field environments by the research team; (3) development of tools that allowed phenotypic evaluation of the large number of samples required by breeding programs; and (4) a long-term commitment both by funding agencies and by the researchers themselves. Many of the challenges faced by physiologists in the past apply to crop biotechnology today. Incorporating biotechnology into a crop improvement program requires a fully integrated research team that includes molecular biologists, physiologists, pathologists, agronomists, and breeders and a research framework that is supportive of cooperative research and long-term commitments.

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Physiological traits for ameliorating drought stress

T. R. Sinclair[1]

Abstract

The pattern of physiological responses to drying soil appears to be closely linked to decreases in soil hydraulic conductivity as the soil dries. Hence, physiological amelioration of stress needs to focus on possibilities for either accessing more soil water or slowing the rate of soil water extraction. Deeper rooting appears to be a critical approach to increasing the amount of water available to plants, although the difficulty of documenting differences in rooting depth among genotypes has resulted in little progress in this approach. Water conservation can be achieved by slightly decreasing plant gas exchange, which results in a decreased growth rate, but depending on the environment, can result in long-term increases in mean crop yield. It is speculated slow-wilting genotypes of soybean that appears to have drought tolerance may result from one of these two traits. Finally, symbiotic nitrogen fixation rates decrease with soil drying well in advance of plant gas exchange. Genotypes have been identified that exhibit substantial nitrogen fixation tolerance to drought stress and this trait in improved genetic backgrounds results in yield increase. On-going research has provided exciting possibilities for ameliorating drought stress in soybean, and the future may be even more promising as these traits are combined in superior cultivars.

Drought stress is a serious limitation of soybean yields, particularly because of the high sensitivity of symbiotic nitrogen fixation system to soil drying. A new monograph on soybean published by the American Society of Agronomy will soon be released and it contains an excellent review of various options for ameliorating the influences of water deficits on soybean growth and yield (Purcell and Specht, 2004). Therefore, in this brief paper only a few of the key physiological traits are discussed as possible approaches to ameliorating the consequences of drying soil. The traits to be considered are plant gas exchange, rooting, the slow-wilting trait and symbiotic nitrogen fixation.

Plant gas exchange

Gas exchange in soybean is sensitive to soil drying and the response (Fig. 1) is the same as reported for virtually all plants. There is no impact of soil drying on transpiration rate (or photosynthesis) until approximately 1/3 of

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the transpirable soil water remains in the soil. (Transpirable soil water is defined as the difference in the amount of soil water between field capacity and full closure of stomata.) At the 1/3 point, stomata closure begins and there is an approximately linear decline in gas exchange rate until the water stored in the soil to support plant gas exchange is exhausted. This endpoint is where the stomata are closed and photosynthesis has essentially stopped so plants are no longer growing. Beyond the soil water content at which the stomata are fully closed (fraction transpirable soil water equals zero) the critical issue becomes plant survival on the remaining limited water reserves. While soybean tends to have low capacity for survival under severe soil drying (Sinclair and Ludlow, 1986), drought survival is probably not a critical concern for many cropping situations. This results because conditions leading to survival-threatening water deficits will likely reflect such low overall amounts of seasonal available water that yields will be very low and the crop will no longer be economically viable even if it survives the drought (Serraj and Sinclair, 2002).

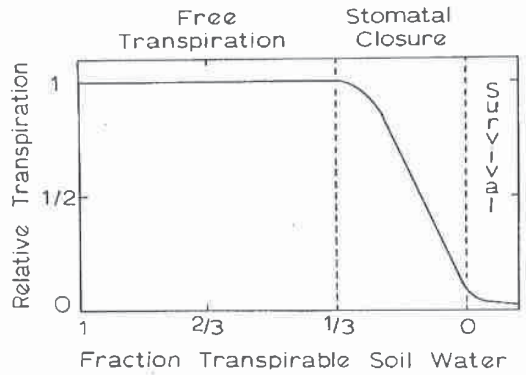


Figure 1. Phases of plant gas exchange response to soil drying.

Recently, an explanation for the consistent decrease in leaf gas exchange at 1/3 transpirable soil water was investigated. A theoretical analysis of changes in soil hydraulic conductivity in response to drying showed that for essentially all soil there are large decreases in soil hydraulic conductivity at volumetric soil water contents equivalent to the 1/3 threshold of transpirable soil water (Sinclair, 2004). Therefore, due to the decreased hydraulic conductance there are decreased rates of soil water uptake by plants. Direct measures of hydraulic conductance in the soybean-soil rhizosphere confirmed that no changes in rhizosphere conductance occurred until approximately the 1/3 threshold was reached (Sinclair et al., 2004). Therefore, the primary constraint on gas exchange can be traced to

decreasing soil hydraulic conductance as soil dries. Stomata closure is essential to avoid plant desiccation as a result of an imbalance between water uptake and transpirational losses.

Given the response shown in Fig. 1, there are a limited number of physiological options to improve crop performance on drying soil. One possibility would be to induce stomata closure before the 1/3 transpirable soil water threshold is reached. If some type of 'early-warning' system was developed in the plant, then early stomata closure would allow soil water to be conserved to delay the onset of severe drought. At this point, this is purely a hypothetical possibility and Hugh Earl (University of Guelph, personal communication) found no variation for the threshold point among soybean genotypes, including the slow-wilting genotype PI 416937 (Sloane et al., 1990). Nevertheless, Sinclair and Muchow (2001) showed in a model analysis that such a trait in maize resulted in a 9% yield gain over a 20-yr span for a hypothetical crop grown in Columbia, MO. Of course, the putative benefit of this trait is highly dependent on the environment in which the crop is grown. If an early-stomata-closure trait is identified in soybean, careful tests over a range of environments will be needed to fully understand the conditions for successful amelioration of drought stress.

Rooting traits

Roots characteristics have received discussion as a basis for crop improvement on drying soil. The soil-wilting genotype (PI 416937) was found to have a much more fibrous root system in the upper 38 cm soil layer than other lines (Hudak and Patterson, 1995) leading to the suggestion that increased root fibrosity allowed increased uptake of water. There is, however, little evidence to indicate that root length density is currently insufficient to recover soil water. Indeed the classical analysis of soil water extraction by Gardner (1960) and Cowan (1965) demonstrated that a root length density of 1.5 cm cm^{-3} was sufficient for water extraction. Soybean root-length density in top soil layers is usually several-fold greater than 1.5 cm cm^{-3} (Coale and Grove, 1990).

Increased rooting depth, however, seems to offer a major opportunity to ameliorate water-deficit stress. Simulation studies consistently indicate major benefits from increasing the depth for extraction of soil water. For example, the simulations of Sinclair and Muchow (2001) in which depth of extraction was increased from 80 cm to 100 cm or 120 cm resulted in mean yield increases for maize at Columbia, MO of 24% and 38%, respectively.

The difficulty in attempting to genetically increase soil water extraction depth is the great difficulty in quantifying rooting depth. Certainly, direct

observations of rooting depth require a huge investment in labor to acquire the required data. Measurements of water extraction depth require large investments in equipment to obtain the required soil moisture data. Consequently, no major study has explored the possibility to genetically alter soybean depth of rooting and water extraction.

Slow-wilting trait

Genotypes that exhibit a slow-wilting trait have been identified and investigated as a basis for ameliorating the affects of drought (Carter et al., 1999). While there is evidence that the slow-wilting trait can contribute to yield increases under water deficit, the explanation of the slow-wilting phenotype remains an enigma. One possible explanation for slow wilting is that the trait results from deeper rooting, which confers the important advantages discussed above. The difficulty is that no unambiguous data have been collected to support this explanation. Most recently, L. Purcell (University of Arkansas, personal communication) measured soil water extraction in the field of a slow-wilting genotype and found that its depth of water extraction was no different from normal lines.

Purcell (personal communication) did find, however, an indication based on one year of data that the rate of water extraction by the slow-wilting line was slightly slower than normal genotypes. This is consistent with the results of Hudak and Patterson (1995) that the growth rate of PI 416937 was slightly less than that of the cultivar Forrest as indicated by the slightly smaller values of the $^{2}_{3}$ coefficient in the logistic growth equation. A slight decrease in the daily potential rate of gas exchange, including both CO_2 and H_2O, will result in a slower loss rate of water from the soil so that there is water conservation.

Sinclair and Muchow (2001) used a crop model to examine the influence on maize yield over a number of years at Columbia, MO for a 22% decrease in radiation use efficiency, i.e. gas exchange capacity from 1.6 to 1.25 g MJ^{-1}. The results of these simulations (Fig. 2) gave a 3% gain in mean yield. Large yield increases were simulated for years with prolong drought while modest yield decreases were simulated in wet years. For soybean, a decrease in gas exchange of only 10% could result in sufficient soil water conservation to delay the appearance of wilting by at least two days. Of course, yield changes in response to changing crop gas exchange depend on the extent of the change in this trait and the specific environment in which the crop is to be grown.

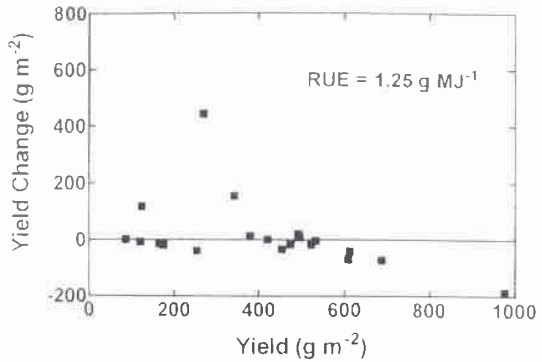


Figure 2. Change in yield of maize crops simulated for each of 20 y in Columbia, MO as a result of a decrease in radiation use efficiency (RUE) from 1.6 to 1.25 g MJ^{-1}. The abscissa is the yield simulated for RUE equal to 1.6 g MJ^{-1} (Sinclair and Muchow, 2001)

Nitrogen fixation

Symbiotic nitrogen fixation of soybean has been shown to be very sensitive to soil drying (Serraj et al., 1999). This is illustrated in the results presented in Fig. 3 from a field study in which the relative accumulation of nitrogen accumulated is graphed against the relative accumulation of plant mass. These results are relative to well-watered treatments and the two-years of experimentation included a range of water-deficit treatments. As seen in Fig. 3, all results in this graph were below the 1:1 line indicating a greater decrease in nitrogen fixation activity than mass accumulation in water-deficit treatments. A direct test of the possibility of ameliorating the negative consequence of soil drying on nitrogen fixation was reported by Purcell and King (1996). In their field experiment, ammonia nitrate was applied to soybean subjected to water deficits in an effort to overcome the loss of nitrogen fixation activity expected under these conditions. Yield was 18% greater with application of ammonia nitrate as compared to those plots that were largely dependent on nitrogen fixation for their nitrogen.

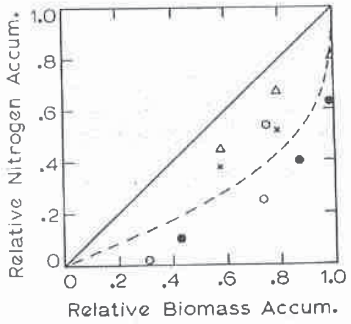


Figure 3. The relative nitrogen accumulations and mass accumulation of soybean subjected to differing water-deficit treatments grown under field conditions (Sinclair et al., 1987).

The basis of the nitrogen fixation sensitivity to water deficit is not fully resolved, but it is related to the accumulation of nitrogen products in the plant associated with soil drying. The main nitrogenous compounds transported from the nodules to the shoots in soybean are ureides. In fact, among the grain legumes it appears that those species that transport ureides have nitrogen fixation that is highly sensitive to soil drying (Sinclair and Serraj, 1995). Several-fold increases in ureides in soybean plants, especially in leaves, have been found when the plants are subjected to drying soils (Serraj et al., 1999). Therefore, uriede transport seems have resulted from an evolutionary tradeoff between using an extremely efficient compound for fixed nitrogen transport and enhanced drought sensitivity.

There is, however, genotypic variability in nitrogen fixation sensitivity to soil drying and in ureide accumulation. The cultivar Jackson was identified early in these studies as having substantial tolerance in its nitrogen fixation to soil drying (Sall and Sinclair, 1991) and much less ureide concentration in plant tissues under both well-watered and dry conditions (Serraj et al., 1997; Purcell et al. 1998). Further, tests in which ureides were fed to Jackson showed that it readily catabolized ureides (Vadez and Sinclair, 2002).

The discovery of the association between nitrogen fixation tolerance to soil drying and ureide accumulation allowed a unique three-stage program to be established to identify genotypes that might be superior to Jackson in their tolerance to soil drying (Sinclair et al., 2000). In the first stage, over 3000 plant introduction lines were screened in the field by harvesting petioles and measuring ureide concentrations. Over 200 lines were selected for the second-stage screen based on low petiole ureide concentrations.

The second-stage screen was also a field study in which plots of each genotype were subjected to approximately 3-wk-drought treatments and the relative nitrogen fixation and mass accumulation during this period were determined. Eighteen lines that sustained high relative nitrogen fixation rates under drought were subjected to the third stage of screen in the greenhouse. Nitrogen fixation was measured by a flow-through acetylene reduction system during a 2-wk, dry-down experiment. Eight genotypes were ultimately identified as having nitrogen fixation tolerance to soil drying that was superior to Jackson. It was subsequently found that all eight genotypes readily catabolized ureides and seven of the genotypes did not require manganese to sustain ureide catabolism as normally required by drought sensitive cultivars (Sinclair et al., 2003). These selected genotypes are now being used as parents in a breeding program at the University of Arkansas to develop commercial cultivars. Already, several progeny based on crosses with Jackson have given mean yields in regional yield tests greater than 5% of commercial check cultivars.

Prospects

Current research progress in ameliorating the influence of water deficits on soybean growth and yield makes this an especially exciting time. Intensive effort has led to the identification of two key traits to improve soybean performance under water deficit. Genotypes have been identified with a slow-wilting characteristic, although the basis of this trait is not yet resolved. Also, genetic material has been identified to overcome the loss in nitrogen fixation activity early in the soil drying cycle. This germplasm will allow soybean to sustain nitrogen fixation activity when soil dries slightly to the levels that lead to losses in activity. A particularly exciting prospect is the incorporation of both of these traits into the same germplasm to obtain complimentary, and maybe even synergistic, amelioration of yield losses resulting from drought stress.

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Mechanisms of flower abortion in water-stressed soybean

M. Kokubun[1]

Abstract

A large proportion of flowers in soybean (*Glycine max* L. Merr.) abort during development even under nonstressed condition. Water stress imposed during this period significantly increases the rate of abortion, and frequent water deficits might decrease grain yield. The physiological mechanisms underlying this phenomenon remain unclear. This report highlights our recent studies on 1) how intra-raceme variation in pod-set percentage is controlled under nonstressed condition, and 2) whether the abortion accelerated by water-stress is attributed to an impairment of the pistil or stamen function. In both studies, a soybean genotype IX93-100, which has long racemes, was grown in an environmentally controlled chamber. In the first study, flowers, which were divided into three floral positions (proximal, middle, distal) on individual racemes, were sampled at intervals after anthesis and the plant hormones (IAA, cytokinins, and ABA) in the samples were identified and quantified. The results revealed that the concentration of cytokinins, particularly *cis*-zeatin riboside (*c*-ZR) and isopentenyl-adenosine (iPA), was greater at more proximal floral positions several days after anthesis, as was the probability of pod set, indicating that pod-set probability was significantly associated with the cytokinin concentration at different floral positions within individual racemes. In the second study, the plants were subjected to water deficit prior to flowering and the abortion rate of flowers crossed between stressed and nonstressed plants was measured. The pistils of well-watered plants, pollinated with either stressed or nonstressed pollen, produced pods at a considerable rate, whereas only a small percentage of water-stressed pistils developed into pods, even when crossed with nonstressed pollen. These results suggest that flower abortion caused by a preanthesis water deficit is not attributed to an impairment of pollen, but was due to an impairment of ovule function.

Introduction

Soybean yield is determined by the number of seeds per unit area and individual seed weight. The seed number depends upon the number of floral buds that initiate pods and attain maturity. Soybean plants produce an abundance of floral buds, but a large proportion of them abscise during

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development to mature pods. A reduction in this abscission might increase pod and seed number, and thereby can lead to an increased yield. However, the physiological mechanism controlling reproductive abortion remains unclear.

Possible physiological factors affecting abortion include quantity of certain plant hormones (Huff and Dybing, 1980; Heindl et al., 1982; Carlson et al., 1987) and deficiency in or competition for carbohydrates and nutrients (Brevedan et al., 1978; Brun and Betts, 1984; Antos and Wiebold, 1984).

There are a number of reports showing that the application of 6-benzylaminopurine to racemes reduced abortion and increased pod-set probability. Endogenous cytokinins detected in the root pressure exudate showed a maximum concentration during a period of 0 to 9 days after the initial flowering, when most flowers are destined to either initiate pods or abort (Heindl et al., 1982; Carlson et al., 1987). These lines of evidence suggest that cytokinins produced in roots and translocated to flowers might be involved in reduction of flower abortion or enhancement of pod initiation. However, data are not available on the ontogenetic changes in the cytokinin contents of racemes, and therefore, whether the cytokinin contents in each portion of a raceme relate to the pod-set probability remains unknown.

Most reproductive abortion occurs at an early stage of embryo development after fertilization (Kato, 1964). A water deficit during this period is a dominant environmental factor accelerating the rate of abortion (Kato, 1964; Westgate and Pearson, 1993). Water stress imposed during flowering reduces photosynthesis and the amount of photoassimilates allocated to flowers, and thereby increase the rate of abortion (Raper and Kramer, 1987). These observations raise the question. Can water deficit prior to flowering cause an impairment of pistils or stamens which are undergoing development, leading to an increased rate of abortion?

This report highlights our recent studies on 1) how intra-raceme variation in pod-set percentage is controlled under nonstressed condition, and 2) whether the abortion accelerated by water deficit is attributed to an impairment of the pistil or stamen function.

Intra-raceme variation of pod-set percentage and its association with cytokinins

First, we examined intra-raceme variation in cytokinin concentrations in relation to days after anthesis (DAA). A soybean genotype IX93-100 was grown in an environmentally controlled chamber at 30/20 °C day/night temperature with a 15-h photoperiod (600 μmol $m^{-2}s^{-1}$ PPFD on the plant canopy). This genotype has long racemes and the pod-set percentage varies

with the position on a raceme. Flowers, which were divided into three floral positions (proximal, middle, distal) on individual racemes, were sampled at intervals after anthesis and the plant hormones (IAA, cytokinins, and ABA) in the samples were identified by enzyme immunoassay (EIA). Forms of cytokinins were further identified by gas chromatography-mass spectrometry (GC-MS).

The total amount of cytokinins in racemes, which was monitored by EIA, peaked one to two weeks after the first flowering on a raceme, when pod development was initiated. Within individual racemes, the total cytokinin concentrations were greater at more proximal floral positions, as was the probability of pod set (Fig. 1). Removal of proximal flowers at anthesis enhanced both cytokinin concentrations and pod set at middle positions on the raceme. The amounts of IAA and ABA changed in a contrasting manner. IAA was more abundant at more distal position, and the contents declined rapidly after initiation of flowering. In contrast, ABA was more abundant at proximal and middle positions on racemes and the concentrations increased after the pods initiated to grow. The GC-MS analysis of cytokinins revealed that *cis*-zeatin riboside (*c*-ZR) and isopentenyl-adenosine (iPA) were predominant forms of cytokinin in soybean racemes. In chickpea, *cis*-isomers were found to be predominant in seeds (Emery et al., 1998), whereas *trans*-isomers are the most commonly reported forms in higher plants (McGaw and Burch, 1995). Thus, pod-set probability was significantly associated with the cytokinin concentration at different floral positions within individual soybean racemes (Kokubun and Honda, 2000).

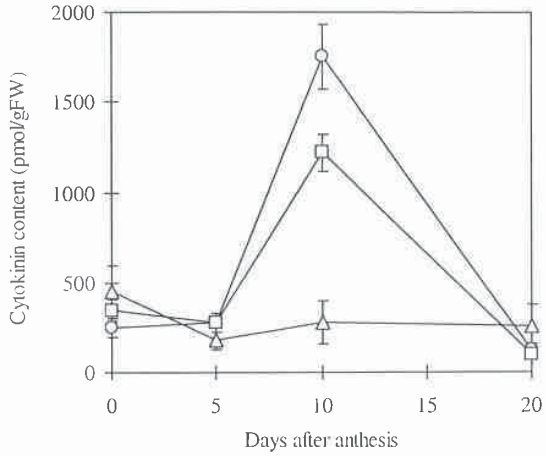


Figure 1. Changes in the amount of cytokinins in various positions (basal, middle and distal) of racemes during reproductive development of soybean.

Each flower on a raceme initiated a pod 4 to 7 days after anthesis. This corresponds to the time when the total cytokinin concentration in the raceme reached the maximum amount (i.e., 5 – 10 DAA). During this period, a fertilized ovule develops embryo and endosperm, and cells are dividing actively (Carlson and Lersten, 1987). Microscopic observations showed that most of the abscised flowers were at the proembryo stage, which is several days after anthesis (Kato, 1964; Abernethy et al., 1977). Abernethy et al. (1977) speculated that the frequent occurrence of abscission at this stage was due to the reduced level of a cell division mediating factor. Although the exact mode of cytokinin action remains unclear, our results showing that enhanced pod-set probability was accompanied by increased levels of endogenous cytokinins, and the observation by other workers that the enhanced pod set resulted from exogenous cytokinin application (for example, Crosby et al., 1981) strongly suggest that cytokinins play a promotive role in differentiation and/or early development of embryos.

Huff and Dybing (1980) attempted to identify substances that cause a difference in abortion percentage among floral positions on racemes of Clark isoline Elt. In their experiment, extracts from various tissues were applied to the basal part (positions 1 - 3) of racemes, and the extract from young pods was found to promote abortion of distal flowers. Among the plant hormones tested in the same way, IAA showed a similar promotive effect, whereas ABA, GA and benzyladenine showed no significant effect on abortion. Yarrow et al. (1988) suggested that ABA might reduce abscission of soybean flowers by favoring reproductive structures with allocation of photosynthate. Investigations on the interactive effects of these hormones, as well as the effects of environmental factors, are needed to clarify the detailed mechanisms controlling reproductive abortion of soybeans.

Viability of pistil and stamen under water stressed condition

It has not been elucidated whether the flower abortion can be caused by preanthesis water deficits, and whether the abortion, if occurs, is due to an impairment of pistil or stamen. To address these issues, experiments were conducted using a soybean genotype IX93-100 grown under environmentally controlled condition (the same as described above). When plants began to flower, the water supply was restricted to a level at which the water potential ($\emptyset_w$) of the leaves fell below –1.5 MPa. This treatment was imposed on half of the plants and lasted for 3 d, then the plants were rewatered to the original level. The water potentials of leaves and flowers, as well as photosynthetic rates of the leaves, were measured daily after the water restriction treatment. Well-watered (WW) and water-deficient (WD) plants were reciprocally hand pollinated (WW × WD and WD × WW) daily. Pod-set percentage of the artificially pollinated flowers was measured.

Water stress caused by restriction of watering for 3 d during the preanthesis stage significantly increased the abortion of the proximal flowers of self-pollinated plants (Fig. 2). The leaf $\emptyset_w$ of water-deficient plants were below –1.1 MPa for a few days, and photosynthetic rates of the stressed leaves were very low compared with well-watered plants. Decreased photosynthate production might have reduced the allocation of assimilates to flowers, which could be partly responsible for the increased rates of abortion in the stressed plants. However, in spite of the recovery of leaf $\emptyset_w$ and photosynthesis a few days after rewatering, the pod-set percentage of water-deficient plants was significantly lower than that of the well-watered plants. This result suggests that the decreased pod-set percentage could be caused by a factor other than a reduction of photosynthetic assimilation.

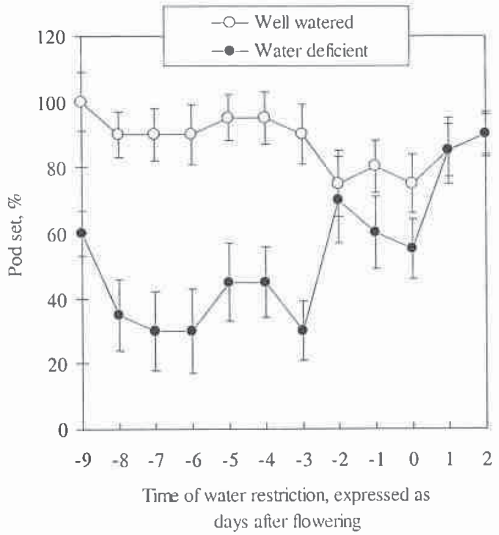


Figure 2. Pod-set percentage at proximal positions (1 - 4) on racemes of self-pollinated flowers from well-watered and water-deficient plants, as a function of time of water restrictions, expressed as days after flowering. Each point represents the mean ± SE of 30 racemes.

The $\emptyset_w$ of flowers were always lower than those of leaves. This phenomenon was previously reported by Westgate and Peterson (1993). The difference in $\emptyset_w$ between flowers and leaves was nearly 0.5 MPa or more, and $\emptyset_w$ of flowers fell to nearly –2.0 MPa under water restricted condition. This characteristics of having a low flower $\emptyset_w$ suggests that an impairment of the reproductive organs is likely to be induced by water stress imposed during the development of these organs. The pistils of well-

watered plants, pollinated with either stressed or nonstressed pollen, produced pods at a considerable rate. However, only a small percentage of water-stressed pistils developed into pods, even when they were crossed with nonstressed pollen (Fig.3). These results suggest that flower abortion caused by a preanthesis water deficit is not attributed to an impairment of pollen, but was due to an impairment of ovule function (Kokubun et al., 2001).

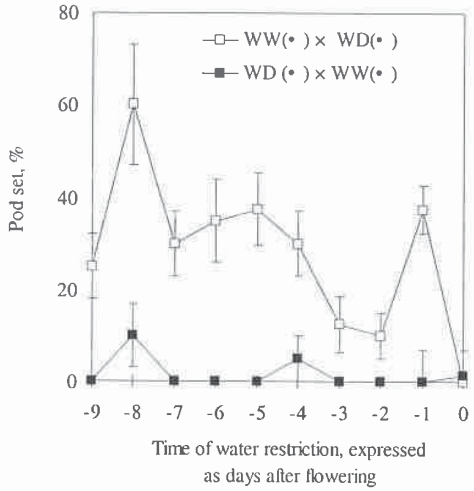


Figure 3. Pod-set frequency of hand-pollinated soybean flowers at proximal positions (1 - 4) on racemes. Reciprocal crosses were made between well-watered (WW) and water-deficient (WD) plants.

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Breeding for soybean drought tolerance: update on U.S. experience

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Abstract

Drought stress is a significant limitation of soybean yields in the U.S. Breeding for drought tolerance has had limited success. The use of canopy wilting scores from field plots has helped identify some sources of drought tolerance. These plant introductions have been crossed with elite cultivars in order to transfer the drought tolerance trait to improved breeding lines. Evaluations based on canopy wilting scores and yield under drought stress at several locations indicates we may be able to make progress in breeding for drought tolerance.

Soybeans are often subjected to drought stress during some period of the growing season in the U.S. As a result, a considerable portion of the year-to-year variation in soybean yield in the U.S. is associated with rainfall. Drought stress occurs more frequently in the southern U.S. but also affects other parts of the U.S. soybean growing area, including the Midwest, as we have seen in the last few years (Purcell et al., 2002). Many authors have discussed the impact of abiotic stresses (including drought) on crop yield. Purcell and Specht (2004) in the new monograph on soybean published by the American Society of Agronomy have a discussion on the impact of drought on soybean seed yield.

There have been numerous research studies conducted on the subject of drought tolerance. Physiologists, geneticists, breeders, agronomists, molecular biologists and many others have addressed the subject in soybean. Over the years there has also been discussion as to what constitutes drought tolerance (Carter, 1989). Successes in breeding for drought tolerance have been limited. Carter et al. (1999) suggested a number of barriers to the development of drought tolerant cultivars. Among the limitations are breeder skepticism, a narrow genetic base, the conventional testing procedures used by breeders and the fact that drought tolerance breeding research in the field is inherently high risk. The discussion in this paper on the topic of breeding for drought tolerance in soybean will be presented from an applied field breeder perspective. Thus many of the points and issues assume there are relatively large numbers of genotypes being grown under field conditions.

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As mentioned earlier, drought stress can occur anytime during the growing season. However, drought tolerance from a breeders perspective, implies that yield be used as one criteria. Thus early season drought (before flowering) generally is not evaluated (Desclaux et al. 2000). Experience has shown however, that drought occurs at different times in different years or it may not persist long enough in a given environment to be the major factor affecting seed yield. Carter (1989) suggested two approaches, the use of physiological traits and a networking effort among breeders, as ways to identify drought tolerant genotypes (parental material as well as lines in segregating populations of crosses involving drought tolerant germplasm) in the field. The use of canopy wilting as a physiological trait has been successful and will be discussed in more detail in subsequent paragraphs. Networking of both public and private breeders interested in drought tolerance has been facilitated and financially supported by commodity groups such as the United Soybean Board and state soybean research boards, federal and state programs, and the private sectors. This networking is a vital part of the breeding effort for developing drought tolerant cultivars since drought tends to be rather unpredictable (except on special sites). In many parts of the U.S. severe drought is experienced only once every three or four years or more with intermediate drought once every two or three years. Thus to ensure field-breeding progress for drought tolerance cooperation among breeders is essential as well as the use of special drought-prone sites.

Research towards developing drought tolerant genotypes was given a significant boost with the identification of Plant Introduction (PI) 416937 as being slow wilting (Sloane et al., 1990). Further information on this PI was summarized by Carter et al. (1999). From previous research the trait that appears to be the most useful in field breeding programs is canopy wilting (or a canopy wilting rating). The use of visual stress ratings to identify drought tolerant genotypes has also been used in peanut (Rucker et al. 1995). Canopy wilting is a visual stress rating made on a whole plot basis (two row or four row plots) for each replicate in yield trials or other plot trials. Because of the unpredictable nature of drought stress, ratings are begun when some (20-30%) of the lines begin to show canopy wilting. A scale of 0 –5 is suggested where 0 = no wilting, 1 = slight wilting (wilting on a few plants), 2 = some wilting (half or more of the plants wilted), 3 = significant wilting (most plants wilted), 4 = severe wilting (leaf scorching or leaf firing on many plants), and 5 = completely wilted (yellow, brown or dead leaves or leaves falling off on many plants). Since plants will recover overnight, ratings should not be started before about 10:00 am and should conclude by 1:00 – 2:00 pm in order to minimize the effects of high temperatures. It is also helpful to look at the plots from the same perspective

(i.e., the sun behind the person scoring or overhead). If the winds are strong or in gusts, it is difficult to make accurate canopy wilting ratings. If the drought stress continues for a long time (several weeks or longer) most, if not all lines may eventually show canopy wilting. Since natural rainfall may occur at anytime it is probably best to take canopy wilting ratings every 3-5 days in order to accurately assess the differential wilting that occurs among genotypes. The successive ratings will provide a way to differentiate which genotypes have slower canopy wilting.

Research trials on special sites where drought occurs frequently is one way to help deal with the unpredictability of drought. Our sites generally have deep sandy soils with very low water holding capacity, such that we can expect some level of drought stress most years, particularly in the late July and August timeframe (although we had no drought stress in MN in 2002 and in NC in 2003). These sites have relatively uniform soils and have on-site irrigation available to be able to have irrigated and non-irrigated trials next to each other. Other locations that have heavier soils with higher water holding capacity have greater risk for lack of drought stress. However by networking with others the chances that some sites will experience drought stress that will provide useful data for assessing the drought tolerance of genotypes in any given year is fairly high. Since drought stress occurs at different times in different years researchers need to pay close attention to maturity dates of genotypes in tests and make yield and canopy wilting comparisons within a limited range of maturities. Of course the timing of the drought stress also affects yield components such as seed number and seed size of genotypes of different maturities. This may be a greater factor in the Midwest than the south particularly as canopy wilting scores are used as an assessment of drought tolerance.

As noted earlier, PI 416937 was identified as a genotype that had low canopy wilting scores (slow wilting) compared to other PI's and cultivars. This PI has been evaluated in many different environments and it always has the lowest (or is among the lowest) in terms of canopy wilting score. This demonstrates that drought tolerance based on canopy wilting is common across environments. This was evident this past growing season when PI 416937 was grown in AR, MN and NE and it was among the best in terms of canopy wilting score at these varied sites.

After working for many years on drought tolerance the USDA-ARS program of North Carolina State University has made substantial progress, although no variety has been released to date which combines high yield potential with a high level of drought tolerance. To briefly summarize this work, slow wilting progeny of PI416937 as well as high yielding progeny were

recovered in U.S. maturity groups (MG) VII and VIII in the early 1990's. Cultivar N7001 was released from this breeding effort with 50% of its pedigree tracing to slow wilting PI 416937. N7001 exhibited yields comparable to the best current cultivars. The contrasting lines and new cultivar developed in initial breeding efforts were used as parents in a second generation of breeding in an attempt to combine high yield potential and drought tolerance which had been identified in separate breeding lines in into a single genetic background. Yield results indicate that the new lines with 50% pedigree derived from PI 416937 have performed very well in USDA regional testing and North Carolina state variety trials. Breeding line N97-9812 (MG VI) yielded only 60 kg/ha less than the control cultivar Boggs in USDA regional trials in three years of testing (2000-02). Breeding line N96-6752 (MG VIII) yielded the same as the control cultivar Prichard in two years of regional testing (2001-02). Breeding line N96-6809 (MG VII), a progeny of N7001, yielded 180 kg/ha greater than N7001 in three years of testing in North Carolina state variety trials. N97-9612 and N97-9638, which are additional progeny of N7001 with only 25% pedigree tracing to PI 416937, have performed even better than the previously mentioned lines, and have consistently outyielded the control cultivars by 8 to 15% and topped the USDA regional trials in 2001 and 2002. The contribution of drought tolerance to these yield advances is a topic of current research.

Through additional screening of exotic accessions and breeding lines, PI471938 from Nepal and adapted breeding line NTCPR94-5157 (no exotic material in pedigree) were identified as slow wilting types. These two have been used in additional rounds of breeding and promising lines have been identified. In 2002, for the first time, lines were yield tested under drought that were derived from two distinct sources of slow wilting, N96-7031 and NTCPR94-5157. N96-7031 traces 50% of its pedigree to PI 416937. Transgressive segregation for wilting was observed and these slow wilting lines tended to yield higher than the controls. The 18 best lines from this test yielded 2166 kg/ha under drought while the control, NTCPR94-5157 yielded only 1800 kg/ha. NTCPR94-5157 outyielded all commercial cultivars that were included in the test. DNA marker studies are in progress to identify alleles for slow wilting and yield performance under drought in this population and in populations derived from PI 471938.

Drought response is difficult to study because drought cannot be predicted well and is quite variable from year to year. To overcome this high risk factor in research, yield trials to identify drought tolerant lines are being conducted as a regional endeavor. In the southern USA, the major growing areas for soybean are the Mississippi delta region and the Atlantic coast and the same

maturity groups can be grown in each. By experience, we have learned that although both areas experience summer drought, they are sufficiently distant that the summer weather patterns are quite distinct. In practical terms, it is rare that both areas receive enough rainfall to avoid drought completely within a single summer. By testing in both regions simultaneously, we usually obtain severe drought on at least a portion of our experiments and, thus, make progress each year. Data collected to date indicate that slow wilting types in the Delta region are usually the same as those identified on the Atlantic coast. This collaborative research involves both the public and private sector, including USDA, the University of Arkansas, the University of Georgia, North Carolina State University, Monsanto Global Seed Co, Pioneer Hi-bred Seed Co, Syngenta, and Delta and Pineland Co.

Even though there has been good progress in later maturity groups in breeding for drought tolerance breeders often ask whether the trait can be transferred to earlier maturing genotypes adapted to the Midwest. The most promising lines derived from PI 416937 and PI 471938 (identified as being drought tolerant) were crossed with maturity group O and II elite cultivars. Plants from four segregating populations that were of the correct maturity were selected in MN and NE. The early lines were planted in 2002 and 2003 in MN and the later lines in 2003 in NE. No canopy wilting scores were obtained in 2002 due to lack of drought stress. However in 2003 canopy wilting scores and yield results from irrigated and non-irrigated plots were obtained in MN and canopy wilting scores were taken in NE. About 250 lines were evaluated in MN plus checks. Canopy wilting scores were taken five times. There were a few lines with low canopy wilting scores even at the last date, which was just before the lines began to show signs of maturing. This demonstrates that the low canopy wilting trait can be transferred into northern germplasm. This also suggests that this trait could also be transferred to other parts of the world.

The lines were also planted at a site with heavier soils that have higher water holding capacity. Canopy wilting data was taken three times at that site. It was noted that almost all the breeding lines had less canopy wilting than current elite cultivars. The lines with the lowest canopy wilting scores at the sandy site also were among the lowest at this site. About 350 plant introductions of maturity groups O-II have also been screened for canopy wilting over the last few years. Several have low canopy wilting scores but their drought tolerance will need to be verified in additional years and at other locations with further yield trials.

A further indication of drought tolerance is in the yield data. Lines from the four populations had yields equal to the elite check cultivars in both the

irrigated and non-irrigated plots. The correlations between yield in the non-irrigated plots and canopy wilting scores were significant (except for the first date of scoring) but were low to moderate (-0.12 to -0.29). There were lines that had low canopy wilting scores and were also among the highest yielding lines (equal or better than the elite checks) in the tests. The lines with lower canopy wilting scores at the site with heavier soils also had yields similar to the elite check varieties. This further illustrates that drought tolerance is similarly expressed in varying environments. Yield data from the PI screening in the sandy soil, non-irrigated plots was also encouraging. Several PI's had low canopy wilting scores and had yields similar to the elite checks. If this is verified in subsequent tests (as we expect), there will be additional sources of drought tolerance, especially in the earlier maturity groups, for breeders to use. It may also be interesting to cross the different sources of drought tolerance (or lines derived from them) to investigate whether there may be different loci contributing to drought tolerance in the different sources and a positive epistatic effect with regard to drought tolerance would be observed.

Breeders, of course, would like to be able to rate material for canopy wilting in populations in early generations. Our experience shows that individual plants cannot be rated for wilting. Individual plant rows might be rated if the rows are sufficiently long (2m or more) to reduce end plot effects, good check genotypes covering the range of canopy wilting response are included often, data on wilting is taken often when the plants are at the right stage, the soil at the testing site is relatively uniform, and the correct rainfall distribution occurs (in other words drought stress may not occur every year).

Considerable research and breeding efforts have gone into investigating genetic variation in nitrogen fixation in response to drought. Purcell and Specht (2004) and Carter et al. (1999) as well as other papers from this conference provide details about this research. Yield evaluation of lines derived from crosses with drought-tolerant nitrogen fixing parents shows that some lines had yields greater than checks under drought stress but not under irrigation (Sneller et al., 2000). Research and breeding continues to explore this aspect related to drought.

Drought avoidance is another way to deal with drought if the timing of the drought is regular and predictable. In the mid-south of the U.S. a system has been developed that uses short-season cultivars to avoid drought or use less irrigation (Edwards et al., 2003). Although this may be a way to deal with drought this probably does not make a major contribution to breeding for drought tolerance.

Drought tolerance is a trait that is of global importance. Soybean breeders all over the world indicate that the lack of adequate water is a major factor limiting soybean yields. Unfortunately progress has been slow, with few soybean cultivars with drought tolerance being released. This is partially due to difficulty in obtaining appropriate field sites and establishing and maintaining networks necessary to do adequate testing. The current work on drought tolerance in the U.S. is encouraging. We look forward to establishing collaborations with colleagues in Asia, Africa, Europe and Latin America for further evaluation of materials we have developed and identification of additional drought tolerant germplasm. This should speed the development of more drought tolerant cultivars for use by soybean growers around the world.

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Breeding for soybean drought tolerance

J.E. Specht[1]

Abstract

Water, when it is scarce, is often thought of as being a plant abiotic stressor. However, water should be thought of as a resource (like CO_2, NO_3^-, etc.) that must be fully exploited, even when crop production is being constrained by its scarcity. It is well-known that the amount of cumulative crop biomass (BM) is a linear function of (1) the amount of transpired (T) water multiplied by (2) a coefficient known as water-use efficiency (WUEb) expressed on a biomass basis: BM = T x WUEb. Note that the WUEb term is a linear regression coefficient reflecting the change (D) in biomass that occurs per unit of change (D) in transpiration (i.e., WUEb = ΔBM / DT). It is also well-known that seed yield (SY) can be described as a function of two entities: BM and harvest index (HI), where HI is defined as the seed fraction of total BM. Thus, SY = BM x HI. By substitution: SY = (T x WUEb) x HI. Note, however: (WUEb x HI) = WUEy. Ergo, SY = T x WUEy, where WUEy = ΔSY / ΔT. The equation SY = T x WUEy illustrates rather profoundly that, if breeders are to genetically increase SY, they must select genotypes that (1) *transpire more of the available annually rechargeable soil water*, or (2) *have a larger WUEy (greater* DSY / DT). Field-based research in Nebraska has demonstrated that both soybean biomass and seed yield respond to variable seasonal water amounts in a highly linear manner, which means that genotypic differences in the WUEy coefficient are estimable. Our drought research has been aimed at discerning the genetic basis of WUEy by trying to detect quantitative trait loci (QTLs) in soybean map populations. Phenotypic WUEy data have now been obtained in five different map populations of 250-300 members each. Only a few WUEy QTLs have been detected, and most have map positions coincident with QTLs governing overall yield performance, and both tend to map to positions of genes known to govern plant growth habit and maturity. We have also used a 13C isotopic composition analysis to obtain an integrated measure of crop transpiration efficiency (TE) on newly emerged soybean leaves, but found that the TE QTLs also tended to map to the same growth habit and maturity genes. Still, one of the most significant findings was that in each mapping population, there was a high correlation between the yield of a soybean genotype in the water-abundant environment and the yield of that same genotype in the water-scarce environment. Coupled with the map

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coincidence of the QTLs for WUEy and the QTLs for overall yield, the implication is that genetic improvement in soybean yield performance in water-abundant environments is likely to result in a concomitant performance improvement (but lesser in degree) in water-scarce environments. Indeed, if one is able to improve the slope of a genotype's yield-to-water relationship (without altering the X = 0 intercept of that slope), then one should also succeed in improving that genotype's yield (on the Y axis) at any water level (on the X axis).

Introduction

Of all of the abiotic factors whose seasonal variability impacts crop productivity, insufficiency in the amount of water available for crop transpiration is fundamentally the most recurrent (if not annually prevalent) factor that reduces crop yield (Boyer, 1982; Waggoner, 1994). Open stomata permit the influx of atmospheric carbon dioxide into the interior of terrestrial plant leaf, but also allow the escape of water vapor from the humid interior of that leaf. Consequently, photosynthesis and transpiration are inseparably coupled as Condon et al. (2002) noted: "Water is the required unit of exchange for the acquisition of CO_2 by plants". Crop biomass accumulation (and seed yield), in any given season, is a linear function of the amount of water made available for crop transpiration (Sinclair, 1994; 1998). If seasonal transpiration is impeded because of inadequate water, crop biomass accumulation (and seed yield) invariably will be lessened.

Water – a crop resource

Many researchers are now recognizing that the value of any plant trait or physiological mechanism in water-limited environments is best measured *vis-à-vis* a framework first proposed by Passioura (1977; 1994, 1996), and recently applied in the reviews by Ludlow and Muchow (1990) and Turner et al. (2001). This framework treats water as a *resource* available for crop production, thus making grain yield (Y) in water-limited environments a function of three largely independent entities, amount of water transpired (T), water use efficiency (WUE), and harvest index (HI):

$$Y = T \times WUE \times HI \qquad [1]$$

Equation 1 can be explained as follows. The term Y is final seed yield (g seed m^{-2}). The term T refers to the total quantity of water (g water m^{-2}) transpired during the season as the crop progresses from emergence to physiological maturity. The term WUE refers to the amount of crop biomass *produced per unit* of water transpired (g biomass g $water^{-1}$), calculated from season ending values. Total crop biomass (BM) is the product of T

multiplied by WUE (technically WUEb). The term HI is defined as the ratio of seed mass to total biomass at harvest, typically expressed without units.

The relationship described by Equation 1 is probably better understood when graphically displayed as a BM *vs.* T plot, as shown in Figure 1. In the figure, the vertical and horizontal axes are expressed in relative terms (i.e., biomass yields obtained for various fractions of crop transpiration possible in a given season, where maximum transpiration is unity). The graph makes it readily apparent that the BM *vs.* T relationship is governed by WUEb, a *linear response coefficient* (i.e., slope) reflecting the dependence of BM on T. It is well-known that a genotypic WUE is constant when T is varied at any given location in any given year (Tanner and Sinclair, 1983; Specht et al., 1986; 2001). Thus, if the amount of water available for transpiration by the crop is increased (genetically or otherwise), the amount of crop biomass is also increased. Of course, breeders would like to see crop biomass production improved over all seasonal transpiration amounts, and this would be possible *if the WUEb coefficient itself could be increased*. In fact, some plant species have accomplished an increase in WUEb by evolving from a C3 to a C4 pathway of photosynthetic CO_2 acquisition. The C4 photosynthetic pathway greatly improves the numerator in the CO2 influx / H2O efflux ratio, such that a C4 WUE is superior to a C3 WUE in Fig. 1. Let us now consider Y *vs.* T. If HI (whatever its value) remains constant *vis-à-vis* variable amounts of T, then WUEy - the response of seed yield to transpiration - will be as linear as that of the response of biomass to transpiration relationship. Note, however, that if breeders can increase in HI, the result is also a steeper WUEy (i.e., compare the WUE slopes when HI = 0.45 *vs.* 0.55).

It is important to point out that researchers can (and have) defined the BM, T, and WUE terms differently than just stated. In most instances, biomass ordinarily consists of only shoots, since it is difficult to recover roots (except in pot studies). In grain legumes, biomass may not include all leaves if some abscise before collection. If crop water consumption is measured without partitioning out evaporative (E) water loss from the soil surface, then an ET term replaces the T term in Equation 1. Crop water input (I), in the form of combined precipitation and irrigation amount (after adjustments for surface runoff and deep percolation), is sometimes used in lieu of T or ET measurements. Relative to the derivation of WUE, the biomass and T amounts measured in treatments that involve genotypes or cultural practices must be assessed on some common time scale, whether this is seasonal, monthly, weekly, daily, hourly, etc. When those measurements are more or less instantaneous, it is CO_2 flux to, and H_2O flux from, the leaf that is measured, and carbon dioxide assimilation (A) then replaces the BM

component of Equation 1, then leaf transpiration efficiency (TE) rather than crop WUE is being measured. The energy costs of producing biomass of differing composition (i.e., different proportions of oil, starch and protein) will strongly affect WUE. For example, production of seed with a high oil and/or protein concentration requires more photosynthate (and hence more water) than production of seed composed primarily of starch, which leads to a lower WUE in the former case (Tanner and Sinclair, 1983). The papers of Tanner and Sinclair (1983), Sinclair et al. (1984), and Sinclair (1994) can be consulted if more detail is desired as to the alternative formulations of Equation 1.

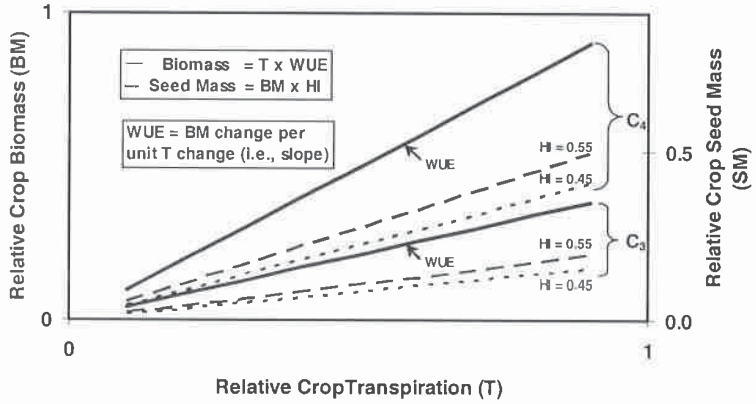


Figure 1. Displayed in this figure is the well-known linear relationship (solid line slopes) between crop biomass (BM) and crop transpiration (T) in water-limited production environments. The linear relationship (i.e., slope) is water use efficiency (WUEb, biomass basis), defined as the change in biomass (DBM) accruing per unit change in crop transpiration (DT). A C4 photosynthetic species (e.g., maize) will typically exhibit a WUE more than twice that of a C3 photosynthetic species (e.g., soybean). The ratio of crop seed mass to crop biomass is defined as the harvest index (HI). In the graph, HI is assumed to be constant over T, so crop seed mass is also a linear function of crop transpiration amount. We have arbitrarily chosen to graph two HI values (0.55 and 0.45) in the two species to illustrate how a change in HI would impact the relationship (WUEy, seed yield basis) between seed mass and crop transpiration (Purcell and Specht, 2004).

Genotypic differences in soybean WUE

To improve soybean WUEy, breeders need to identify genotypes whose WUEy is higher (i.e., steeper). To enhance WUE, one could simply increase

the WUEy numerator - the increment of biomass (i.e., ΔBM) generated per incremental unit of transpiration (i.e., ΔT). The evolution of C3 to C4 photosynthesis is an example of a WUEy improvement achieved via an increase in the WUEy numerator. Inversely, one could try to decrease the WUEy numerator - the transpiratory increment (i.e., ΔT) associated with a given incremental unit of biomass (i.e., ΔBM). In general, it is difficult to enhance WUEy via decreases in the WUEy denominator term. This is because such decreases almost invariably involve decreases in stomatal conductance, which is effective in reducing water efflux from the stomata, but it also depresses carbon dioxide influx to the extent that the water "saved" from transpiration may not be worth it, given the coincident biomass reduction. Space limits my discussion of WUE and QTL data obtained in each of the five mapping populations, but the results obtained in other mapping populations do not differ significantly from the results obtained with the first mapping population, so the key conclusions will be re-iterated here. Specht et al. (2001) conducted a QTL analysis of soybean response to differing water levels generated by a sprinkler-gradient system that replenished 100, 80, 60, 40, 20, and 0% of weekly crop ET loss. The genotypic material was a population of 236 recombinant inbred lines from a 'Minsoy' x 'Noir 1' mating. Because of the fortuitous occurrence of a severe drought during one of the years, tissue samples from the genotypes grown in the 100% ET (water freely available for transpiration) and in the 0% ET (water supply severely limited) treatments were collected for carbon isotopic composition (d) analyses and TE calculations. Plots of seed yield *vs.* crop water input in the 0, 20, 40, 60, 80, and 100% ET treatments were quite linear, and for each genotype a WUEy coefficient (termed *beta*) was derived. Significant genetic differences were observed for *beta*, which is an empirical, seed yield-based estimation of genotypic WUE, and for δ, which is purported to be a mechanistic, leaf tissue-based indication of TE. The genotypic correlation of *beta* and d was positive confirming that the δ - based measure of TE was indicative of WUE; however the correlation was low ($r = 0.26$). The major QTL identified as affecting these two traits turned out to be coincident with two major genes for maturity and one major gene for stem growth habit. Such genes have pleiotropic impacts on many traits. While some minor QTL were detected for *beta* and δ, their effects were too small to be of use in a marker-assisted selection program. The strong positive relationship ($r = 0.71$) between genotypic *beta* and genotypic mean yield did indicate that selection for a high yield potential would likely produce cultivars with a high *beta*, and because of the latter's positive correlation with δ, probably a higher TE as well.

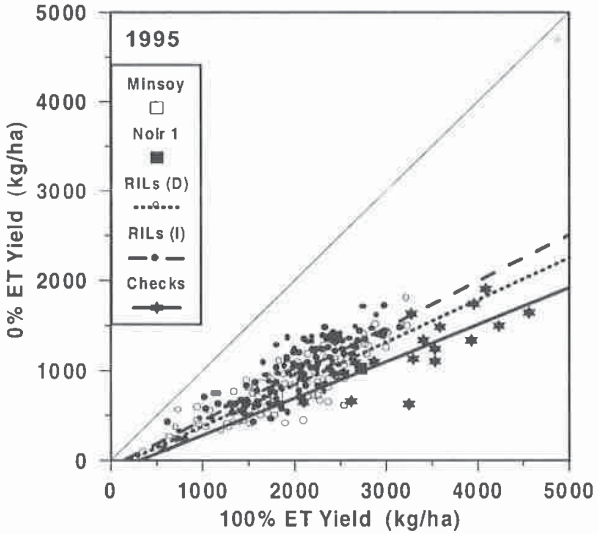


Figure 2. Yields of mapping population recombinant inbred lines (RILs), parents, and checks when grown in water-scarce (0% replenishment of weekly ET losses) *vs.* yields in water-abundant (100% replenishment of weekly ET losses) environments (Specht et al., 2001).

The results of Specht et al. (2001) confirmed in soybean that there is a close association of seed yield and transpiration (i.e., compare Fig. 3 in Specht et al. 2001 with Fig. 1 in this chapter). Moreover, the close association between genotypic *beta* (a field-based proxy measure of WUE) and genotypic mean yield provides some credence that soybean WUEy (and thus drought tolerance *per se*) is improved coincidently by selecting for higher genotypic mean yield. It was also clear in every mapping population that the highest yielding entries when water was abundant (100% ET) were also the highest yielding entries when water was scarce (0% ET), as is clear from the above Fig. 2. This would be consistent with the observation that most of the QTLs that detected for WUEy in the various mapping populations were positionally coincident (in terms of genome position with the QTLs that we detected for yield *per se*. Whether this coincidence turns out to be linkage or pleiotropy is probably irrelevant, given that the highly positive correlation between the two traits is a desirable one.

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Zero tillage challenges and solutions in Brazil

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Abstract

Brazil´s farmers and scientists, having risen to every challenge that ZT has presented, are now world leaders in this technology. ZT conditions are more environmentally friendly than conventional tillage and favour some diseases, pests and weeds, but reduce the levels of others. Therefore, ZT requires a holistic approach towards crop rotations, biological controls and environmental impacts, where farmer-led research priorities lead to quicker and more cost effective results. The public image of the farmer as " Steward of the Land" is crucial to receiving environmental services payments. Farmers and their organisations can contribute considerably to research, especially if they have some effective control in deciding research priorities.
A list of research topics is presented in key areas for future development
Key words: Zero Tillage, farmer-led priorities, biological controls; environmental services payments.

Areas of challenge for zero tillage in Brazil

Introduction

The Zero Tillage (ZT) revelation has outpaced the old Green Revolution in terms of sustainable agriculture and its practice has engendered a holistic view of farming systems, emphasizing pluri-annual crop rotations, which complicates research. For this reason, but also due to the individual characteristics of each farm and an evolution of the technical level of Brazil´s farmers, systems need to be tailor-made on-farm. Research must present building blocks (e.g. functional crop successions or IPM recommendations) to local technical advisers and farmers for them to synthesise these local systems; Brazil has at least one consultant agronomist and/or extensionist in most agricultural municipalities and today many farmers are agronomists. Also, the farmer-led Brazilian Zero Tillage movement, headed by the Federação Brasileira de Plantio Direto na Palha (FEBRAPDP) and supported by the Associação de Plantio Direto no Cerrado (APDC) and a network of Clubes Amigos da Terra and farmer research/extension foundations represents a formidable level of grass-roots support for research, especially on-farm.

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The ZT soil environment is radically different from that of conventional tillage (CT). Higher moisture and oxygen levels, more organic detritus, micro-organisms/soil meso-fauna no longer exposed to sterilization by the sun´s rays and more amenable soil temperatures for life processes, translate into much more intense biological activity. This has proved negative in favouring certain diseases and pests (e.g. late season leaf diseases) and given positive controls in others (e.g. *Sclerotinia scleriotorum and Rhizoctonia* by reduced spore dispersal and antagonic actions of other fungi (Nasser et al., 2001) and reduced attacks of *Elasmopalpus lignocellus* stem borer, which is favoured by bare soil (João Celso Rauber, Formosa-GO, personal communication). The same is true of weeds – those inhibited by mulch cover or with little capacity to germinate on the surface, tend to disappear and others predominate, requiring new control strategies. This emphasizes the great importance of residue generation, where the contribution of soybeans is inadequate under mono-cropping´. It is necessary to prove to farmers the additional costs, in terms of the extra controls required, when not generating adequate biomass in their systems : average annual biomass targets should be established.. ZT was fundamental to the introduction of true pluri-annual crop rotations and the biological controls of weeds, pests and insects, which these generate. It also generated a sea-change in farmers´ perception, making them more system-oriented and environmentally responsible.

But the complex interactions of soil flora and fauna in different crop successions is a determinant for the yield level of the following crop, as shown by the results of Gianluppi (2002) in Roraima, which also illustrate the principle of rotating different crop families. Broch et al. (1997) from the Fundação MS have developed a table of recommendations for the most appropriate crop successions for soybeans and other crops, principally taking into account disease aspects. This could be expanded and further disseminated. But these are the outward manifestations.

What are the scientific reasons for these differences and how can we harness them? Vilela et al. (2002???) have shown that a pasture phase in the soybean rotation can generate a tenfold reduction in parasitic nematode populations, increase the availability of soil P, increase the water-stable aggregates and increase the biodiversity of arbuscular mycorrhiza, which improve the absorption of plant nutrients. This is one of the reasons why the next quantum leap in Brazilian ZT technology is under way : integrated crop x pasture rotations are showing surprising gains in productivity and lower production costs of both enterprises. A pioneer farmer in MS has shown that a one year ley is more efficient than a two or three year ley, since with an exceptionally high carrying capacity, he can maintain 75% of

his area in crops, as opposed to 68% with a two year ley, and still fatten 1000 head on 290 ha of pasture (Ake van de Vinne – personal communication, 2003 and Broch et al., 1997), The *Brachiaria* genus of pasture grasses, which features prominently in these rotations, has shown a remarkable potential for reducing *Fusarium* sp. and other soil diseases, plus regeneration of soil structure and re-cycling of nutrients: the *Panicum* genus may also have these properties.

New challenges, like Asian Rust, Stem Canker and new weeds and pests will always present sudden plant breeding and field control challenges. Investment is required in pre-empting such disasters with acquisition of new resistant material, gene transfers of resistance and testing of agricultural chemicals. So far, Brazil´s public and private sector plant breeders have risen magnificently to the challenges posed by ZT, but they need support to invest in the future, in order to pre-empt the negation of the tremendous gains of ZT as a system. Besides plant breeding, other means of biological controls need to be addressed, with further development of practical guidelines for farmer implantation. Why have farmers been so slow to adopt the use of parasitic wasps and yet embrace IPM more readily? A commercial enterprise has recently changed tack from selling eggs to teaching farmers to rear and multiply the wasps. And how is it that Herbert Bartz (Brazilian ZT pioneer) has not applied insecticide over his soybeans for 18 years but has few followers? Are the bottlenecks located in extension guidelines, perception of physical and financial results, practical management difficulties or in supply/cost problems? There is a need for more anthropological studies on farmers´ attitudes to new technology so that researchers can direct themselves to non-technical limitations, which hamper the uptake of good research.

Table 1. Effects of preceding cover crop on soybean yields

Preceding cover crop	Yield (kg/ha)
Brachiaria	3775
Native grass + *Stylosanthes guyanensis* cv. Lavrado	3632
Pigeon pea	2815
Stylosanthes guyanensis cv. Lavrado	2828

Source: Gianluppi (2002).

Social considerations

ZT has annual impacts outside the farm gate of over US$ 1 billion per year (Landers et al., 2001), directly reducing the cost of living (cheaper farm

products) and, indirectly, by reducing social costs, e.g. silting of hydro-electric reservoirs, massive carbon sequestration and a plethora of other impacts. But the public doesn´t know. Chicken and pork comes from supermarkets, nobody sees the soybean meal! In order to generate public policies to reward the farmer for his environmental services as Steward of the Land, we urgently need economic and environmental impact studies comparing the sustainability and cost-effectiveness of ZT, conventional tillage (CT) and organic agriculture (OA) in terms of producing agricultural products to supply an increasing world demand. Indicators such as the cost/ton produced (tp), nutrients consumed/tp, irrigation water/tp and the SOM balance/tp need to be standard practice in research and economic evaluations. While organic farming is subliminally considered as sustainable, this is not always true, given the difficulties of maintaining fertility and naturally controlling weeds, diseases and pests. There is much woolly thinking about organics, which are laudable but only represent a niche market for the rich, because they cost more to produce (the real economic costs of fallows and imported organic matter need to be computed). Nobody in their right mind would be against organics, but the trade-offs must be evaluated, especially when erosion-provoking CT is employed. In the United Kingdom, an affluent society, organics only represent 1.5% of total food sales (The Economist Dec. 13 – 19, 2003). But the proponents of organics are vociferous and often decry the use of (very low impact) herbicides in ZT, instead of analysing the net total benefits to the environment and society. This distorts the decisions of policy-makers away from supporting ZT, a proven, cost effective solution. Supporting this practice as a BMP (or as a half brother and not an illegitimate one), but possibly not an IMP (ideal management practice) would do good for both sides. Where economic research is now required is in environmental accounting for all farm practices and into the pros and cons of different incentive mechanisms to farmers, to adopt more ZT and continue practising it, especially ICLZT systems, which can be an important factor in mitigation of de-forestation (Landers and Freitas, 2001 and Landers et al., 2001).

Harnessing the farmers

First of all, focus. The social capital of these inquisitive ZT farmers is tremendous. Let the farmers decide ZT research priorities, with control over the allocation of say, 50% of all official research funds : who is this research for, anyway?. The other 50% would be for more basic research, mostly underpinning the farmers´ priorities, in areas where scientists have a better insight. With such devolution can be expected financial support. Our Brazilian Clubes Amigos da Terra (CATs) are carrying out on-farm research with large plots and farm equipment and this means immediate

adoption of results, without wasting time on large-scale validation of small plot results. Farmer foundations also locally and sub-regionally carry out technology development and dissemination, with much on-farm experimentation. Often they are picking up on practical aspects of on-station research, or it is their own initiative, as with the introduction of double-row planting by Homero Fuzaro, member of CAT Uberlândia-MG or the development of an implement to incorporate broadcast cover crop seed without disturbing the residue, or the use of maize intercropped in sugar cane as a soil cover to control weeds (F. Cardoso personal communication). Strategic collaboration of these farmer organizations with state, university and federal government research generates scarce operating funds for researchers to achieve useful results. This is an effective supplement to overstretched official budgets, raised from private sector grants and farmer contributions. But the *quid pro quo* is that the priorities are set by farmers and this leads to very cost-effective and quick results. Finally, if experiments were analysed at the 10% error level, many results would be clearer to farmers and technicians – this is a risk level which they can easily handle and is especially justified with on-farm experiments with farmer equipment, which eliminate the need for technology validation trials.

Solutions

This section is a pot-pourri of areas of potential for research, presented as a brain-storming effort in the form of the list which follows, with little elucubridation and in only an approximate order of priority:

1) Sources of resistance to Asian Soybean Rust - the comparative risks of infection under ZT and CT need to be assessed;
2) trap crops e.g. the planting of an early-flowering variety at field edges which would attract pests and be selectively sprayed, or for small farmers, the push-pull effect as used in Africa, with trap crops plus repellent crops;
3) improved over-sowing technology with seed coatings for ICLZT systems: (i) improved moisture retention, (ii) critical nutrition, (iii) protection against diseases and pests and (iv) delayed germination;
4) disease resistance and avoidance for Amazônia where disease pressure under ZT is even higher;
5) better understanding of release and fixation mechanisms under ZT and comparison of NPK fertilisation and liming efficiency with CT and OA;
6) improved yields for early-planted short season varieties to improve second crop cash and/or biomass generation via earlier planting;
7) a resumé of ZT research results in soybeans to upgrade the farmers´ technical knowledge of ZT and awareness of the environmental services he provides, so as to encourage: (i) a long-term view of investments in soil improvement, and (ii) assumption of the role of "Steward of the Land";

8) studies to facilitate labelling of ZT products as "Sustainable";
9) easily-adopted biological control methods for shield bugs and other pests where these are currently not effectively available and IPM for cover crops;
10) testing of new planter mechanism e.g. cross slot, with a view to placing the fertilizer to the side of the seed instead of directly below and better moisture conservation with less disturbance of residues;
11) continued public comparative evaluation of planters and other equipment to provide farmers with unbiased data for comparison;
12) definition of soil conditions which allow use of double fertilizer discs instead of the high-power-requirement shanks and boots commonly used, detrimental to soil cover;
13) better definition of the effects of plant populations x yield and disease incidence, uniform in-row spacing/emergence, double-row planting and seed depth control, with evaluation of devices such as seed firmers, under regional/local conditions;
14) further development of the concept of fertilising the system, with simple research-based criteria, including the elucidation of the availability of micronutrients and broadcast P, as affected by broadcast application of maintenance liming, with recommendations for minimising fixation;
15) new technology using permanent cover crops and the attendant competition-checking practices, such as safe sub-doses of herbicides or mechanical means;
16) revision of soil capability classes (developed for CT) to incorporate ZT soil management, which will reduce erodibility and improve agronomic performance, besides bringing marginal textures and slopes into the aptitude class for annual crops;
17) an extension publication resuming the comparative risks of disease, pest and weed species under both ZT and CT situations and different crop successions;
18) widespread screening of allelopathic and suppressant actions of crops, cover crops (and weeds?) as a means to reduce herbicide dependence – this must initially be a broad spectrum, low intensity evaluation to separate species with high potential;
19) alternative summer crops to soybeans to break weed/disease/pest cycles, diversify risks and increase total biomass generation;
20) a holistic farm model economic analyis, which shows returns to the system, crop succession interrelationships and the returns to winter pasture on the crop area, taking into account loss of yield through loss of biomass;
21) nematode control with cover crops or parasites and slug control with parasitic nematodes or other biological means (more important in ZT under irrigation);

22) selection/breeding of new rhizobia strains with high fixing capacity at the time of maximum N utilization by new higher-yielding varieties;
23) alternative application technologies for separating seed treatment chemicals from inoculant e.g. furrow application.
24) effectiveness of insecticide application concomitant with dessication;
25) evaluation of cocktails of cover crops and their effects on the subsequent soybean crop;
26) quantification of drought resistance mechanisms engendered by ZT ; (i) reduced evaporation less from soil surface, (ii) increased infiltration of rainfall, (iii) increased water-holding capacity due to higher SOM and (iv) ratio of yield/m^3 water consumed;
27) definition of control measures for root-eating white grubs ("Corós") e.g. *Phyllofaga* spp. and elucidation of disease epidemics in beneficial species;
28) Better definition of the epidemiology of *Macrophomina sp.* in soybeans and evaluation of biological controls e.g. with *Brachiaria spp.*or antagonic fungi
29) use of inoculation with residue-digestion-efficient bacteria and/or fungi to liberate nutrients at the time of maximum crop growth;
30) idem with P-solubilizing bacteria and/or fungi;;
31) use of worm counts to indicate soil health and pesticide residue levels

Conclusion

Without farmers, agricultural research would be meaningless; without research, farming would be rudderless. If given a greater degree of control over research priorities, farmers would participate more actively in fund-raising and in on-farm research. Good farmers are also good observers and always present in the field – they are a very useful resource for research scientists. The list of research topics is a contribution to the discussion of research priorities.

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The no till cropping system and its evolution toward the achievement of the moshpa model principles (modern sustainable high productivity agricultural model)

R. A. Peiretti[1]

Abstract

During the last twenty years and as a result of a quest to improve cropping efficiency, and to counteract soil erosion and deterioration, the Argentinean producers, introduced, improved and extensively adopted the No Till cropping system principles. The No Till approach is inspired in emulate nature as much as possible regarding those plant growth basic mechanism on which the cropping and general farming processes are pillared on. No Till is a cropping system aimed to improve the agro-ecosystem functioning protecting soils against erosion and degradation by means of avoiding soil tillage and keeping the topsoil covered with crop residues -and living plants- as much, and as longer as possible.. Utilizing No Till and the consequent soil and agro-ecosystem "health" improvement as a general framework, the Argentinean cropping system evolved toward the achievement of the MOSHPA model goals: namely a higher level of productivity, profitability and competitiveness within the short term, and a proper level of sustainability within the medium-long run. This goals are very much in coincidence with those pertaining to Conservation Agriculture.The result was a deep transformation of the Argentinean grain production and general farming system. Within the analysis of this farming evolution, the Argentinean agro-biotechnology adoption and the soybean production case are considered to better exemplify the agricultural transformation and evolution process.

Keywords: No Till, tillage avoidance, soil covered, erosion control, stubble, biotechnology, MOSHPA, productivity, competitiveness, sustainability.

Introduction

"The world is not globalizing but instead it is already functioning as a global unit". Based on this assumption, and heavily exposed to the international and internal challenges and opportunities, the Argentinean farmers started to work toward the counteraction of the challenges and at the same time attempting to take advantages of the opportunities. The

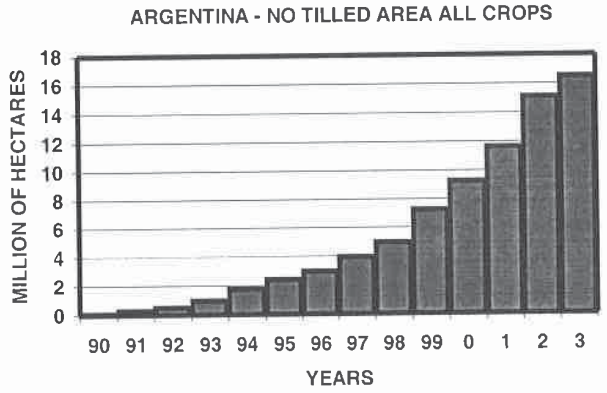
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main challenges or pressures were (and are) basically derived from: 1.) a strong economic pressure originated on both an international subsidized competition and a domestic very high and somehow farming sector discriminatory taxation policy; and 2.) an unavoidable necessity to overcome the serious soil -and agro-ecosystem- degradation mainly caused by the erosion derived from the conventional tillage on which the intensification of agriculture was based on the past. The traditional or conventional till practice is to disturb the soil surface by plowing and denude of vegetation from the fallow field, rather than to keep it covered with vegetation or crop residues as is done when no-till is practiced. Plowing the land, from the standpoint of soil structure and its biological environment is "equivalent to an earthquake". Through this operation, the topsoil –in most of the cases and in terms of soil science terminology, the "A" layer, is turned upside down. All existing vegetation is killed by burring it together with previous crop residues. (Peiretti, Roberto A., 2001). Among other negative impacts, soil tillage (based on plough and other tillage tools) denudates soil and pulverize it heavily exposing the resulting "bared topsoil" to the rain and wind forces that act as strong eroding forces. Also, this procedure normally decreases water infiltration capacity, increases water run off and consequent eroding force, increases water contamination, decreases soil organic matter by an enhanced oxidation that finally decreases soil organic matter and liberates carbon to the atmosphere, etc. At the bottom line, the process ends degrading agro-ecosystem and soil quality and fertility understanding it as the basic ability or aptitude to produce. Focusing on the opportunities, it was first clearly recognized that they always were, are, and will be, located on the future but never in the past. The possibility to increase total production and supply both the domestic and the international market was perceived by the Argentinean farmers as an important opportunity. To be able to take advantage of this opportunity, and also to be able to sustain it, it was understood that the simultaneous achievement of a higher level of productivity, profitability and competitiveness on the short run, and a proper level of sustainability -and even improvement- of the natural, economic and human resources involved in the process, constituted a must. The starting point for the new cropping/farming model have to be located on the reversion of the serious soil deterioration originated on the cropping intensification based on conventional tillage that had taken place specially during the second part of last century. No-till, the farming system introduced first in the United States in the 1960, that replaces traditional plowing and general soil tilling, was recognized and seen as the basic tool. The biggest difference separating different cropping/farming systems involves tilling the land or avoiding tillage. Under No Till, instead of tilling/ploughing the land, after harvesting a crop, residues are left on the field to protect the soil against erosion and to obtain other benefits. When it comes time to plant the new crop, special machinery cut a very thin groove into which

the seed and fertilizer are dropped. This groove is then covered by the same machine. Weeds are controlled through the simultaneous application of herbicides and integrated weed management strategies derived, among others, from rotational principles, soil seed bank managerial strategies, allelophatic effects; etc. Similar criteria are also applied to control insects and diseases. Finally, the adoption of No Till as the general framework, and the strong development and utilization of the MOSHPA Model principles are in coincidence with Conservation Agriculture principles and represented the "simultaneous valid and realistic answer" they found to "properly satisfy" both the short, and the medium and long run necessities or model constrains.

The Argentinean no till adoption process

The first trials started on the seventies; however, it was not until after the end of the eighties, and beginning of the nineties, that the adoption process boomed. The evolution of the adoption process can be seen on next graphic N° 1. From a couple of hundred thousand hectares in 1990; the adoption jumped to more than sixteen millions hectares, which accounts for around 65 % of the Argentinean grain cropped area.



The drillers and planters improvement allowed to properly and efficiently operate under a no tilled and mulch covered soil condition. Among other factors, the irruption of a new generation of better herbicides and the improvement of other managerial strategies are some of the important facts that allowed the No Till adoption process to take place. However, a clear farmer proactive attitude was a key factor and allowed them to realize that the No Till cropping system offered a true possibility to simultaneously evolve to a superior cropping/farming system. This fact, definitely should be considered as one of the main driving forces that shaped the adoption process. The strong domestic and international interactions among farmers

promoted by CAAPAS (American Confederation of Organizations for a Sustainable Agriculture) and developed by farmers that belongs to AAPRESID (Argentinean No Till Farmers Association) and to similar institutions created in other countries, represented a valid mechanism to keep improving, adjusting, evolving, and scaling up, the No Till and MOSHPA model principles adoption in America all, and in other parts of the world. (Peiretti, Roberto A., 2003)

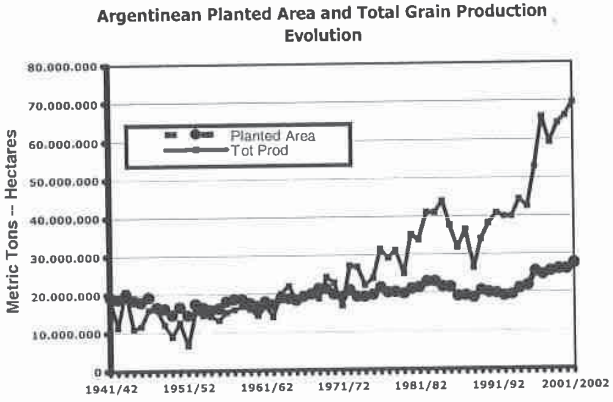
The new Argentinean farming approach

The Argentinean farmers were aiming, and found in No Till, a valid (realistic and applicable) mechanism to improve productivity and profit but at the same time to counteract the evident soil erosion (water and wind born) and degradation process, that was becoming more and more evident and worrisome. (Casas, Roberto R. 1997), (Buschiazzo, D. Aimar, S.B., 1998). From the beginning of the farming transformation process the leader farmers started to look at the soil as "A marvelous lab, and as an entity able to contain and sustain the extraordinary complex phenomenon up to the present time only known in planet earth: LIFE". Also a somehow revalorized and more realistic hierarchical array was established: "First human necessity then the environment; however, a sustainable and even improvement of the "relationship between human been and the environment", ought to strictly be considered and achieved to ensure the sustainability of the human future food provision. Based on these principles, an "evolutionary process" of the farming/cropping system was started. At bottom line the whole idea is pivoting in evoluving from a "mining relationship with the environment" to a "more balanced" and sustainable one. However, it always ought to be kept in mind that this goal can only be aimed after matching the unavoidable necessity to increase total production as the only realistic way to properly supply the almost ever increasing human food demand. Also, the total production mainly would have to be obtained trough a productivity increase rather trough a cropping area enlargement. This is absolutely needed as a way to alleviate -or diminish- the necessity to keep expanding the agricultural boundaries and transforming "natural still untouched ecosystems" into agro-ecosystem. Nowadays, most of the "still untouched potential new farming areas" are located on those tropical and sub-tropical forests of the world. Within them, we can find the largest biodiversity reserve on earth, so that, the productivity increase -as a mean to increase total production- turns to be an absolutely strategic approach to keep biodiversity. It constitutes the more "environmentally friendly" way to enlarge total production. (Avery, Dennis T., 1997). Following all this principles, a deep redefinition of the pillars that were going to shape and guide the new Argentinean cropping/ farming system functioning, were introduced. A more systemic, interactive and balanced approach was established as the new framework. A

revalorization of the interactions in front of the individual system component effects, played an important role on the process. This revalorization, do not only relate to the agronomic, agro-ecological and technical matters if not also to the contractual relationship between land owner, farm manager and other actors involved on the complexity of the grain production and general farming process. Based on this innovative farmer attitude, and on a relevant intervention of agronomist and other related professionals, an intensive and deep utilization of the whole spectrum of knowledge were brought into the new farming model. A somehow entirely new cropping and farming system was developed and intensively applied. The "wide scope", "open minded and proactive farmer attitude", allowed a wide spectrum of human knowledge (from agro-ecology to biotechnology, from ancestral knowledge to latest scientific findings) to be accepted, incorporated and utilized. Science, and to some extent pragmatism and empirical knowledge, were widely accepted but ideological and related principles fully neglected. A clear shift from the farmer typical figure (operating their own land with their own machinery mostly based on traditional knowledge), to a sort of entrepreneurial array that in most cases operates rented land and contracts and have custom made services of all types (from the drilling/ planting operation to the harvesting and grain hauling ones), constituted an important tool for the Argentinean farming sector transformation and evolution. Within this different farming sector array, the best of each system actor, and factor, were enhanced and offered an opportunity of be fully expressed. This change yielded significant benefits from all standpoints. The transformation was very deep so that, nowadays, around sixty five to seventy percent of the grains produced in Argentina are been obtained mostly under No Till with a much higher efficiency by non-land tenants (farmers that operates rented land) that use rented (custom made) machinery and other services of all types.(Ordoñez Hector, 2003. Personal Communication). This "evolved attitude" characterized the cropping and farming system transformation. It yielded significant benefits that even with different intensity reached all the farm size and type of farming operation spectrum; from those large scale market oriented ones to the small scale mostly subsistence operations. Also, it is worth to be mentioned that leader domestic machinery factories working together with leader farmers, made a big effort and a significant contribution designing, developing and supplying a new generation of very efficient drillers and planters specially adapted to work under No Till and soil covered condition, which at the bottom line is also a key factor to be able to adopt and properly apply the No Till principles. All this transformation allowed to achieve a significant increase in productivity, economic competitiveness and sustainability to the Argentinean grain production and general farming sector functioning. (Peiretti, Roberto A., 2001).

About other benefits

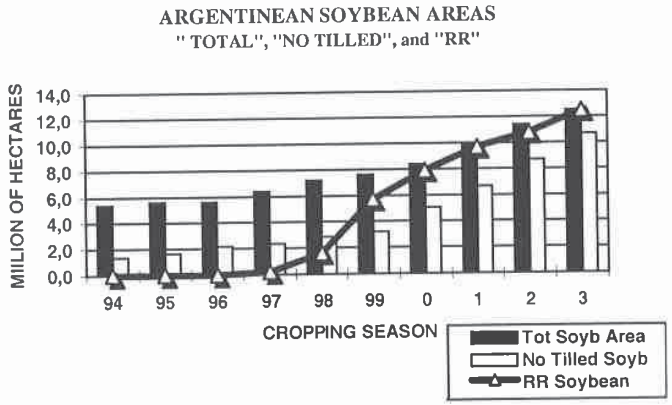
Under a properly implemented No Till farming/cropping operation, most of the agro-ecosystem key functioning factors are significantly improved. (Beck, Dwayne L., et.al., 1998). Both wind and water soil erosion can be almost totally controlled by the No Till approach. Mainly due to a great reduction of the water run off and water born soil erosion, the silt and nutrient contamination of creeks, rivers, water reservoirs etc, are significantly reduced and controlled when the adoption happens at a regional level and is accompanied of other actions. (Papendick R.I., 1995). Water management is also an issue largely improved. The soil water capturing and saving capacity are significantly enhanced. For a given crop, rainfall and evapo-transpiration condition, several "extra millimeters of soil available water" are added. Under No Till, and for a given rainfall and evapo-transpiration condition, according to some central Argentinean area measurements, sixty millimeters of soil available water for the crop were added. (Dardanelly J., 1998) .It was also measured a significant reduction of the amount of millimeters needed to produce a given amount of grain. At the bottom line, it means an increase of the water use efficiency by the crop. This and other related phenomena allow the cropping system to achieve a higher buffering capacity in from of the very common water stress that regularly happens under rain-feed crop condition. Related to the soil organic matter issue -specially when located on temperate climate- an increase of it is commonly found under No Till cropping operated agro-ecosystems. (Lal, R., 1998(a) and (b)). This phenomenon correlates with a soil C fixation and positively interacts and enhance many biochemical and chemical phenomena that have to be with a "better and healthier" soil and agro-ecosystem functioning. Also, the utilization of a proper crop rotation, and the principle of "feeding the soil rather than fertilizing a crop", greatly enhanced several beneficial biological processes. Some allelophatic effects utilized to control weeds, as well as some symbiotic interactions, as legume-rhizobium for nitrogen fixation and others; constitutes only a few examples of this type of benefic related effects. All these phenomena together with the "structural changes" and with the better economic domestic environment that prevailed in Argentina during the nineties, yielded a significant increase of the total production in a sustainable manner. Focusing on next graphic that includes the area cropped and the evolution of total Argentinean grain production between 1941/42 and 2001/02; we can see how much the total production grew especially during the last fifteen years in coincidence with cropping/ farming system transformation.



Even so new areas were incorporated into the cropping system, the biggest part of the explanation for the total production increase can be found in a relevant productivity increase. The productivity increase (paired with a significant reduction in production costs of all type), allowed a profitability and competitiveness improvement, but now obtained within a sustainable frame.

No till, biotechnology and the Argentinean soybean case

Agro-biotechnology that was introduced in Argentina in 1996, as RR soybean also played an important role in the process. After 1996, other biotechnological events as Bt corn, Bt cotton, and others, were also introduced and followed a steady farmer acceptance and adoption.



A positive correlation between "No Till" and "biotechnology", in this case with soybean, can be clearly detected by the analysis of upper graphic N° 3; and a kind of synergy between both strongly suspected. This synergy speeded up the adoption rate, and yielded economic and environmental benefits. The control of insects, weeds, and diseases by a combination of pesticides and biological means as biotechnology, rotations and other related strategies represents and offer us a clear example of a favorable trend that at the bottom line allows to reduce costs, and to generate farm products obtained trough a much more environmentally friendlier farming process. Furthermore, they are obtained not paying a penalty in terms of productivity (as other approaches like the organic one implies), if not with an increased productivity.

Conclusions

A few years ago, Dr. Norman Borlaug, the agronomist's father of the Green Revolution and Novel Peace Price Laureate 1970, stated: "One of the most serious challenges that humanity will be faced with during XXI century, will be to develop the capacity to produce enough food but conserving the environment at the same time". Most of the actions and phenomena associated with the Argentinean grain/farming production evolution are oriented to offer a "valid and realistic" answer to this challenge. Even it should not be considered as a final stage because surely it will keep evolving on the future; it clearly constitutes a proper reaction of the Argentinean farmers to both the challenges imposed and the opportunities offered by the globalization process. Until after other attempts can prove in reality to be superior to the one described in this paper, it could be consider as a successful attempt and could be followed by other farmers of different regions of the world to further improve the world grain and farming production system.

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No-tillage challenges on multiple cultivating systems of soybean production in China for today and tomorrow

W. Li[1]; J. Wang[1]; Z. Lu[2] and J. Wang[3]

General situation of no-tillage agriculture in China

Over thousands years, tillage agriculture have played an overriding role in the traditional farms of China, Including the manipulation of plough, harrow and mid-plough. One of the advantages of the tillage system is the inhibition of weed and insect in soil. However, plough system is considered to be an inefficient use of time and fuel and caused much "wear and tear" to the machines (Waydelin, 1994). The unavoidable negative effects of intensive and repeated soil tillage in China are apparent on soil loss, diminishing of organic matter content, soil erosion, ecological environment (such as city slums and marginal populations), soil moisture, water infiltration, soil microbial population, loss of nutrients, and the degradation of soil physical structure. These results lead to a poor productivity of soil and poor yields over time. Soil in many places of China appeared to be poor, out of water and low nutrition. In order to maintain and improve soil fertility and water supplement no-till system possesses a specific implication for the sustainable agriculture of China.

No-till and reduced tillage have been used since ancient times by indigenous cultures, simply because man has not the muscle force to till any significant area of land to a significant depth by hand. As long as the advent of modern herbicides, no-till agriculture initiated in the United States in which the no-till field has reached one third of its total acreage. Since 1960s, no-till agriculture has been widely adopted in Canada, Brazil, Paraguay, Australia, Russia, India, etcÿ but there is no official data about no-till cultivation in China by FAO. Regardless of this information gap, the study and practice of conservative tillage is actually practiced in China since centuries ago. In the book of 'Essential Elements for the People in Qi Dynasty (479-502)', no-tillage is defined as 'cultivation method of preserving moisture of soil'. In the years of the Post-Wei Dynasty (220-265), soybean and wheat were planted with no-till. Up to the years of the Ming Dynasty (1368-1644) and the Qing Dynasty (1644-1911), no-till cultivation was expanded to rice. However, the no-tillage assembled by modern techniques

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such as machines, herbicides and pesticides emerged in China around 1970s. Since 1992, a broad practice of no-tillage in Sanxi Province was undertaken and now extended to 31 counties containing 20 thousand hectares, yield increased 15-30%, the cost of manipulation was only 60% of tillage cultivation, soil organic matter content increased 0.05%-0.06%, soil water increased 24%-35%, management expenses reduced 60% (Xiao et al. 2001). In Inner Mongolia, no-till is extended to 25 counties in 2003. Results indicated that no-till benefits the increment of soil water up to 20%. The less-till and zero-till fields in China increased to 13 million hectares in 1990s. The characteristics of conservative cultivation system include the utilizations of deep-loosening soil and the feedback of straw into soil. In China, there is a very big potential to bring this soil conserving technology to this part of the world, especially in western and north parts of China, although limiting climatic and socio-economic factors have to be taken into account.

The study and practice of no-tillage soybean in China

Soybean originated in northeastern China and was domesticated about 2500 B.C. in China. As a traditional crop soybean is the fourth in production after rice, wheat and corn, and third in value after rice and corn in China. The production of high quality soybeans is concentrated in the three Provinces of north part of China, e.g. Heilongjiang, Jilin and Liaoning. Of them Heilonjiang Province alone possesses one third acreage for soybean production and produces 50 percentage of total soybeans in China.

In Chinese crop cultivation history, soybean cultivation was centered by the tillage system of plough, harrow and mid-plough combination. Recently, as long as the over exploitation of field and the frequent ploughs, the soil structure was seriously damaged with the loss of soil and water (especially in western and north parts of China), deficiency of organic nutrition, which addresses the decrement of soybean yield over years (Ju 1995). To improve soil condition and environmental condition and to increase soybean yield and seed quality, no-tillage has been proved to be a critical technology.

There have been some studies and practices on the no-tillage and minimum tillage system for soybean in China. The major Provinces that adopted no-tillage for soybean production include Sanxi, Sandong, Hebei, Inner Mongolia, Jiangshu and Heilongjiang (Ma et. al., 2002). The country-wide practice indicated that soybean yield could be increased for 22.8%-30% by the adoption of no-till technique (Xie 1995; Chen 1998). The advantages and benefits from this system were in many other cases observed in terms of labor/management costs, soil erosion, and soil nutrition and water maintenance.

The major types of no-tillage for soybean production in China

For spring soybean production

• Planting on the originated rows without plow

Plant residues of corn or wheat were shattered by machine and the rows were recovered simultaneously by a ploughshare. Subsequently, soybean was sowed on the rows.

• Planting soybean in double lines beside corn residues

After harvesting corn, the residues were left in the field. In next spring double lines of soybeans were planted in both sides of the corn residues (Fig. 1).

• Planting soybean without row and with changed direction

After the harvest of corn that was grown without rows, soil was retained without plow. In next spring soybean was planted in the direction of 30° angle against the planting direction of last year (Fig. 2). The distance between the two lines was 35-45cm. In the third year, wheat was planted instead of corn and soybean.

For summer soybean production

In central of China, after the harvest of winter wheat, soybean was sowed on the wheat field directly without plow by the grain drill with airflow system and the residue-shattering system. The distance between rows was settled as 35-40cm.

For fall soybean production

In southern China, fall soybean is usually seeded in the dry season. The soil water is a limited factor for the effective germination of soybean. To solve this hinder, after the harvest of rice or 15-20 days earlier before harvesting, soybean is sowed in the middle of two rows directly without plow. The distances between rows and seeds are 36cm and 18cm respectively.

Reducing labor/management costs

Conventional plough system for soybean production includes a complicated procession, e.g. plough, harrow, mid-plough and the preparation of rows. More cost was spent on the usage of mechanisms and fuel. However, 40-80% cost on it could be reduced by adopting no-till or minimum till system (Table 1).

Controlling soil erosion

Soil loss is a serious problem in western China and north China. Specially, in Heilongjiang Province, the plough was followed by the windy seasons in

fall and spring, which caused a huge loss of surface soil and fertility. Xiao et al (2001) compared the loss of soil and fertility after the use of tillage and no-tillage operation.

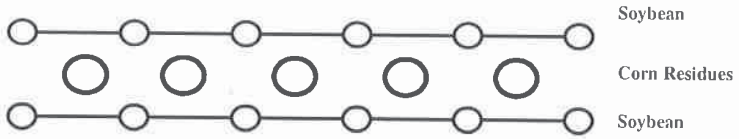


Figure 1. Planting soybean in double lines beside corn residues

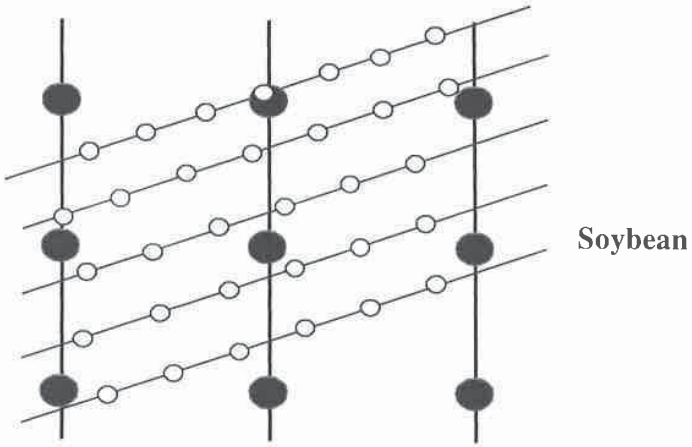


Figure 2. Planting soybean without row and with changed direction

Table 1. The expenses on the usage of mechanism and fuel for till and no-till systems (one hectare)

Tillage System	Cost of Mechanism (USD)	Cost of Fuel (USD)
Conventional Tillage	46.87	3.75
Minimum Tillage	28.13	2.25
No-Tillage	9.38	0.56

The results illustrated that soil loss from conventional tillage was 7.1 fold higher than the loss from minimum tillage and that the loss of soil was zero from no-tillage. Meanwhile, the loss of fertility from conventional tillage was 7.1 fold higher than minimum tillage (Table 2).

Table 2. The comparison of tillage and less-tillage on the loss of soil and fertility

Tillage System	Weight of Soil (Kg/hm^2)	Erosion N	Loss of Fertility (Kg/Hectare) P_2O_5	Organic
Conventional Tillage	74250.0	150.0	142.5	3341.3
Minimum Tillage	10424.8	21.1	20.0	468.1
No-Tillage	0	0	0	0

Varieties that is suitable for no-till cultivation

Weed, disease, insect and low soil temperature are the weakness for no-till system. The soybean varieties that is used in no-till system in China popularly possess the characteristics of earlier maturity, broad adaptation, resistant to insects and diseases, resistance to lodging, high yield potential. Some qualified varieties include Zhao Shu 18, Hefeng 25, Jidou 12, 9588, Ludou 10, Sanning 4.

The controls of weed and insect for no-till soybean

The practice of no-till in soybean caused the increase of insect harmfulness in certain areas. Ma (1997) introduced an effective method to control insects. That uses mixture of the phoxim and wheat bran and applied on soil at evening (122.5kg/ha). In addition, the control of weeds is as well an important task in no-till soybean production. The popular herbicides that is broadly utilized in soybean no-till include: 1) 300g of 50% acetochior plus 60g of 20% chlorimuron per hectare; 2) Soil regulator 'New Century' 3000g per hectare that is effective for the control of weeds and the softening of soil.

Constraints for no-till soybean adoption in China and the prospect

As it is known, China has a long traditional agriculture history. It cost time to change the mind of farmers to switch to the no-till agriculture system. The slow development of no-tillage soybean production in China has been closely related to: the shortage of appropriate no-till machines at adequate price; the shortage of efficient low-cost herbicides and pesticides; the availability of appropriate knowledge under different agro-ecological and socio-economic conditions; the shortage of no-till research and the specific knowledge of the no-till system; requiring of important policy and institutional

reforms and more financial support from Government. Meanwhile, before farmers accept no-till agriculture in China completely, it is necessary to have an effective method to control the soil and environmental pollutions from the application of herbicides. Besides, In Northeast of China, the relatively lower temperature of soil caused by no-till is another constraint for the extension of this new cultivation system in China. Despite of these restrictions, no-till, as one of the biggest breakthroughs in agricultural technology of 20's century, is the most competent cultivation system in the world for the sustainable agriculture. Consequently, no-till soybean production will lead all other systems as a main cultivation system in this century.

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No tillage challenges and solutions for soybeans in Australia

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Abstract

Increased acceptance of no-till and controlled traffic has renewed interest in soybeans in Australia. This papers reviews how soybeans are being utilised in three farming systems in Australia that are dominated by sugarcane, perennial pasture and rice. The farming systems, termed tropical coastal sugarcane, subtropical coastal beef and Murrumbidgee irrigated have had complete or extended periods of monoculture. Resultant soil health and disease problems have caused yield decline comparatively to uncultivated soil. Soybeans are an attractive "break" crop that is being used for green manure or grain production. As a grain crop it can provide an alternative income to subsidise the cost of land improvement techniques. With the adoption of no-till techniques establishment costs are reduced, crop frequency increased and land sustainability improved.

Introduction

Australian soybean production peaked at 130,000 kt in 1988-89. Production has always been variable and well below expectations for an industry that started in the 1970's. Soybeans have never become a dominant crop within a farming system like wheat in dry land areas, cotton or rice in irrigated areas or sugar cane in coastal areas. However in the last 10 years interest in growing soybeans has moved away from a 'competitive' main crop to a 'complimentary' rotation crop. Researchers and leading farmers have always acknowledged the benefits of including legumes in rotations. However, the availability of no-till and controlled traffic techniques has changed the outlook for soybeans as they can be easily established and harvested within a dominant crop rotation.

At the same time oil availability in Australia has increased with the development of canola as a break crop in the inland wheat cropping areas of Australia. As a consequence crushing soybean for oil was reduced and centralised to Brisbane and Newcastle. Soybean is now a less profitable crop to grow where freight costs have substantially increased. This has necessitated a change in marketing direction and soybeans are now being grown for specialised human food uses. Soybean breeding and marketing is now focused on colourless hilum soybeans used for milk, tofu.

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The industry challenge now is to revitalise grower interest by focusing research and development opportunities in production areas where soybeans are a preferred break crop or where production for a niche market can be sustained over a long period. Specifically, the utilisation of soybeans under no-tillage is reviewed within three farming systems in Australia. See Table 1.

Tropical Sugarcane

Background – Sugarcane production is confined to about 450,000 ha of land located along the coast of Queensland and northern NSW. In most districts sugarcane is grown largely as a monoculture. Normally 4-5 ratoon crops are produced before the stool is ploughed out. Since the 1970's a system of plough out/ replant has been employed, resulting in virtually no fallow period. There is extensive use of inorganic fertilisers, insecticides and herbicides. As well, the technique of green cane harvesting has increased soil compaction through the use of heavy machinery. There is a soil based production constraint referred to as yield decline (Y.D).

Farming System Rotation – In order to improve sugarcane productivity a legume rotation 'break' crop is recommended at the end of the cropping or ratoon cycle to reduce detrimental biota and restore soil fertility. Soybeans have proved to be the best legume available to use in terms of both dry matter production and nitrogen input. Very little else changes in the farming system except cane replanting is delayed by 6 months. Adoption of soybean first occurred on the NSW North Coast and more recently on the Southern Qld coast. Research has also clearly shown that in high rainfall environments it is best to leave plant residues on the soil surface that soil incorporate to maximise nitrogen carry-over. Sugarcane yield improvement has ranged between 15-25% in trials.

Preferred Crop Establishment – The adoption of no till and controlled traffic has improved cane profitability and reduced soil compaction caused by green cane harvesting. Soybeans can be directly sown into sugarcane stubble which is subsequently removed by herbicides.

Leading growers spray out the old cane crop, use minimum cultivation to develop wheel tracks and then direct plant two or three rows of soybeans to one side of the old stool rows. Usually 1.8m beds are formed. Grass and any volunteer cane control is achieved using an initial spray of a pre-emergent herbicide and a post-emergent grass selective herbicide. Alternatively low rates of a knockdown herbicide are applied with a hooded sprayer. Similar methods to establish sugarcane are then used, although light cultivations take place to prepare seedbeds.

d) Problems Encountered –
- The monoculture of sugarcane has led to pesticide residues (OC) and heavy metal accumulation (cadmium) putting the alternative legume peanuts at risk.
- The wetter tropical north Queensland coast (north of Ingham) has proven more difficult to grow soybeans for grain.
- There is a lack of specialised harvesting equipment and crop management experience in soybeans.
- There is no focused R&D program on soybeans.

Subtropical beef

Background – There is an increasing number of beef producers on the NSW north coast adopting the Beef'n Beans scheme and integrating soybeans into their overall farming system. Beef'n Beans is a pasture improvement program that utilises no-till soybeans. The concept is not a new one and some innovators have had it in place for the last twenty years with very successful results. Beef'n Beans provides an opportunity for beef producers to increase on-farm income and diversification as well as developing a sustainable crop production system. Recent expansion has occurred within the Grafton, Kempsey and Wauchope areas on the NSW north coast.

Farming System Rotation – Soybeans are the engine-room in the Beef'n Beans program. They are no-tilled into run down or unimproved pasture (mostly on soils with low fertility levels) as part of a pasture improvement program. Due to the high rainfall of the region and the opportunity to double crop, the soybean crop is followed by either a forage crop (ryegrass, oats) or a grain crop (barley or triticale) during the cool season. These can be aerially sown into the soybean crop prior to harvesting or direct drilled after harvest. This program is repeated for a period of 2-3 years and then in the final year returned to an improved pasture mix. The final pasture mix may include setaria, Rhodes grass, kikuyu, white clover, lotus or other long term pasture species. The cropping phase is repeated when the pasture becomes rundown and requires revamping, usually after 5-6 years.

All fertiliser, usually phosphorus and potassium, is applied during the soybean cropping phase, which means the return on investment is rapid.

Preferred Crop Establishment – Many of the farms undertaking this program are situated on undulating country and the application of direct drill technology allows cropping to take place in a sustainable manner. With ploughing, this country would be prone to erosion and nutrient decline. Using the Beef'n Beans program this scenario is avoided. The no-till

technology helps to improve/maintain soil structure. Leading growers will establish a pioneer crop, such as oats, in the winter prior to the first soybean crop. This crop as well as all subsequent crops in the rotation will be no-tilled using mostly coulter/tyne combine planters (sometimes triple disc) with narrow rows, 250-350mm apart. Some of the winter crops and pasture mixture will be dropped by aircraft into a senescing soybean crop. At no stage is the ground fully cultivated.

d) Problems Encountered - Whilst the Beef 'n Beans scheme has been utilised very successfully for increased profits in these areas of Australia for a long time, the level of adoption has been affected by a number of factors:
- Low prices for soybean grain and high prices for cattle have a large affect on the new areas under Beef 'n Beans
- Cropping skill levels of traditional cattle producers can dampen the enthusiasm of some to commence a Beef 'n Beans program. However, good advisory services and a large core of experienced soybean growers in the neighbourhood can assist this situation greatly.
- In newer areas the lack of availability of suitable no-till planting equipment for contract work.

Irrigated murrumbidgee

Background - The Murrumbidgee Irrigation Area (MIA) is situated in south west of New South Wales. The Irrigation water is supplied via the Murrumbidgee River and a series of large reservoirs. The main farming enterprise of the MIA has been rice-based farming systems with up to 50,000 hectares been grown in one season. In the past, continuous rice cropping, and rice pasture or winter cereal (followed by a fallow period) were the main rotations used by broad acre irrigators. However in recent times with irrigation water restrictions and thus the effects on price of water/mega-litre, opportunities have arisen for other shorter growing season summer crops such as soybeans. Soybeans have not only proved to be a very profitable crop, it has also proved to be complementary to rice and all other winter cropping rotations. Soybean has helped break disease cycles, improve soil structure and fertility, reduce weed seed-banks, and improve total water use efficiencies.

Farming System Rotation - Rice is grown in flooded bays following the land contours, where as soybeans (and other summer crops) are best grown on raised beds down the slope (across the contour). This allows rapid drainage after irrigation of excess water, and reduces the detrimental effects of water-logging. Paddocks are usually land formed to an ideal slope factor

before summer cropping. Raised beds are generally 1-2 m (depending soil lateral water movement abilities or termed "subbing"). Once established, these beds are left permanently for cropping until rice is regrown (on contour layout). Soybeans are no-tilled into raised beds, generally 2 or 4 plant rows/bed depending on bed width and soil subbing ability.

Following the soybean crop, a winter cereal is again no-tilled into the soybean stubble/ residue. No-till enables the crop to be sown on-time and the paddock cropped 52 weeks a year. Double cropping continues for 2-3 years.

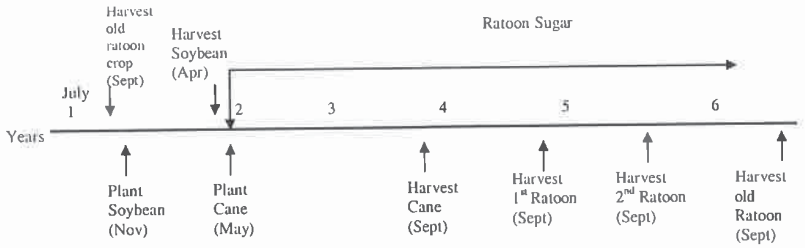
Farming implements and equipment size is generally 6 or 8 rows. Implements are generally all the same size for ease of operation and to enable control traffic or "tram-lining". Tractors and harvesters are fitted with narrow row-crop tyres to reduce furrow and bed shoulder compaction. Harvesters are fitted with open fronts, ideally flexi-fronts to follow the land contours and prevent yield lose and soil contamination of seed.

Preferred Crop Establishment - Soybeans are ideally direct drilled into pre-watered raised beds. Disc-openers are currently the best available cultivating tool for handling large quantities of crop residues and also providing minimal to zero soil disturbance. Either 2 or 4 plant rows per bed depending upon soil subbing abilities. Row-crop tractors for sowing are used by leading farmers to reduce compaction. Volunteer cereals are controlled with low-rate Glyphosate application at post-sowing pre-emergence. Later emerging weeds can be controlled with a range of post-emergent selective herbicides can be applied throughout the entire season with ground coupes or by plane if conditions are unsuitable. Harvest commences early April, followed directly by no-tilling planting of winter cereals.

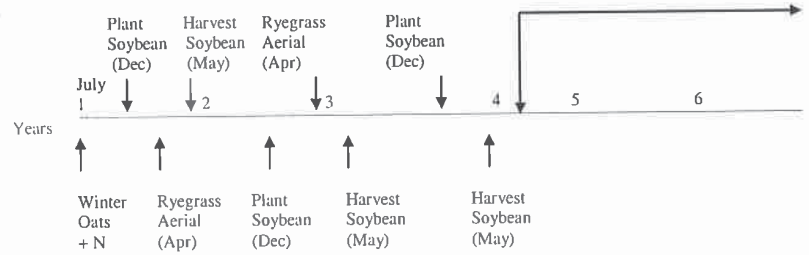
Few Problems Encountered -
- No-till has improved soybean establishment and suppressed weeds through minimal soil disturbance and residue cover.
- If cereal stubbles are heavy there is a decreased ability to inter-row cultivate and straw will lodge in furrows and significantly reduce water flow. A cool burn is utilised or straw is baled.

Table 1. Three Farming Systems utilising Soybeans

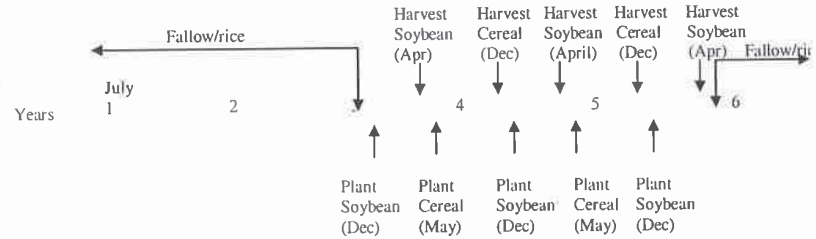
A. Tropical Coastal Sugarcane – Fallow Legume System



B. Coastal Beef – Soybean Inclusion Before Pasture



C. Irrigated Riverina – Double Crop



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Soil management for soybean production and its effect on soybean cyst nematode

G. R. Noel[1]

Abstract

Soybean cyst nematode [SCN], *Heterodera glycines* Ichinohe, is established through out the world where ever there is intense soybean [*Glycine max* (L.) Merr.] production or a long history of production. The nematode is adapted to both tropical and temperate environments and causes crop loss in soils that range from light textured sands to heavier textured soils with organic matter of 6-8%. Research on no-till soybean production and its effects on SCN population dynamics has been studied in both the southern and northern production areas in the USA. Results are contradictory with some research indicating reduced numbers of SCN in no-till production and other research demonstrating increased numbers of SCN following establishment of no-till soybean production. Data indicating that higher pH and calcium levels result in larger numbers of nematodes was developed recently in the USA and exists anecdotally in the People's Republic of China and in Brazil. Higher levels of organic matter are associated with lower populations of SCN, presumably by increasing nematode antagonists. Soybean production using narrow row spacing has no long-term effect SCN numbers.

The various tillage practices cause different effects on carbon, nitrogen, and phosphorous cycles and the myriad of aerobic and anaerobic organisms in soil (Aon et al., 2001). Of interest in pest management are effects of tillage practices on pathogens, insects, and nematodes and their interactions with plant roots and soil colonizing microflora and microfauna (Roane et al., 1975; Rothrock et al., 1985; Sturz, et al., 1995; Workneh et al., 1999a). The presentation outlined in this paper will focus on the interrelationships of tillage, cropping systems, and their effects on various soil nutrients and soybean cyst nematode (SCN) population dynamics. Conservation tillage is practiced to reduce soil erosion, preserve soil moisture, improve water quality, increase organic matter, and to reduce inputs of fuel, labor, and machinery. Conservation tillage is defined as those tillage practices that leave > 30% crop residue after planting and consists of no-tillage/strip-till, ridge-till, or mulch-till soil preparation (CTIC, 2003). Since 1989, conservation tillage has increased from approximately 27% of the hectares planted in the United States to 37% or nearly 42 million hectares in 2002.

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Although 55% of the 28 million hectares planted to full season soybean in the United States in 2002 received conservation tillage, there is little information available on the effects of cropping sequences and conservation tillage on population dynamics of soybean cyst nematode (SCN) and associated yield loss in soybean (Baird and Bernard, 1984; Edwards et al., 1988; Hershman and Bachi, 1995; Howard et al., 1998; Koenning et al., 1995; Noel and Wax, 2003; Schmitt and Nelson, 1987; Tyler et al., 1987).

Most studies on the effects of conservation tillage and SCN have been done in southern growing regions where soybean production has decreased in recent years, but the percentage of hectares planted no-till has increased. Those studies have not provided consistent results that have allowed region-wide recommendations on utilization of various tillage practices to reduce numbers of SCN and crop loss. One difficulty has been the use of different life stages of SCN to define population dynamics. Numbers of second-stage juveniles (J2) were higher in July in soil that received conventional tillage (CT) in absence of wheat in the cropping sequence than in no-till (NT) or CT with wheat in the cropping sequence (Baird and Bernard, 1984). Numbers of cysts and eggs were not determined. In another study in the southern USA, long-term NT resulted in lower numbers of cysts when compared with certain CT treatments (Tyler et al., 1987). However, NT of 1 and 2 years did not affect numbers of cysts. During the first year of another study, numbers of eggs were lower in subsoiling plus NT when compared with NT and CT (Schmitt and Nelson, 1987). No differences were found during the second year. In the third year of the study fewer eggs were recovered from NT plots when compared with the subsoiled/NT and CT treatments. Numbers of J2 were lower only in the first year of NT. No differences were observed among treatments in years 2 and 3. In another study in the southern region, numbers of J2 were higher in August under CT than under strip tillage and NT (Edwards et al., 1988). However, under soybean monoculture numbers of J2 were similar when CT was compared with NT. Another study evaluated NT and minimum tillage and found no effect of tillage on numbers of cysts or eggs at planting or at harvest, but there was a tillage x wheat residue interaction in which larger numbers of cysts were found in minimum tillage plots with residue than no-till plots with residue (Hershman and Bachi, 1995). Numbers of eggs plus J2 at harvest were lower in NT plots 4 of 6 years (Koenning et al., 1995).

In contrast to soybean production in the southern USA, production in the northern region has increased in both the number of hectares and the number of hectares in no-till production. As with the southern region, results in the northern region also have been variable. Workneh et al. (1999b) conducted a survey in the northern region of the USA and reported numbers of SCN

eggs were greater in fields under CT than those under NT. A 4-year regional study found no differences in numbers of SCN eggs recovered from soybean produced NT versus those produced using CT (Atibalentja et al., 2001). That study also found no effect on SCN numbers when soybean was grown in narrow rows. A 7-year study found NT production supported larger numbers of SCN eggs at harvest when compared with CT (Noel and Wax, 2003; Fig 1). However crop rotation with maize negated that effect, resulting in no long-term differences in SCN populations between the two production systems.

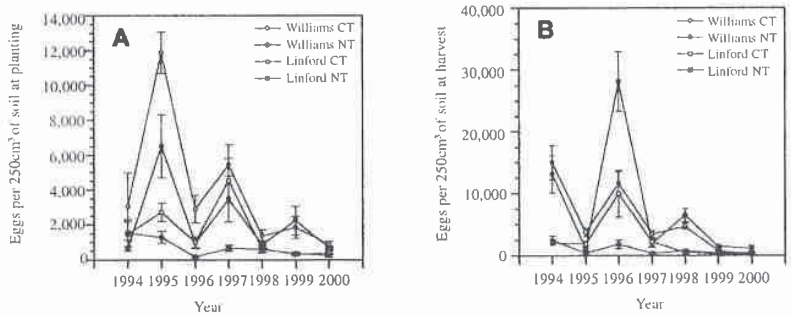


Figure 1. Population dynamics of *Heterodera glycines* in a soybean-corn rotation planted either with conventional tillage (CT) or no-tillage (NT). A) Numbers of eggs at planting, and B) numbers of eggs at harvest. Soybean cvs. Williams 82, susceptible to *H. glycines*, and Fayette or Linford, resistant to *H. glycines*, were planted in 1994 (Fayette) 1996, 1998, 1999, and 2000. Maize was planted in 1995 and 1997. Each datum is the treatment mean, and bars represent the standard error of the mean.

The interrelationships of micro and macronutrients, pH, organic matter, cropping systems, and SCN have received little attention. These interrelationships are complex and may be site specific. A study sponsored by the North Central Soybean Research Program examined these factors. Two examples are high lighted below. The first is the effect of soil pH on initial SCN populations following cropping of soybean and maize the preceding two years (Fig .2). The SCN population available to infect the soybean crop in year three of the rotation was larger when the pH was higher. In the second example, the converse is true for organic matter (Fig. 3). As the percent organic matter increases the population of SCN present at planting decreases. Micronutrients that were important at the various sites of the study included boron, calcium, iron, manganese, and zinc. Interestingly, the macronutrients potassium and phosphorous seldom were significant factors in SCN population dynamics. However, in the southern

state Tennessee, a multiyear study planted NT found that lower rates of potassium and phosphorous were associated with higher numbers of SCN cysts (Howard et al., 1998).

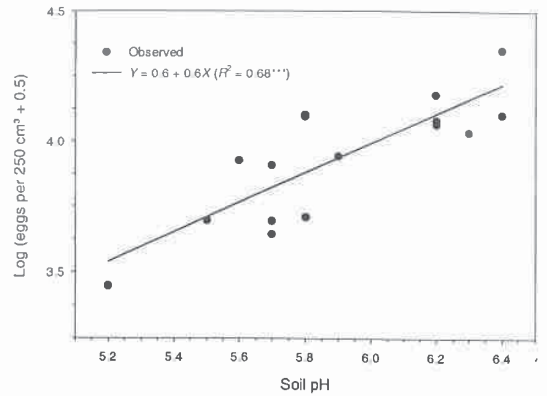


Figure 2. Effect of soil pH on *Heterodera glycines* initial population on the susceptible soybean cultivar Williams 82 in the third year of a soybean-maize-soybean-maize rotation (1997-2000) under no-till in Illinois, USA.

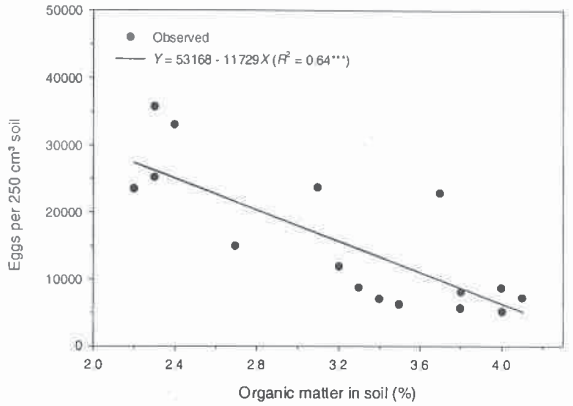


Figure 3. Effect of organic matter in soil on *Heterodera glycines* initial population on the susceptible soybean cultivar Kenwood 94 in the third year of a soybean-maize-soybean-maize rotation (1997-2000) under conventional tillage in Michigan, USA.

Soybean cyst nematode is highly adapted and is a successful parasite in a myriad of environments. Very few of the interrelationships among tillage systems, micro and macronutrients are understood with any certainty.

Additional research is needed to determine whether soil nutrients can be managed to reduce crop loss caused by SCN while not causing plant stress that reduces yield of the soybean. One promising area of research is the planting of crops allelopathic to SCN. Wheat may be a potential crop as indicated by the studies of Baird and Bernard (1984) and Hershman and Bachi (1995). The management practice with the best likelihood of success in the near term is to increase the organic matter content of soil, provided the economics of production are feasible.

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Soil acidity, pH and aluminum management in tropical soils: the Brazilian experience

C. A. Rosolem[1]; M. Miyazawa[2]; J.C. Franchini[3]; M.A. Pavan[2] and A. Costa[2]

Introduction

Soybean is grown in Brazil from Parallel 33° S to the Equator, under climates ranging from temperate to equatorial. Climate and geology diversity of these regions led to formation of very different soils. Within the diversity, practically all of the soils are acidic but the main limiting factor is different in each region. In the South, besides Al toxicity, Mn toxicity is very important as a consequence of low pH. On the other side, in most of the Cerrado region, Mn deficiency, and not toxicity, is a problem, but Al toxicity is important, as well as the low Ca and Mg contents.

No-tillage has become very popular in Brazil and soybean is the main crop under no-till throughout the Country. Where soybean is not under no-till, several degrees of conservation tillage are employed, always leaving some crop residues over the soil surface. This practice has an important impact on soil acidity management.

Considering that soil, climate and cropping system diversity lead to different soil acidity management practices, this chapter will consider the different management practices to obtain high soybean yields in each region.

Soil acidity and nutrient acquisition by soybeans

To meet nutrient requirements of the plant, nutrients must be available in the soil at the right time, amount and quality. Moreover, there must be a root for uptake to occur. Soil pH is paramount in regulating nutrient availability in soil and hence root and plant growth. A pH_{water} from 6.0 to 6.5 is required for most nutrients to be at the highest availability in the soil solution. As soil pH is increased beyond that, all micronutrients but Mo and Cl will became more and more unavailable. Considering that a pH above 6.0 favors N mineralization and nitrification and that usually tropical soils are in regions where rainfalls are abundant and heavy, this N may be lost by leaching if the pH is to high (Rosolem et al., 2003). Conversely, as soil

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pH_{water} is decreased below 5.4, Al toxicity will occur, as well as Mn toxicity in some tropical soils.

Generally soil amelioration in the arable layer has been enough to provide for nutrient acquisition by soybean, but Al toxicity in the subsoil has been a problem because it impairs soybean root grow in this region. This is not a problem for nutrient acquisition but it is determinant in water acquisition, mainly during dry spells (Black, 1993) frequently observed in tropical regions. Besides been free of Al toxicity, the subsoil must have adequate Ca levels for root grow. Noble et al. (1988) found no response of soybean roots to Ca when there was no Al in the solution, but in field conditions Ca content must be 8,5 $mmol_{(c)}\ dm^{-3}$ or higher for normal soybean root growth (Rosolem e Marcello, 1998). Moreover, when Ca levels were increased up to 20,0 $mmol_{(c)}\ dm^{-3}$, soybean root distribution in the soil profile was modified, allowing for a better exploration of the subsoil (Rosolem et al., 1995).

Therefore, soil acidity in tropical soils must be managed to avoid N leaching and Al and Mn toxicities but, at the same time, assure root growth in the subsoil and nutrient availability to soybeans. This implies in amelioration of the soil arable layer and, not rarely, also in amelioration of subsoil.

Soil acidity management in conventional tillage systems

The first thing to consider is the main limiting factor for the region. In some regions, besides Al, Mn is found in toxic levels. In other regions, Al neutralization will be enough to obtain high soybean yields. When there is no Mn toxicity, Mo deficiency may also be a problem if the pH_{water} is relatively low. Always it is important to check on Ca and Al contents in the subsoil in order to have a good root growth in this region of the soil.

Liming to raise soil base saturation up to 70 % has been the major tool in dealing with Mn toxicity in São Paulo State (Mascarenhas et al., 1982) and in Southern Brazil (Vidor & Freire, 1972). In this case, the amounts of lime to be applied for maximum economic yields is higher than those recommended to neutralize toxic Al. However, Rosolem and Nakagawa (1990) showed that Mn deficiency may be induced in soybean when lime rate is high or lime incorporation into the soil is not adequate in sandy soils. In this kind of soil, Mn deficiency may be worse if high K rates are applied following liming.

More recently, Lantmann et al. (1985) in the State of Paraná and Quaggio et al. (1998) in the State of São Paulo, showed that the high soybean requirement in lime was associated not only to Mn toxicity but also with

Mo availability. It has been shown that when Mo is applied to soybean seeds the amount of lime to be applied can be lower. According to Quaggio et al. (1998), maximum soybean yield was observed when the soil base saturation was increased to 70 % only in Mo absence. When Mo was applied to the seeds lime requirement was lowered.

In Mato Grosso do Sul, working with various soils ranging from 100 to 800 g Kg^{-1} of clay, Wong (1999) showed that for clay and very clay soils, lime must applied to raise base saturation up to 70 %, for medium texture soils base saturation must be around 60 % and for sandy soils, base saturation must be raised to 50 %. This reduction in lime rates is very interesting because the application of high lime rates to soils with low organic matter contents, in conventional systems, decrease the aggregate stability, favoring compaction of these soils (Westerhof et al. 1999).

As regarding lime recommendation methods, the following have been in use: Al neutralization, correction of Ca and Mg contents, base saturation increase to 50, 60 or 70 %, depending on the region and the soil. According to Wong (1999) methods based on Ca and Mg contents and on Al neutralization are not adequate for Mato Grosso do Sul, because lime requirement is underestimated in soils with medium to low organic matter e little Al. On the other hand, they overestimate lime rates in soils with high Al. Therefore, base saturation showed to be more reliable in estimating lime rates, provided soil texture is taken in account.

Low Ca and high Al levels in the subsoil can impair soybean root growth. Mechanical incorporation of lime would be not economic in most cases, but Ca and Mg from lime applied into the arable layer can eventually be leached through soil profile enhancing Ca levels and neutralizing toxic Al. This can happens in short on long periods, depending on the soil nature, lime rates and quality, rains, fertilizer management, etc. Rosolem et al. (1995) in an clay hapludult of São Paulo noticed Ca and Mg leaching down to 40 cm 50 days after lime application at regular rates. However, Wong (1999) noticed very little Ca and Mg leaching down to the 20-40 cm layers of 7 soils from Mato Grosso do Sul ranging from 100 to 800 g Kg^{-1} of clay, even with excessive liming, during one soybean crop. In the long range, regular lime application followed by a fertilizer program including sulphates and nitrogen can ameliorate the subsoil, but gypsum application has been the easiest and economic way to deal with subsoil acidity in soils where base leaching from lime does not occur easily. Gypsum must be applied when Al saturation is higher than 20 % or Ca saturation is less than 60 % in the subsoil. Gypsum rate can be calculated using clay content (Kg g^{-1}) times 4 (Lopes et al., 1999).

Soil acidity management in no-till systems

Cropped land under no-till has been grown very fast in Brazil in the last years and it is estimated around 20 millions ha. Without plowing there is no way to incorporate lime into the arable layer and soil acidity could be one impeding factor for high yields. One alternative was to try lime application to soil surface, but how would soil and plant respond to this application? Lime reactivity in soil depends on specific surface contact and carbonate leaching was shown to be very slow in most cases, so the response was predicted to be small.

That was not the case. Crop responses to surface applied lime had been observed in very acidic soils (McMahon e Thomas, 1976; Blevins et al.; 1978), when Al saturation was very high (Sá, 1999) or when Al sensitive species were cropped (Sá, 1999; Pöttker e Ben, 1998). Caires (2000) showed that there was soybean yield response to lime applied to soil surface in no-till systems with increases ranging from 4 to 42 %. Moreover, soybean yields exceeding 4,000 Kg ha^{-1} are observed in soils with base saturation as low as 40% (Fundação MT, 2002), in no-till systems.

Soybean responses to lime in consolidated no-till systems have been small or non-existent (Caires et al., 1998; Caires et al., 1999), even when the soil base saturation is as low as 32 %, with a $pH_{(CaCl)}$ of 4,1. When a response is observed, lime rates to optimum yields are lower than in conventional systems (Sá, 1999, Pöttker and Ben, 1998). In conventional systems, lime must be applied to raise soil base saturation to 50 to 70 %, but in no-till systems base saturation to be reached is lower, 50 % at most, as shown by Costa (2000), or even lower (Caires et al., 1998, 1999, Fundação MT, 2002).

In a three-year study, Costa (2000) showed that the application of 4.5 t ha^{-1} of lime incorporated into the arable layer in the first year resulted in a accumulated soybean yield of 9,942 Kg ha^{-1}. Soybean yield for the same lime rate applied to soil surface was 8,999 Kg ha^{-1}, i.e., 10 % lower than when lime was incorporated. Therefore, when the no-till system is consolidated, lime can be applied to soil surface, but by the time no-till is started, lime must be incorporated in the soil arable layer for best results.

Soil acidity problems in tropical soils comprehends Al and Mn toxicities and low nutrient availability. Low Ca levels in subsoil may impair root growth and eventually water acquisition by soybean. In no-till systems, the maintenance of the previous crop residues on soil surface is one of the main factors for success. Moreover, in tropical regions, the soil must be

covered throughout the year, which implies in more than one crop in the season. The result is an accumulation of straw in the soil surface. The contact soil-straw is poor, decreasing the decay rate of the straw, what allows for a continuum flow of organic matter decomposition products to soil.

Toxic Al in the soil can be neutralized by organic acids coming from decaying straw. The more effective are citric, oxalic and tartaric. Malic, malonic and salicylic acids have a moderate action and succinic, lactic, formic, phtalic and acetic acids have little effect on Al (Hue et al., 1996). Species usually grown as cover crops or green manures have these organic acids. Franchini (2003) found citric, malic, acconitic and fumaric, among the aliphatic acids, and cafeic, p-coumaric and ferulic among the phenolic acids in tissues of black oats and oil seed radish. The contents of these organic acids decreased in the tissues as the plants matured, from 60 to 120 days after plant emergence.

Cations require soluble anions as Cl^-, NO_3^-, NO_2^-, SO_4^-, OAc^-, $OFor^-$, and HCO_3^- to be leached through soil profile. The retention of M+ in the superficial layers of the soil only would be possible if negative charges of clay and humic acids were free, what seldom occurs. To minimize this kind of loss, soil chemistry must be modified favoring a higher mobility of M^{2+} by maintaining plant residues in soil surface (Oliveira and Pavan, 1996, Pavan, 1994). Under no-till and perennials an increase in pH, Ca and Mg levels and a decrease in toxic Al were observed beyond the region of the soil where lime was applied (Pavan, 1994, Oliveira and Pavan, 1996, Caires et al., 1999). Ziglio et al. (1999) added crop residues mixed with $CaCO_3$ to the soil surface and found that in treatments with residues of black oats and black mucuna, maximum leaching of Ca and Mg were observed before the maximum K leaching. Franchini et al. (1999) also observed that Ca and Al were the main cations mobilized after application of plant extracts to the soil.

The main chemical characteristics associated with cation mobility in acidic soils are those related to the formation of metal-organic complexes and their stability. When a complex ML (L= organic ligant) is transported by water through the soil profile the M^{n+} cation is substituted by H^+, increasing pH and base levels. This reaction occurs because, in general, HL affinity is higher than ML affinity (M= Ca and Mg) (Martell and Smith, 1989).

The neutralization of subsoil acidity by organic ligants can be expressed as:

$$R\text{-}COOOM + H^+ \leftrightarrows R\text{-}COOH + M^{n+}$$

by analogy, Al toxicity can be reduced by hydrolysis:

$$R\text{-}COOM + H_2O \leftrightarrows R\text{-}COOH + OH^- + M^{n+} \text{ (increase in pH)}$$
$$Al^{3+} + 3OH^- \leftrightarrows Al(OH)^3 \text{ (precipitated)}$$

or by organic acid complexation:

$$R\text{-}COOM + Al^{+3} \leftrightarrows R\text{-}COOAl + M^{n+}$$

The high stability of the organic complexes responsible for Al toxicity decrease is determined by chemical bounds with rings with 5 or 6 C atoms. Some examples of organic acids presenting this characteristics are citric, oxalic, tartaric salicylic and gentisic. Organic acids with three carboxylic groups, as citrate, show the highest affinity with polyvalent cations.

Citrate complexed K^+ or Ca^+ are quickly exchanged for Al that was adsorbed to exchange sites in the soil organic matter. This substitution occurs because Al-citrate stability is higher than that of Al-humate. Considering the leaching of a K maloate solution through the first layer of soil, usually K is exchanged for Ca^+ that was adsorbed to the colloids. Thereafter, Ca^+ bound to maloate is exchanged for free H^+ and/or Al^+ in soil solution. Consequently, there will be an increase in K content in the superficial layer, an increase in pH and Ca levels and a decrease in Al toxicity in the subsoil.

So, the use of plant residues presenting high amounts of organic acids more efficient in polyvalent cation transport in the soil profile and in neutralizing soil acidity is an important tool in managing soil acidity in no-till systems. Some exemples are: oil seed radish, black oat, feijão bravo do Ceará (*Canavalia brasiliensis),* jackbean (*Canavalia ensiformes)*, etc. Some spontaneous plants as *Synedrellopsis grisebachii* and *Amaranthus hybridus)* show the same characteristic (Meda et al., 2002), but crop residues as those from soybean, wheat, maize and pearl millet are poor in organic acids.

In field conditions, the soluble organic compounds of vegetable tissues are the first to be consumed by microorganisms. Conventional and no-till systems may produce similar amounts of dry matter, but Ca and Mg leaching are higher in no-till. This happens because in conventional systems crop residues are mixed to the arable layer, enhancing the contact of the decaying straw with moist soil and favoring its decomposition by microorganisms. In spite of the low amounts of soluble organic acids in crop residues as soybean, maize and wheat, they have considerable amounts of lignins, celluloses and hemicelluloses, which, with time, will be decomposed generating organic acids. This effect may be significant in the long range.

Concluding remarks

In conventional systems soil pH, Al and Mn toxicities can be managed by applying lime at rates calculated to raise base saturation to 50, 60 or 70 %, depending on soil texture and region. When Mo is applied, lime rate may be lowered in most cases. The residual effect of lime application is expected to be 4 or 5 years, depending on fertilizer management. To ameliorate Al toxicity and Ca levels in the subsoil, gypsum application seems to be the economical option.

In no-till systems, lime incorporation is required as in conventional tillage in the first year. Thereafter, lime can be applied to soil surface and the base saturation to be reached is lower than in conventional systems. Surface lime application must be associated with an adequate management of cover crops and/or green manures to assure Ca and Mg distribution in the soil profile. How low can be the soil base saturation and how much lime to apply are open questions. There are recommendations ranging from 1/3 to full rates as compared to conventional tillage.

Gypsum application in no-till systems will be seldom required because Ca and Al will be leached through the soil profile due to the action or organic acids from crop residues, ameliorating the subsoil.

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Organic soybean production: challenges and perspectives of an increasing trend

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Abstract

Annual organic soybean [*Glycine max* (L.) Merr.] production in the U.S. has risen to more than 60,000 ha in less than 10 yr. This presentation reviews current organic soybean research being conducted in Iowa and elsewhere throughout the U.S. It examines challenges that producers must deal with and shares our perspectives on this increasing trend. Critical challenges associated with organic soybean production include weed control, crop rotation, bean leaf beetles (*Ceratoma trifurcata*) – primarily as vectors for the seed-staining bean pod mottle virus (BPMV) and other seed-staining fungi such as *Cercospora kikuchii* and *Fusarium* – and the near-by use of genetically modified (GM) soybean cultivars. An economic evaluation based on three years of research at the Neely-Kinyon Long-Term Agroecological Research site near Greenfield, IA revealed that food-grade organic soybean could return $1160 to 1250 ha^{-1} compared to $235 for conventional soybean grown in a 2-yr rotation with corn (*Zea mays* L.). Weed control challenges are site-specific, being closely associated with the weed seed bank populations and crop management strategies used prior to converting to organic crop production. The amount of stained soybean seed is variable generally increasing from NE to SW Iowa because of warmer winter temperatures. At the Neely-Kinyon site, staining ranged from 12 to 22% and 7.5 to 10% while yields averaged 2.4 to 2.8 and 3.5 to 3.8 Mg ha^{-1}, in 2001 and 2002, respectively. Stained soybean seed is currently rejected for food-grade markets (e.g. tofu), but increasing demand for organic meat and a small premium for organic feed-grade soybean has encouraged producers to continue growing the crop. The primary concern that organic producers have regarding GM crops is the increased risk of their crop failing a GM test when sold because of pollen drift or other contamination sources. Despite the many challenges, increasing consumer demand for certified organic soybean certainly warrants more aggressive and participatory research around the world.

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The USDA National Organic Program (NOP) defines organic agriculture as "an ecological production management system that promotes and enhances biodiversity, biological cycles, and soil biological activity. It is based on minimal use of off-farm inputs and on management practices that restore, maintain, or enhance ecological harmony. The primary goal of organic agriculture is to optimize the health and productivity of interdependent communities of soil life, plants, animals and people" (USDA-NOP, 2002). U.S. organic production in 2001 approached 1 M ha (USDA-ERS, 2001) with approximately 60,000 ha planted to organic soybean (Fig. 1). In Iowa, organic soybean production (Fig. 2) exceeded 9,700 ha in 1999 (IDALS, 2000) and has been growing steadily ever since. The majority of organic soybean grown in Iowa is destined for the Japanese and domestic tofu and soymilk market. The dominant varieties are bred for seed size and protein content.

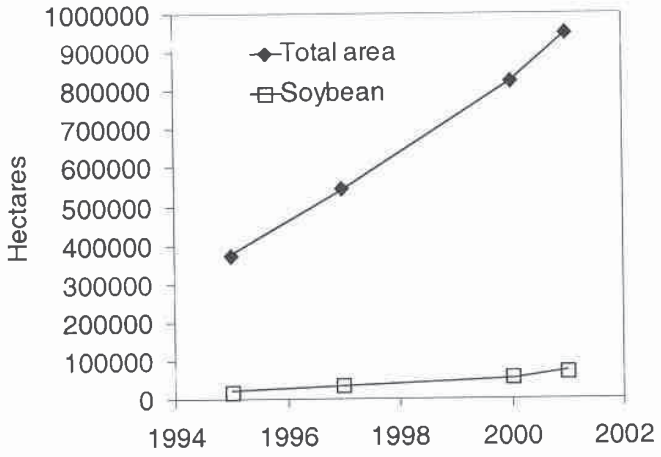


Figure 1. U.S. organic crop production 1995 to 2001.

Organic farmers avoid the use of synthetic chemicals and many completely shun all external inputs. Only naturally occurring materials are allowed in certified organic production or processing operations, and as a result, all inputs are reviewed by independent, third party inspectors. Rotations, cover crops, and natural products are used to maintain or enhance soil fertility rather than synthetic fertilizers and pesticides. Organic farmers rely on biological, cultural and physical methods to limit pest expansion and increase populations of beneficial insects on their farms. Livestock are usually integrated into farming operations and on-farm compost is produced to meet fertility requirements. All rely on conventionally developed insect- and disease-resistant crop varieties and encourage the activity of beneficial

insects through conservation of food and nesting sites. Some organic farmers import their fertility and pest management inputs but follow organic certification regulations in selecting approved products. Philosophically, organic agriculture seeks to use practices that lead to better water, nutrient, energy, and labor use efficiencies and to avoid those that have or may lead to negative impacts on society (e.g. decreased community structure and/or viable economic options).

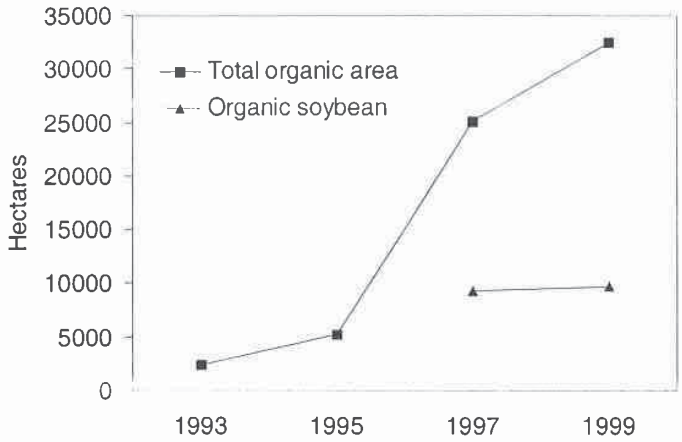


Figure 2. Iowa organic crop production 1993 to 1999.

A challenge that emerged in 1997 was the presence of stained seed in the harvested crop. In 1998, staining caused rejection of approximately 5% of Iowa's large-seeded, high-protein organic soybean crop for the purpose of making silky tofu, a soft, custard-like product that is eaten raw or used in soups and desserts. The rejection rate at some locations increased to nearly 50% by 1999 and continued to show an increasing trend in 2000. The requirement for a seed color free from staining is more of an aesthetic than food quality distinction, but soybean that has purple, brown or tan staining from one or more of the factors associated with the complex will be rejected from food-grade uses (e.g. tofu or soymilk) and diverted to the organic feed market at a reduced price. A suite of diseases, either working in concert or individually, can cause soybean seed staining. Four fungal groups (*Fusarium* spp., *Phomopsis* spp., *Alternaria* spp. and *Cercospora kikuchii*) and two viruses (bean pod mottle and soybean mosaic) have been identified as potential contributors to the suite. Organic farmers typically rely on biological insect control for managing most insect pests so a variety of approaches may be needed to minimize the problem. Reducing the amount

of soybean staining is of great economic importance however, because many organic producers rely on premiums associated with unstained seed to maintain their profitability.

The insect responsible for transmission of bean pod mottle virus is *Ceratoma trifurcata* or bean leaf beetle. The larvae of this beetle live in the soil and feed on soybean roots with relatively few natural enemies. There are generally two generations each year in Iowa with second-generation beetles emerging from the soil to feed on pods in late summer and fall. This second generation then hibernates through the winter before emerging from leaf litter to attack soybean seedlings in spring (Lam et al, 2001; Lam and Pedigo, 2000a,b). Females of the overwintering generation oviposit eggs in the soil near soybean plants. The eggs develop into first-generation beetles that emerge in late June or early July. The first-generation beetle populations usually peak when soybean is in the late vegetative or early reproductive growth stages. Feeding by the first generation on soybean leaves seldom results in an economic yield loss, but second-generation feeding on pods in late summer can be very significant. Both generations, however, can transmit the bean pod mottle virus.

Winter survival of bean leaf beetles is negatively impacted by low temperature (Lamb and Pedigo, 2000a) but due to insulating effects of leaf litter and possibly a heavy snow pack, many hibernating beetles can survive by avoiding extreme temperature fluctuations during winter. From 1997 through 2000 winter temperatures in central and southwest Iowa were generally 3 to 5° C above average. This apparently enabled a large number of beetles to survive, since high infestations of over-wintered beetles were observed on early-planted soybean in the spring of 2000.

To address this problem for organic soybean growers, a cooperative project involving Iowa State University (ISU), Heartland Organic Marketing Cooperative, and the NSTL was conducted. We evaluated certified organic pest management treatments that might help growers manage bean leaf beetle populations and transmission of virus or fungal agents responsible for seed coat staining. Studies were conducted from 2000-2002 at the ISU Neely-Kinyon Farm near Greenfield, IA. The Northrup King '2412' variety was planted in 2000 and Pioneer Brand '9305' was planted in 2001 and 2002. The treatments in 2002 included: Garlic Barrier™ with a mixture of fish oil and molasses applied at rates of 103 and 21 L ha^{-1}, 3% hydrogen peroxide with baking soda at 84 L ha^{-1} and 5 kg ha^{-1}, respectively, Neemix 4.5™ at 1.2 L ha^{-1}, and Surround™ at 28 kg ha^{-1}. All treatments were applied every 2 weeks beginning in late June and compared to a non-treated control. Bean leaf beetle populations were measured every other week by taking

20 sweeps with a 38-cm diameter net across each 6 by 9 m plot. Insects were placed in Zip-Loc™ bags and transported in coolers to ISU where they were frozen until enumeration in the laboratory. Soybean yield was determined with a plot combine and the percentage of stained soybean was determined by counting the number of stained seeds in a 200-gram sample from each plot.

As shown by Lam et al. (2001), beetle populations in 2001 (data not shown) and 2002 (Fig. 3) increased substantially following emergence of the second generation. There were no significant differences among treatments in 2002 with the percentage of stained soybean ranging from 7.5 to 10%. This was lower than in 2001 when staining ranged from 12 to 22% (Fig. 4) and the maximum bean leaf beetle populations averaged more than 40 beetles per 20 sweeps (data not presented). The yields in 2001 and 2002 averaged 2.4 to 2.8 and 3.5 to 3.8 Mg ha^{-1}, respectively, levels that were comparable to conventional soybean production practices both years.

Weed control and nutrient management are two other challenges frequently identified by organic producers (OFRF, 2003). In a 3-yr, 10-ha study conducted at the Deep Loess Research Station near Treynor, IA to evaluate three cropping strategies for transitioning from conventional to organic agriculture, a very large residual weed seed bank at this long-term research site (Karlen et al. 1999) caused direct transition to herbicide-free production of either corn or soybean to fail with regard to weed control, despite multiple rotary-hoeing and cultivation operations. Planting alfalfa (*Medicago sativa* L.) and/or red clover (*Trifolium pratense* L.) with an oat (*Avena sativa* L.) nurse crop and harvesting the forage as hay for three years appeared to substantially reduce the population of several weed species, but the study had to be terminated before a subsequent certified (i.e. 3-yr without herbicide or other prohibited material applications) organic corn crop could be harvested. Surprisingly, a sweet corn (*Zea mays* L.), fall-planted triticale (*X Triticosecale* spp.) and soybean rotation provided the best weed control during transition from conventional to certified organic management practices. With regard to nutrient management, use of liquid swine manure or compost as nutrient sources was successful. However, additional efforts are needed to develop better equipment for more rapid analysis and accurate application of these materials to be sure appropriate amounts of nutrients are being applied.

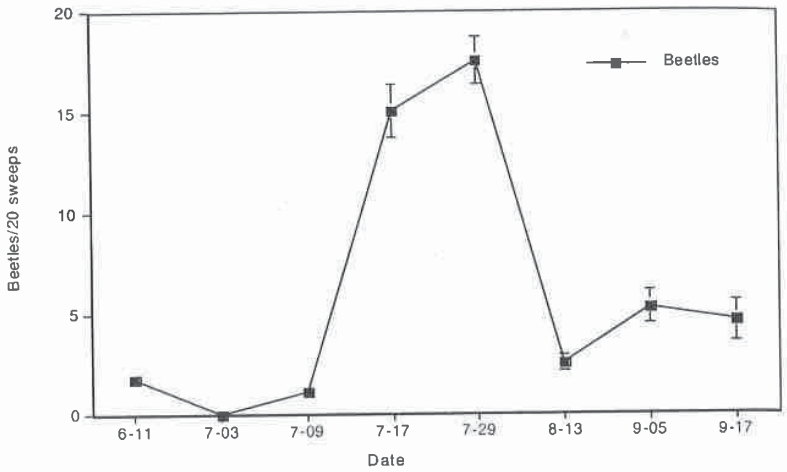


Figure 3. Beal leaf beetle population at Neely-Kinyon site, 2002.

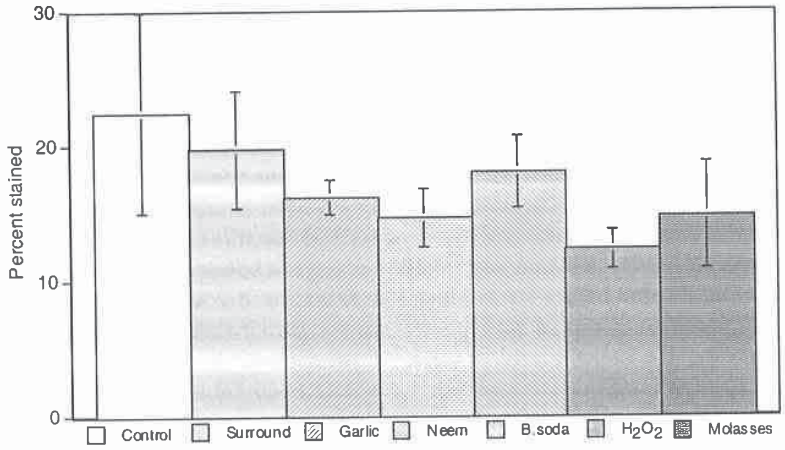


Figure 4. Treatment effects on percent of stained soybean at the Neely-Kinyon research site.

Increased economic viability, especially for small-scale producers, is often an incentive for transitioning into organic crop production. An analysis using three years of data (1999–2001) from the Neely-Kinyon site revealed that returns for organic corn within corn-soybean-oat or corn-soybean-oat-alfalfa rotations ($652 or $672 ha^{-1}, respectively) were significantly greater

than for corn in a conventional corn-soybean rotation ($126 ha^{-1}). Food-grade soybean returns within the organic rotations were not significantly different at $1160 or 1247 ha^{-1}, respectively, but were significantly greater than for conventional soybean which returned $235 ha^{-1} (Delate et al., 2003).

An uncontrollable challenge affecting U.S. organic soybean producers is the real and/or perceived risk associated with agricultural biotechnology or more specifically the development of genetically modified (GM) crops. This emerging technology has substantially altered the allocation of uncertainty and risk within the marketplace. Prior to the introduction of GM crops, the market for organic crops was driven by supply, demand, quality and price. Processors and consumers had no real or perceived concern regarding risk associated with the genetic profile of the seed itself. Since the introduction of GM soybean into conventional production systems, a non-quantifiable risk profile has been introduced across the soybean production-consumption spectrum. Each entity in the chain (seed companies, producers, processors and consumers) is struggling to assess and account for risks associated with GM crops. Producers, particularly in the Western Hemisphere have demonstrated a high level of acceptance of the risk associated with GM soybean. The reasons for increased use of GM soybean appear to be those associated with convenience, familiarity, and advertising. The majority of GM soybean is planted for its resistance to glyphosate, the active ingredient in Roundup™ and similar herbicides. GM crop producers, and apparently the seed companies as well, failed to accurately assess the risks associated with consumer resistance to the new technology. For the U.S. producer, this failure has resulted in the loss of markets and approximately $189 million in annual revenue from 1997 to 2001 (Benbrook, 2003). Herbicide-resistant crops also continue to suffer from a "yield drag" compared to non-transformed varieties (Duffy, 2001).

With annual worldwide consumption of organic products increasing at a rate equal to or exceeding 20%, the increased use of GM soybean by conventional producers also affects those producing certified organic crops. Motivating factors for organic production include economic viability, environmental concerns, and food safety. Much of the increase in consumption of organic products has been fueled by consumers' demand for GM-free products. As a result, genetically modified organisms (GMOs) are not allowed in organic production or processing, so organic products are automatically identified within the marketplace as being GM-free. Testing of crops for GM residues is not required for USDA certification of organic products, but to ensure worldwide marketability producers and processors of organic corn or soybean must anticipate contamination, pay for the GM testing, and bear the risk of losing a certifiable organic product at the point

of sale. This increased risk for organic producers has profound economic consequences and is generally beyond his/her control. Therefore, a recent survey by the Organic Farming Research Foundation (OFRF) reported that GM contamination is a major concern for many U.S. organic farmers and that a majority of respondents believed efforts by the U.S. government to protect them from this contamination were inadequate (OFRF, 2003). In the same survey, 11% (233 respondents) of those who have paid for this testing received a positive result. Furthermore, because GM contamination can prevent the crop from entering the organic stream of commerce, many cases may go unreported because of the fear of losing future business. GM contamination of organic crops can occur through several pathways, so it is difficult to isolate blame. Seeds used on organic farms may contain traces of biotech varieties, as seed companies will only certify that no GM processes were used to develop the variety – not that GM contamination was prevented. Contamination can also occur within the harvesting, storage, and transport equipment, where traces of GM seeds from previous operations may remain. Organic farmers are required to document clean-out procedures to assure a GM-free environment, but accidental contamination can still occur.

Organic consumers prefer a 100% GM-free product; but due to pollen drift and accidental contamination, the organic market must tolerate contamination levels of 0.1% to 5%, depending upon the country and specific market. The most common level tested is 0.1%. More sophisticated, expensive tests can detect levels down to 0.01%. The net result is that the organic consumer now bears the risk of GM contamination. The organic industry believes that labeling allows informed choices and is a reasonable approach to address the potential risks associated with GM crops.

In summary, the steady increase in consumer demand for organic soybean and other commodities during the past decade should be viewed as a long-term continuing opportunity for soybean producers throughout the world. The entire soybean industry including those developing cultivars, producing soybean, purchasing, transporting, processing, and marketing soy products should embrace this opportunity. We anticipate that 21st century consumers are going to become more demanding and less willing to accept whatever the market offers as their education and income increase. As demonstrated by our experience with the bean leaf beetle, there will be opportunities to develop certifiable natural-based materials that will help organic producers overcome the typical crop production challenges associated with fertility, weeds, insects, and diseases. We suggest that the soybean industry should be sensitive to consumer concern regarding GM crops and not assume that simple economics or market share alone will dominate. Finally, one of the most important facts that the entire soybean industry might learn from

organic producers is the long-term benefit of crop rotation. Required for organic certification, more diversified crop rotations ultimately increase yield, reduce the risk of crop failure, and have community benefits far beyond the farm gate (Karlen, 2004).

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VII WORLD SOYBEAN RESEARCH CONFERENCE

MARCH 3

Soybean diseases report in China

F. Feng[1]; Z.-H. Ma[2] and H.-G. Wang[2]

Soybean diseases, which occurred in the agro-ecosystems of China, affected the soybean production. Based on the collection and analysis of published data that described the diseases, which there are 30 diseases (table 1), the diversity and the distribution pattern of the fungal diseases were examined. The effects of the planting acres and geographical factors on the disease diversity and distribution pattern were analyzed. The primary possible reasons that lead to the similarities and differences were also discussed. The result is important to quarantine the soybean fungal diseases and also to estimate the pest risk assessment.

Similar aspects of diversity and distribution pattern of soybean fungal diseases in USA and China were found (1). Among the 44 kinds of soybean fungal diseases reported in the world, there were 33 recorded in USA, 27 in China (Table 2). Among them, 16 recorded both in USA and China. (2). The distribution pattern was similar for the 16 kinds of diseases both in USA and China. Their latitude centers were mainly located between 35° ~ 39°N. (3). Significant linear relationships between disease diversity and planting area were found in both agro-ecosystems. (4). Both agro-ecosystems have their special regions, which had the highest disease diversity. The Delta area, which is an area that includes the Mississippi, Missouri, and Ohio Rivers, had the highest disease diversity in USA. In China, the regions located on 41 ~ 46°N for south-north and 122 ~ 131°E for ease-west had the greatest soybean planting acre and this includes the Shonghua, Mudan, and Nen River. (5). Disease diversity measured by number of diseases/longitudinal breadth decreased from east to west both in USA and China. (6). The planting acres, annual average temperature and annual precipitation were significantly correlated with the diversity of the fungal diseases in both agro-ecosystems.

There were different aspects of diversity and distribution pattern of soybean fungal diseases in USA and China. (1). For the 17 kinds of diseases reported only in the USA, the diseases centered at higher latitude and had greater geographic ranges in latitudinal dimension. For the 16 kinds of diseases

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Table 1. The pathogens of soybean diseases in China

No.	Disease name	Causal organism
1	Thielaviopsis root rot	*Thielaviopsis basicola* *Thielavia basicola*
2	Soybean web blight	*Pellicularia sasakii (Shirai) ito*
3	Soybean rust	*Phakospora pachyrhizi*
4	Downy mildew	*Peronospora manschurica*
5	Purple seed stain	*Cercospora kikuchii*
6	Frogeye leaf spot	*Cercospora sojina*
7	Anthracnose	*Colletotrichum glycines* *C. destructivum*
8	Brown spot	*Septoria glycines*
9	Fusarium wilt	*Fusarium oxysporum* *F. tracheiphilum*
10	Botrytis mildew	*Botrytis cinerae*
11	Pythium root rot	*Pythium debaryanum* *P.aphanidermatum* *P.myriotylum* *P.ultimum*
12	Pellicularia blight	*Pellicularia filamentosa*
13	Powdery mildew	*Erysiphe polygoni*
14	Sclerotinia stem rot	*Sclerotinia sclerotiorum*
15	Fusarium blight	*Fusarium avenacerum* *F. oxysporum*
16	Pod and stem blight	*Phomopsis sojae*
17	Ascochyta leaf spot	*Ascochyta glycines*
18	Target spot	*Corynespora cassiicola*
19	Phyllosticta leaf spot	*Phyllosticta sojaecola*
20	Soybean leaf spot	*Mycosphaerella sojae*
21	Alternaria leaf spot	*Alternaria brassicae var. phaseoli* *A. ulata* *A. alternate*
22	Soybean pot rot	*Macrophoma mame*
23	Soybean sleeping blight	*Septogloeum sojae*
24	Rhizoctonia root rot	*Rhizoctonia solani*
25	Soybean stem rot	*Phoma glycines*
26	Soybean scab	*Sphaceloma glycines*
27	Phytophthora root rot	*Phytophthora megasperma* f.sp. *gly* *P. meyasperma* f. sp. *sojae* *P. sojae* *P. cactorum*
28	Bacteria spot	*Pseudomonas syringae* pv. *blycinea*
29	Soybean mosaic disease	Soybean mosaic virus
30	Soybean cyst nematode	Heterodera glycines

Table 2. The soybean diseases, their distribution in China.

Disease name	Province																														
	HN*	GD	GX	YN	GZ	SC	JX	FJ	AH	ZJ	JS	HUN	HB	HEN	SD	SX	HEB	NM	LN	JL	HLJ	SHX	NX	GS	XJ
Thielaviopsis root rot					Yes		Yes				Yes														
Soybean web blight					yes	yes	yes	yes			yes	yes	yes							yes					
Soybean rust	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes		yes	yes	yes	yes		yes	
Downy mildew	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Purple seed stain	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Frogeye leaf spot	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Anthracnose	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Brown spot	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Fusarium wilt	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Botrytis mildew	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pythium root rot	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pellicularia blight					Yes	Yes	Yes				Yes	Yes	Yes							Yes					
Powdery mildew						Yes								Yes						Yes					
Sclerotinia stem rot			Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes				Yes	Yes	Yes				
Fusarium blight				Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pod and stem blight				Yes									Yes												
Ascochyta leaf spot							Yes	Yes	Yes	Yes	Yes	Yes		Yes		Yes	Yes	Yes	Yes	Yes	Yes				
Target spot						Yes	Yes	Yes	Yes	Yes	Yes			Yes	Yes				Yes	Yes	Yes				
Phyllosticta leaf spot						Yes										Yes	Yes	Yes	Yes	Yes	Yes				
Soybean leaf spot						Yes	Yes	Yes	Yes	Yes	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes				
Alternaria leaf spot						Yes				Yes	Yes		Yes							Yes					
Soybean pot rot						Yes																			
Soybean sleeping blight													Yes												
Rhizoctonia root rot																									
Soybean stem rot																									
Soybean scab																									
Phytophthora root rot																									

found both in the USA and China and the 11 diseases reported only in China, the diseases centered at a higher latitude and had smaller geographic ranges in the latitudinal dimension. (2). Disease diversity measured by the number of disease per latitudinal breadth had a south-north gradient as the diversity decreased with latitudinal increase in USA, but the diversity increased with latitudinal increase in China.

The diversity and distribution pattern of soybean fungal diseases were related with many factors both in the USA and China. The climate, cultivation approach, variety resistance and even the culture had influence on diseases. The origination and transmission of diseases may be different in both countries. These variations have contributed to both similarities and differences in the diversity and distribution pattern of soybean fungal diseases in these countries.

With the similar distribution of common diseases in USA and China, and the different distribution of diseases reported only in USA or China, the common diseases in USA and China may have originated in the same region. This is a scientific question that needs to be investigated further. Also, it is necessary to probe into the factors related to the disease diversity center in both agro-ecosystems in USA and China.

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Country report: how soybean rust is managed in Thailand

S. Poonpolgul[1]

Introduction

Soybean rust is one of the important disease of soybean in Thailand caused by *Phakopsora pachyrhizi*. Reports for crop losses were up to 80% on susceptible cultivars. The principle of disease spreading of the spores by means of wind borne. The condition that promoted the severity of the disease was in late rainy season which temperature in range of 18 – 28 C, dew period at least 3 – 4 hr. in the morning. The windy day encourage spore dispersion once the infection occurred.

Disease situation and losses

There are around 10 soybean cultivars that are recommended farmers to grow in Thailand. Each cultivar has degree of susceptibility and its own characters to other diseases. Most of them susceptible to soybean rust at different planting location. Some cultivars seem to resistance to soybean rust but it has some character that farmers refuse to accept such as shattering, small seed size and susceptible to virus disease.

The occurrence of soybean rust in Thailand were in the northern and northeastern parts especially in late season, August to November. The soybean produced from this season would be used as seed for dry season due to storage and seed germination problem. Then the seed production from this season faced the quality problem and become one of limiting factor for soybean production in Thailand.
Average yield for non infected soybean of late season was about 280 – 300 kg / rai (1780 – 1875 kg / ha). The infected field, Chiang Mai 60, would give seed yield as low as 180 kg/ rai (1125 kg / ha).

Physiological races

The physiological races of soybean rust fungus, *Phakopsora pachyrhizi*, was determined at Kasetsart University, Kamphaeng Saen, Nakhon Pathom during 1994-1996. Ten soybean cultivars Ankur, CM60, PI200492, PI230970, PI230971, PI459024, PI459025, TK#5,TN#4, and Wayne, were used to

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differentiate physiological races by observing the symptoms of reddish brown (RB) and tan (TAN) type reaction. Sixty nine isolates from 6 districts in 5 provinces were grouped into 59 races. It was found that interaction between hosts and pathogens, RB type and Tan type, gave different uredospore per uredium on hosts. There was no significant difference in uredospore production of 4 representing rust isolates on 10 soybean cultivars. TK#5 produced the highest number of uredospore in 2 weeks from race LOI-1(2626 uredospore/ uredium) while LOI-1 on PI459024 produced the least (195 uredospore/ uredium). (Poonpolgul,1997) Fifteen physiological races of *Phakopsora pachyrhizi* were further analyzed by random amplified polymorphic DNA (RAPD). Reaction types on 10 differential cultivars were compared with the RAPD patterns. The amplification pattern obtained from 57 primers have no correlation with reaction types on soybean hosts. (Poonpolgul *et al*,1997

Host range

Legumes species have been identified as host of *Phakopsora pachyrhizi* in Thailand were *Canavaria gladiata, Carjinus sp. Centrosema pubescens, Pachyrhizus erosus, Vigna mungo, Dolichos lab lab, Phaselus aureus,P. lathyloides, Pueraria thunbergiana, Pisum sativum, Vigna sinonsis* (Poonpolgul and Surin, 1980)

Soybean rust resistance gene

The inheritance of RB and Tan type reaction of 4 soybean crosses indicated interrelationship between specific resistance genes in cultivars CM60, PI230970, PI459025 and Wayne. Each cultivar carried a different single dominant gene for resistance and these genes were located at different loci. PI 230970 and Wayne have the same resistance gene for rust reaction at the same locus. The genes for rust reaction were assigned to the four soybean parental types as followed ; CM60 : Rpp_1 Rpp_1 rpp_2rpp_2 rpp_3rpp_3; PI230970 and Wayne : rpp_1rpp_1 $Rpp_2Rpp_2rpp_3rpp_3$; and PI459025 : rpp_1rpp_1 rpp_2rpp_2 Rpp_3Rpp_3 .(Poonpolgul,1997)

Evaluation of soybean lines for rust resistance

One hundred and twenty five soybean lines have been evaluate for rust resistance by means of RB and Tan type lesions at Samueng District, Chiang Mai Province during 1999-2001. The results showed 3 lines that were resistance to the fungus, CM60-10kr-71-PS-21, 9518-5 and 9519-5. All lines showed RB type lesions. (Srisombun *et al*, 2002)

History of Chemical Research

Since the disease has been reported in Thailand, according to the first report in 1972 (Sangawong,1973),the fungicide experiment had been concerned in oxycarboxin, mancoeb, benomyl and mixed fungicides. The results came out that oxycarboxin and oxycarboxin + benomyl reduced defoliation significantly but benomyl has no appreciation effect when used alone. Meanwhile mancozeb has slightly reduced the defoliation than the non fungicide treatment but non of the treatment increase grain yield.(Sangawonge,1973)

In 1977, the fungicide field trial of triazone and triadimefon were conducted at Chiang Mai Research Center. The results revealed that 5 applications of the either fungicide spraying at flowering stage and 7 day interval increased yield of soybean SJ5 cultivars up to 300 %. (Kittisin, Nanthapun and Surin, 1977)

Efficacy of fungicides to control soybean rust

Recently there were many fungicide have been reported that could be used to control soybean rust such as propiconazole, chlorotalonil hexaconazole and bittertanole, etc.

Efficacy of chlorothalonil 5%SC

The experiment had been conducted at Prao District, Chiang Mai Province of northern Thailand during July to October, 1999 and soybean CM60 had been used in the experiment. Four concentrations of chlorothalonil at 20,25,30 and 35 gm.product / 20 lt water compared to mancozeb 80%WP at 30 gm.product / 20 lt. Water and non fungicide treatment with RCBD and 4 replications. The first application was at 35 days after planting and 4 applications at 7 day interval. The results showed that infection decline after the second application when compared to non fungicide treatment, especially when high concentration was observed. However, at the fifth application, all fungicides but the non fungicide treatment gave a little different in grain yield. (Ruengwiset and Poonpolgul,1999)

Hexaconazole 5%W/V SC

The experiment had been conducted at Prao District, Chiang Mai Province during August to November, 2002 and soybean SJ5 had been used in the experiment. Three concentrations of hexaconazole at 20, 30 and 40 cc. Product / 20 lt water compared to mancozeb 80% WP at 30 gm. Product / 20 lt water and non fungicide treatment with RCBD and 4 replications. The first application was at 35 day after planting and 4 application at 7 day interval. The results came out that disease severity decreased after

second application (42 DAP) when spray at 40 cc / 20 lt and showed significant difference to mancozeb, 30 gm. /20 lt and non fungicide treatment. After the last application (63 DAP) the disease severity had trend to decrease and still showed significant difference to non fungicide treatment. However, although the yield increase from 154 kg / rai (kg / ha) from non fungicide plots to 207 kg /rai (kg / ha.)from 40 cc/ 20 lt treatment (53 kg different) the percentage of yield looses didn't show significant difference when compared by DMRT at 99%.(Surapongchai and Poonpolgul, 2002)

Integrated disease management

In extension program for, soybean CM 60 was recommended to farmers in the area that have been reported of severe rust infection. Three time of mancozeb 80%WP at 40 gm / lt. applications at 7 day interval when the first symptom was found on the unifoliate leaves at flowering stage or when there were continuos shower for 3 days or when there were dew on leaf surface at least 3 - 4 hours in the cool morning. Spraying manner should be concentrated at the lower part of the soybean plant and under leaf surface.

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Zimbabwe – a country report on soybean rust control

C. Levy[1]

Abstract

Soybean production in Zimbabwe has been limited by three diseases in the last decade, namely, red leaf blotch, frog-eye leaf spot and soybean rust. *Phakopsora pachyrhizi* was first recorded in Zimbabwe in February 1998. Pathologists and breeders have adopted a practical, integrated control strategy, which includes an early-warning of rust infection, the use of effective fungicides, and the development of resistant, agronomically-suitable cvs. The need for the accurate timing of sprays during reproductive growth is highlighted.

Introduction

Soybeans have been produced on heavy, clay-loam soils in Zimbabwe since the late 1960s, and predominantly by large operators, with access to sophisticated machinery, high-yielding cvs, irrigation, fertilizers and finance. National production increased steadily from 1993 to 2001, when a record crop of 162 500 t was marketed (COPA, 2003). Although better cvs became available, and agronomic practice improved over the preceding decade, the decontrol of marketing from a state-controlled monopoly was the major cause of this increase. Since 2001, production has declined rapidly – falling to approx. 65 000 t in 2002, and to 36 000 t last summer. The demise of the industry has been caused solely by on-farm chaos resulting from the Zimbabwe Government's controversial land reform program (CFU, 2003).

Recent disease epidemics and rust epidemiology

During the past 15 years, soybean farmers have been faced with controlling three pathogens, which have caused severe yield losses. During the late-1980s, red leaf blotch (*Pyrenochaeta glycines* Stewart = *Dactuliochaeta glycines* (Stewart) Hartman & Sinclair) depleted the yield of unsprayed, susceptible cvs by 10% in the north-western areas. Successful breeding of resistant cvs controlled this organism by the early 1990s, and did away with the need to spray. As this problem dissipated, an aggressive biotype of *Cercospora sojina* Hara (frog-eye leaf spot) entered Zimbabwe, and caused severe yield reductions of 50 – 60% over a wide area (Levy *et al.*, 1997).

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An integrated national strategy utilizing resistant cvs, fungicides, crop rotations and soil management, controlled this biotype, and by 1997, frog-eye leaf spot was of little significance.

In mid-February 1998, Tichagwa, soybean breeder, noted a leaf-spot, which resembled bacterial pustule (*Xanthomonas axonopodis* pv. *glycines* (Nakano) Vauterin *et al.*) on several soybean lines known to be resistant to the bacterium at the Rattray Arnold Research Station (RARS), Enterprise. Two days later, this infection had spread throughout the plants to the uppermost trifoliate leaves – which was most unlike bacterial pustule. This 'new' pathogen was identified as *Phakopsora pachyrhizi* Syd. & Syd., and confirmed as such by CABI Bioscience, United Kingdom.

By early March, numerous reports of soybean rust were being received from farmers in the north-western and central growing areas, with the first outbreaks occurring in the northern parts. The epidemic coincided with the southward movement of the Inter-Tropical Convergence Zone (ITCZ) from East Africa into Zimbabwe in mid- to late-January. Disease severity increased very rapidly to defoliate plants in 4-5 days, and this was especially noticeable in the later plantings. Farmers attempted to save their diseased crops with various triazole and benzimidazole + triazole fungicides, but most sprays were too late, and a widespread, 40 – 80% yield loss resulted. Rust was present south-west of Harare by the end of the season, but it had little impact on these maturing crops.

In late-January 1999, rust was initially observed on soybeans growing in two eastern border areas, which had been previously unaffected. The movement of the ITCZ differed greatly from the previous season, and only when a cyclone moved southward down the Mozambique Channel was rust introduced into Zimbabwe. The pathogen was then dispersed westwards, to again affect the main Mashonaland production region. Although this epidemic was far more widespread, major losses were contained by extensive spraying, and only a few farmers, who chose not to spray, suffered the consequences.

In the following three seasons, soybean rust was reported initially from Glendale in the Mashonaland Central Province during early- to mid-January, but its appearance did not coincide with any complimentary weather movements. It is likely that by 2000, *P. pachyrhizi* had found another leguminous host(s) in the natural flora of Zimbabwe, and had become endemic; thus, external epidemics became of lesser significance. By 2001, rust was present throughout all soybean growing areas, but the level of severity differed greatly. Spraying had become routine among most large

operators, but many small-scale farmers, who had been encouraged to grow soybeans in the late-1990s, were having trouble with the disease. Their losses were estimated at between 0.3 - 0.7 t ha^{-1} (30 – 80%) (Mpepereki, *pers. comm.*).

Political imperatives led the Zimbabwe Government in early 2001 to systematically acquire commercial farms for resettlement. With little consideration for the economic consequences, farm owners were evicted from their properties, without compensation, and destitute peasants, who had little farming knowledge or financial support, were moved onto these highly productive lands. As the chaos developed, these peasants were displaced by other more affluent persons, who had little desire to plant soybeans - those who did, generally failed to control the rust. Getting details of their soybean production and the losses is virtually impossible. Rust severity in the last two summers has been low – probably due to the reduced national crop size, the significantly drier weather, and the effective control being achieved by the few 'original' farm owners.

Control strategy

Shortly after soybean rust was discovered at RARS in 1998, a number of meetings were held between scientists from the Commercial Oilseeds Producers Association (COPA), the Seed Company Ltd. and several of the agro-chemical companies. Due to the rapidity at which this disease developed and its amazing devastation, farmers had to be very quickly taught to recognize the early symptoms. Thus, the details were described, and distributed by post and on radio to all growers. The Zimbabwe Chemical Registration Authority also allowed the temporary registration of selected rust fungicides, so that farmers could try to contain the disease in that season. Contact was made also with respected international soybean rust scientists (Drs. Shanmugasundaram, Tschanz, and Hartman), to benefit from their experience.

These informal meetings led COPA to convene a multi-sectorial workshop in June 1998, to which Dr. Shanmugasundaram was invited. From this, a co-ordinated research program was developed whereby: a) pathogen avoidance, b) the use of suitable fungicides, and c) resistant, agronomically-adapted cvs., would be used to control *P. pachyrhizi* in Zimbabwe. General farmer education was viewed as a priority, and a rust early-warning mechanism, a necessity. At another rust workshop in March 1999, soybean scientists from Zimbabwe, South Africa, Zambia, Uganda and Nigeria gathered to discuss the rust situation in Africa, the field research done the preceding 15 months, and to share the knowledge gained. Aspects of the

continental movement, over-season survival of *P. pachyrhizi*, and possible methods of control were also debated.

'Rust traps'

One strategy employed was the establishment of a country-wide 'rust trap' network (also known as 'indicator plots' in South Africa and 'sentinel plantings' in the United States). Not only did these 'traps' warn that rust had arrived in a specific area, but they could be used to demonstrate the early characteristics of the disease, and the benefits of fungicidal control. Small, 0.25 ha plots were planted to susceptible cvs on-farm about a month earlier than normal, and left unsprayed. Co-operators were asked to scout the plants daily from flowering onwards for rust symptoms. When rust was observed (and confirmed by a competent pathologist) other soybean farmers in that area were notified to start spraying their crops as soon as possible, and in so doing, alleviated the need for early disease identification. Several co-operators chose to spray portions of their 'traps' thereafter, to demonstrate the relative benefits of single and multiple applications. This early detection method has worked - but its success was dependent on the correct siting of these 'traps', regular contact with the co-operators, and a sound knowledge of the initial disease symptoms.

Pathogen avoidance

The effect of planting date in relation to later disease development was investigated. It was hypothesized that earlier planting may provide a partial disease control, viz. the plants would become susceptible when the environment was unfavorable to infection, and/or mature beyond their most susceptible stages, when the weather was conducive. A replicated experiment in 1998-99 showed that this was not the case, and that host susceptibility was more linked to physiological age than the prevailing environment, as reported by Tschanz (1982). Plants that had been planted in early-November, flowered at approx. the same time as those seeded in late-December, and developed similarly thereafter. Hence, all the plants became susceptible during similar reproductive growth and under the same environment. This experiment, however, was confounded by the influence of different day lengths and changing light conditions, which resulted in the natural decline in productivity of December plantings.

Use of suitable fungicides

During the 1998 meetings, it was agreed that the local agro-chemical companies would be responsible for evaluating fungicides for efficacy and promoting any suitable products. As a result, the Chemical Registration Authority has approved seven fungicides as controls of *P. pachyrhizi* in Zimbabwe – five triazoles, a benzimidazole-triazole mix, and a morpholine.

COPA would investigate i) the number and timing of sprays required for optimum control; ii) make an independent comparison of the registered chemicals, and iii) the persistence of chemical efficacy. However, in the 2002-03 season, COPA screened six fungicides provided by several American manufacturers, under a USDA-United Soybean Board contract. This program has been extended to test a broader range of chemicals this summer.

Some farmers achieved substantially higher yields following spraying - even under low rust severity, and believed that the fungicides were stimulating the plants. An experiment to test this premise since 2001-02 has shown this not to be the case - any differences in the physiology and maturity were due solely to field variations, and upon harvest, no significant yield improvement could be attributed to the spraying.

i) Number and timing of sprays

The optimum number of sprays and their timing, have been studied in two replicated experiments since 1998-99. In the first, two cvs, 'Sonata' (determinate) and 'Soprano' (indeterminate), were sprayed differentially with an increasing number of 50 g flusilazole + 100 g carbendazim ha^{-1} applications, at 20-day intervals. The earliest treatment was made on plants with three trifoliate leaves (V3 growth stage, Fehr *et al.*, 1971; 28 days after planting (dap)), and the latest, when they were physiologically-mature (R7 growth stage; 108 dap). A five-spray treatment was included to protect the plants throughout their growth, and to provide a potential yield estimate. A rapid sigmoidal decrease in kernel yield occurred when fewer and later sprays were applied - three sprays were necessary to maintain the yield under high rust severity (Levy, 2003). Although, this study showed that *P. pachyrhizi* could be chemically-controlled, and that several sprays were necessary to maintain the yield potential, it did not indicate when these sprays would be most beneficial.

Another experiment was designed, whereby one, two or three sprayings were applied at different times after flowering, so that the relative benefits of several early- and late-applications could also be compared. The kernel yields harvested at RARS in the 2000-01 season represent the control and trends achieved (Fig. 1). The 70 dap single-spraying is of particular interest - it provided the best control of the single treatments of 'Sonata' plants, equivalent to the multiple-sprayings, and the 80 dap and 70 + 90 dap treatments of 'Soprano' plants. Early, single-sprays (50 dap and 60 dap) did not adequately protect the plants from late-season infection, and those applied after 80 dap, did not contain rust infection that developed at early flowering (R1 growth stage; 55 dap). The benefit of earlier spraying was

evident - 'Soprano' plants sprayed at 50 + 70 dap produced significantly higher yields than those sprayed at 70 + 90 dap (*P* d"0.05).

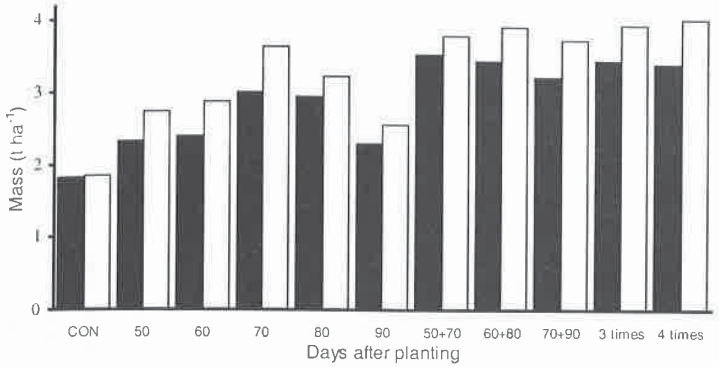


Figure 1. Kernel yield (t ha^{-1}, at 11% moisture) of two soybean cvs, 'Soprano'(O) and 'Sonata' (G), either left unsprayed (CON), or sprayed with 50 g flusilazole + 100 g carbendazim ha^{-1} at various days after planting at the Rattray Arnold Research Station, Zimbabwe in the 2000-01 season. ($S.E._{(d)}$(treatments) = ± 0.1332 t ha^{-1}; 3 times:50 + 70 + 90 d; 4 times:50 + 70 + 90 + 110 d)

These two experiments show that soybeans need to be adequately protected from rust throughout their reproductive growth (approx. 50 to 120 dap in Zimbabwe), that more than one spray is necessary to contain the infection, but yield potential is maintained only when the plants are initially treated during late-flowering and early podding.

ii) Comparison of registered fungicides

COPA evaluated the registered fungicides at several sites between 1998-2001. Early research suggested that mancozeb consistently controlled *P. pachyrhizi* in Australasia (Quebral, 1977; Sinclair, 1977; Bromfield, 1984; Shanmugasundaram, 1998), and therefore, it was included at 1.6 kg ha^{-1} in these trials. Plants were either left unsprayed, or sprayed three times at 18- to 21-day intervals. However, the times of these applications differed in the three seasons, as knowledge was gained from other research. In the 1998-99 season, they were applied at 21, 42 and 63 dap. Although the fungicides controlled infection, and the sprayed plants produced significantly higher yields than those unsprayed (*P*d"0.05) (Fig. 2), the differences were not as great as when spraying was done during reproductive growth (e.g. at 50, 70 and 90 dap, in the 2000-01 season).

iii) Persistence of fungicide efficacy

This experiment has been hampered by a low incidence of rust in the past two seasons. Differences within the yields, kernel masses, and the resulting economics, did not follow patterns relative to the spray treatments, and therefore no meaningful conclusions can be drawn as yet. The trial is being repeated this summer with the hope that higher rust severity will provide discriminatory results.

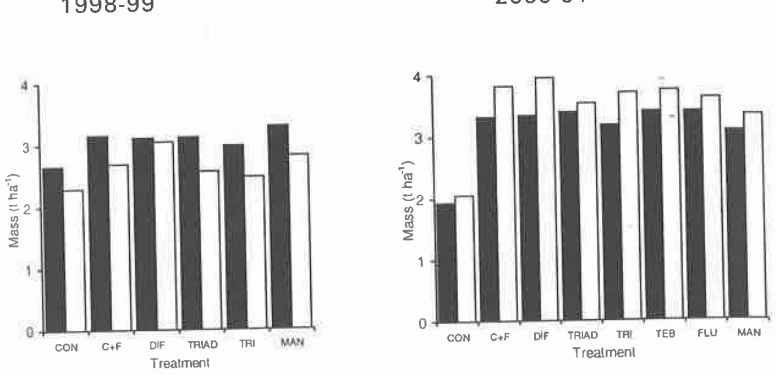


Figure 2. Kernel yields (t ha^{-1}, at 11% moisture) of two soybean cvs, 'Soprano' (O) and 'Sonata' (G), either left unsprayed (CON), or sprayed differentially with various fungicides at the Rattray Arnold Research Station, Zimbabwe in the 1998-99 and 2001-02 seasons. (S.E.$_{(d)}$(treatments) 1998-99: ± 0.134 t ha^{-1}; 2000-01: ± 0.099 t ha^{-1}; C+F: carbendazim + flusilazole; DIF: difenoconazole; TRIAD: triadimenol; TRI: triforine; TEB: tebuconazole; FLU: flutriafol; MAN: mancozeb)

Breeding for resistance

Shortly after soybean rust appeared in Uganda, an arrangement was made for all the Zimbabwean commercialised cvs to be screened for resistance there in the 1997-98 season. All were found to be susceptible, but the popular cv., 'Solitaire', showed a "slow-rusting" trait, which indicated that it may possess a form of partial resistance.

Field research became easier when rust arrived in Zimbabwe in 1998. Seventy nine soybean lines, reported elsewhere to be resistant to *P. pachyrhizi*, were obtained from South East Asia, India, Australia, South Africa and the USDA, and planted initially in a field screen under natural infection for two seasons. Although all were susceptible to the local biotype(s), and none was suitable for direct commercialization, 25 were

considered well-enough adapted to be used in a breeding program. Using a single-seed descent propagation, 1960 F4 progenies were derived from these lines, and then field-tested for resistance and agronomic suitability; subsequently, 57 were selected, and included in three preliminary cv. evaluations. Although, low rust severity has hampered this selection in the past two seasons, 18 better-adapted lines are being assessed at three sites in Zimbabwe, two sites in Malawi, and a site in Zambia this summer (Tichagwa, *pers. comm.*)

The International Working Group on Soybean Rust's method to determine severity (Shanmugasundaram, 1977) was found unsuitable for a differentiation of multiple phenotypes. Therefore, a comparative method was employed, whereby half of the test material was sprayed three times with a triazole, and the other half was left unsprayed. While the disease severity was still recorded, other parameters (i.e. differences in 100-seed mass, kernel yield, protein and oil contents) were taken into account when determining the phenotype.

Tichagwa will present further details of this program in his paper

Epidemiology

The meteorological data recorded at RARS between 1997-2003 has been analyzed to determine the parameters that influence field infection. A preliminary conclusion is that a max. temp. of 26-29 °C and a min. temp. of 15-17 °C, are required, with a diurnal range of 11-12 °C, and a dew point of 16-18 °C. Additionally, an early-morning humidity of > 80% is necessary, with 85%, optimal. These conditions were present at 42-43 dap in four seasons, when the plants were in late vegetative growth (Levy, 2003).

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Breeding for resistance to soybean rust in Zimbabwe

J. S. Tichagwa[1]

Introduction

The first outbreak of soybean rust (*Phakopsora pachyrhizi* Syd. & P. Syd.) occurred in 1998/99 in Zimbabwe. The most severe outbreaks occurred in 1998/99, 1999/2000 and 2000/2001. All the locally bred soybean cultivars are susceptible to this disease and fungicides have to be used to protect them against the disease. Breeding rust resistant cultivars is necessary to cut down on the use of fungicides and reduce cost.

Materials and methods

The first step in developing rust resistant genotypes is to identify sources of resistance to the disease. The next step is to cross these with local cultivars to create genotypes that are well adapted to local conditions. The third and final step is to identify from these new genotypes those that are resistant to rust and suitable for production.

Identification of soybean rust resistant genotypes

Rust resistant exotic soybean genotypes were sourced from Taiwan, Thailand, India, United States of America, Japan, China, Australia and Uganda. To date 75 of these have been tested in replicated trials for their response to fungicide in the presence of rust.

Visual assessments of disease lesions on the leaves are made to determine the presence and severity of rust. Fungicides are then used to determine the damage, if any, that the disease may cause.
Replicated trials are conducted using a split plot factorial design with genotypes as the main plot and fungicide as the sub-plot. Punch Xtra [Flusilazole (12.5%)/ Carbendazim (25.0%)] is the fungicide that has been used in these trials. The fungicide was applied to the sprayed plots of the trials at 6, 9 and 12 weeks after planting at the rate of 400ml of fungicide in 425 litres of water.

Visual assessments of the disease were carried out at the R6 stage of growth (after Fehr).

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Visual assessments of leaf rust

These were carried out on a two digit system (adapted from Shanmugasundaram) as follows:-

First digit: Position of leaf rust on the plant.
1. Bottom third of the leaves measured from the ground level.
2. Middle third of the leaves measured from the ground level.
3. Upper third of the leaves measured from the ground level.

Second digit: Density of rust lesions on the leaf.
1. No lesions noticeable.
2. Light lesions noticeable.
3. Medium lesion density noticeable.
4. Heavy lesion density noticeable.

Results

Results of some of the measurements made in Trial 1 in 1999/2000 are shown in Table 1 and those made in Trial 1 in 2000/2001 are shown in Table 2.

Discussion

The two seasons 1999/2000 and 2000/2001 were characterized by outbreaks of severe soybean rust. They were a good opportunity to identify genotypes that are resistant or tolerant to rust. One lesson learnt from the results of these trials is that the best indicator of resistance or susceptibility of a genotype to rust is the reaction of seed mass. The seed mass or seed size of a resistant genotype does not alter much in the presence of rust even if the rust is not controlled with fungicide. In addition, the crude protein and crude oil content of the seed of a resistant genotype do not change in the presence of rust even if the rust is not controlled. In contrast, the seed of a susceptible genotype gets badly shriveled by rust. Its crude protein content increases and the crude oil content decreases in the presence of rust.

Entries in Trial 1 of 1999/2000 tabulated in Table 1 include three genotypes that have been reported to carry dominant genes for resistance to rust. These are PI 230970 with the gene Rpp2, PI 462312 with the gene Rpp3 and PI 459025 with the gene Rpp4. As can be seen from Table 1, all the three are not resistant to the biotype of rust that occurs in Zimbabwe.

Ten of the entries in Trial 1 of 2000/2001, whose results are tabulated in Table 2, were tested for the second time, having been tested in the previous season. Nine of the entries showed the good resistance that they had

Table 1. Seed mass, crude protein content in the seed, crude oil content in the seed, seed yield and rust scores from Trial 1 in 1999/2000.

Genotypes	Fungicide Applied	Leaf rust Position	Leaf rust Scores	Mass of 100 seeds	Percent crude protein	Percent crude oil	Seed Yield Kg/ha	Percent Yield change
Solitaire	1	3.0	2.7	15.5	49.1	15.3	1532	
	2	1.0	1.0	22.6	43.1	20.1	3592	134
GC 86049-35-2-1-1-8 IN B	1	3.0	2.3	19.5	44.8	18.2	1675	
	2	1.0	1.0	24.0	44.4	18.4	2314	38
GC 86018-427-3B	1	2.3	2.3	20.2	44.5	18.2	1998	
	2	1.7	1.3	20.9	44.2	18.9	2629	32
GC 84058-21-2	1	2.3	2.7	24.3	45.2	19.1	1739	
	2	1.0	1.0	31.0	45.3	18.9	2111	21
Ankur (PI 462312)	1	1.7	2.0	13.1	51.9	14.1	1355	
	2	1.0	1.0	20.9	48.0	17.0	3109	129
S5-UG	1	2.0	2.0	16.0	48.0	16.2	1787	
	2	1.0	1.0	18.5	48.1	16.5	1967	10
GC 84058-18-4	1	2.3	2.0	24.3	45.4	18.8	2083	
	2	1.0	1.0	26.1	44.9	18.8	2287	10
Sonata	1	3.0	3.0	14.3	49.9	14.2	916	
	2	1.0	1.0	28.3	44.0	18.6	3352	266
GC 86049-35-2-1-1-8-IN A	1	3.0	2.0	19.3	44.0	18.8	1709	
	2	1.0	1.0	22.1	44.7	18.3	2335	37
GC 86004-9	1	3.0	2.3	15.1	46.5	17.1	1744	
	2	1.0	1.0	20.3	45.9	18.0	2462	41
PI 459025	1	3.0	3.7	18.6	51.2	14.1	754	
	2	1.0	1.3	27.5	47.7	16.8	1786	137
GC 84051-32-1	1	3.0	2.7	11.8	47.2	15.4	1709	
	2	1.0	1.0	16.2	45.8	17.2	2219	30
AGS 190	1	3.0	3.0	23.0	47.9	16.6	1455	
	2	1.0	1.0	34.4	46.6	18.4	2188	50
PI 230970	1	2.3	2.3	10.8	50.5	14.6	1056	
	2	1.7	1.7	15.6	45.9	17.2	1344	27
GC 00138-29A	1	1.0	1.0	20.8	47.1	17.2	2542	
	2	1.0	1.0	21.8	46.4	17.4	2425	-5
GC 84040-27-1	1	3.0	2.0	13.9	48.0	16.2	1674	
	2	1.0	1.0	18.5	47.5	17.2	2286	37
SRE-C-56A	1	2.0	1.7	20.0	48.4	16.1	1608	
	2	1.7	1.3	23.2	47.8	16.8	2112	31
AGS 229	1	2.7	2.7	15.2	45.0	17.1	1959	
	2	1.0	1.0	16.9	44.7	17.2	2362	21
GC 00138-29B	1	2.3	1.7	20.4	47.5	16.8	2558	
	2	1.0	1.0	20.8	46.6	17.4	2482	-3
GC 84058-21-4	1	1.0	1.0	28.9	46.2	18.6	1921	
	2	1.0	1.0	28.9	45.7	18.4	1791	-7
GC 86018-427-3A	1	3.0	2.3	17.4	44.5	18.4	1971	
	2	1.0	1.0	20.4	44.1	18.8	2430	23
SS 86045-23-2	1	3.0	3.0	22.0	44.6	17.5	1431	
	2	1.0	1.0	29.0	44.6	17.9	1875	31
Means		1.8	1.7	20.7	-	-	2014	
S.E. (mean) ±		0.39	0.34	0.69	-	-	124.0	
Coefficient of variation %		37.2	35.5	5.8	-	-	10.7	

Notes.
Fungicide applied
1 = No fungicide applied.
2 = Punch Xtra applied at the rate of 400ml of fungicide in 425 litres of water per hectare applied three times, at 6, 9 and 12 weeks after planting.

shown the previous season. The tenth entry, L 87-0482, showed less resistance than it had shown the previous season. The other entries in this trial were commercial cultivars. These are all susceptible to rust.

As a way of identifying resistance to rust, the use of fungicide is reliable and consistent.

Table 2. Seed mass, crude protein content in the seed, crude oil content in the seed, seed yield and leaf rust scores from Trial 1 in 2000/2001.

Genotypes	Fungicide Applied	Leaf Rust Position	Leaf Rust Scores	Mass Of 100 Seeds	Percent Crude Protein	Percent Crude Oil	Seed Yield Kg\ha	Percent Yield change
	1	1.5	1.7	20.1	47.1	17.3	2248	
GC 00138-29B	2	1.0	1.0	20.3	46.9	17.3	2109	-6.2
	1	3.0	3.3	15.2	47.5	16.2	2219	
Soma	2	1.5	1.3	19.7	45.7	17.6	3472	56.5
	1	2.3	2.7	15.0	47.0	17.1	1887	
S5-UG	2	1.0	1.0	18.3	47.0	17.0	1984	5.1
	1	1.5	1.5	23.9	47.0	16.4	2135	
GC 60020-8-7-7-18B	2	1.0	1.0	26.2	46.6	16.8	1993	-6.7
	1	1.3	2.0	21.9	46.5	17.9	2448	
PI 200466	2	1.0	1.0	22.0	47.2	17.6	2413	-1.4
	1	1.5	1.7	24.3	45.7	18.9	2434	
GC 84058-18-4	2	1.0	1.0	27.0	45.6	18.7	2427	-0.3
	1	1.7	2.3	19.7	47.6	17.1	2222	
GC 00138-29A	2	1.0	1.0	22.0	47.5	17.1	2116	-4.8
	1	3.0	3.3	17.0	46.8	16.7	1606	
Sonata	2	1.0	1.0	29.7	45.0	18.2	3450	114.8
	1	1.5	2.0	28.5	46.3	18.4	2241	
GC 84058-21-4	2	1.0	1.0	30.0	45.9	18.7	2271	1.3
	1	1.5	2.0	17.3	44.8	17.6	2050	
CM-60-10KR-71	2	1.0	1.0	19.2	44.6	17.5	2002	-2.3
	1	1.5	1.5	21.8	46.9	16.5	1884	
GC 60020-8-7-7-18A	2	1.0	1.0	22.6	46.7	16.7	2040	8.3
	1	3.0	4.0	12.7	47.2	15.0	1969	
Storm	2	1.0	1.0	20.5	45.2	17.4	3583	82.0
	1	2.5	2.3	20.1	46.0	18.5	2076	
L 87-0482	2	1.0	1.0	21.5	45.9	18.6	2498	20.3
	1	3.0	4.0	17.1	45.8	17.8	2378	
Solitaire	2	1.0	1.0	20.7	43.7	19.6	3632	52.7
	1	3.0	4.0	13.7	47.1	15.4	1964	
Safari	2	1.0	1.0	20.3	44.8	17.8	3604	83.5
Means		1.6	1.8	20.9	46.3	17.4	2379	
S.E. (mean) ±		0.21	0.19	0.96	0.29	0.16	110	
Coefficient of variation %		27.0	20.9	9.2	1.2	1.8	9.2	

Breeding for soybean rust resistance

To date twenty-five genotypes have been identified as resistant to rust. These have been crossed with local genotypes that are adapted to Zimbabwean conditions. Generations of the created populations are advanced by the single seed descent method to produce pure lines. Two generations are grown per year, one in summer and the other in winter.

At the end of the 2000/2001 season, 1960 selections were taken from the F_4 rust resistant populations. These were evaluated in progeny plots in 2001/2002. Fifty-seven selections were taken from these at end of the season for testing in replicated trials in 2002/2003.

In 2002/2003 the season was dry and not much rust developed. In spite of this, however, eighteen of the fifty-seven selections were deemed to be as

good as the control variety Solitaire on agronomic traits. Some of the results of the measurements made on these eighteen selections in the progeny rows of 2001/2002 are shown in Table 3. These have been included in a trial to evaluate their reaction to fungicide in the presence of rust. This trial will be grown at four sites in Zimbabwe, two sites in Malawi and one site in Zambia.

Table 3. Number of days to shatter, number of days to mature, seed yield in grams per plot and leaf rust scores of progeny plots of 2001/2002.

Genotypes	Leaf Rust Position	Leaf Rust Scores	Number Of days To shatter	Number Of days To mature	Seed Yield In g/ plot
Solitaire(mean of 20 plots sprayed)	2	2	35	120	852
S676/4/31	3	3	30	117	805
S676/4/37	3	3	34	120	901
S684/4/3	2	1	24	130	852
S684/4/27	2	1	38	128	830
S688/4/17	3	1	43	121	906
S696/4/77	3	1	23	132	805
S696/4/78	3	1	25	126	817
S696/4/104	3	1	36	115	802
S696/4/118	3	1	16	124	884
S697/4/11	1	1	35	124	807
S697/4/22	1	1	34	135	871
S697/4/25	3	1	25	126	874
S697/4/26	3	1	27	124	947
S697/4/53	1	1	35	124	842
S700/4/4	3	2	41	110	901
S700/4/17	2	2	40	111	999
S701/4/19	3	1	27	124	819
S701/4/20	3	1	22	124	895

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Soybean rust research in South Africa

P.M. Caldwell[1] and N. W. McLaren[2]

Abstract

South Africa produces approximately 200,000 tons of soybeans annually on 193,000 hectares of land. Over 600,000 tons of soybeans are imported each year. Soybean rust, caused by *Phakopsora pachyrhizi* Syd., was first reported in South Africa in February, 2001 in the Vryheid, Piet Retief, Karkloof, Greytown and Winterton/Bergville farming areas of KwaZulu-Natal. A National Soybean Rust Task Team including the Department of Agriculture and Environmental Affairs, Protein Research Foundation, Agricultural Research Council, seed and chemical companies and several universities was formed to control the disease. Current research programmes include studies on germination, epidemiology, electron microscopy, risk analysis, loss assessment, cultivar resistance/tolerance, agronomic and fungicide control programmes. Influence of temperature, leaf wetness duration, humidity and leaf age are being investigated under controlled environmental conditions. Data from 2001/02 and 2002/03 field trials showed that rust development is closely related to host development stage, with a rapid increase in disease severity from growth stage R3 onward. Disease severity differed with planting date but this was dependent on weather conditions and results were not consistent. Row spacing had a significant effect on disease severity. In unsprayed plots, rust was up to 16% more severe in 45cm rows compared to 90cm rows. However, in fungicide treated plots rust was up to 40% more severe in 45cm rows than in 90cm rows. This was attributed to the penetration of chemicals. Timing of sprays was critical and late spraying, after symptom development, did not significantly reduce disease severity or associated losses. Yield loss was directly related to disease severity/area under disease progress curve. Comparison of yield losses in sprayed and unsprayed plots indicated that losses were generally smaller in the short season cultivars tested, increasing with maturity group. Losses were locality (weather) dependent with losses being more extensive in the more humid trial areas. The 1000 kernel mass of harvested seed followed a similar tendency with the greatest mass reduction associated with the longest season cultivar. Changes in kernel oil and protein content with rust were minimal. Initial results suggest a yield loss threshold when approximately 30% of the leaf area is infected at growth stage R6. A number of effective fungicides, mainly triazoles, have been registered for rust control. All local cultivars are susceptible to the rust but several lines from Zimbabwe have been found to be resistant to the South African race of the pathogen.

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Introduction

South Africa produces approximately 200,000 tons of soybeans annually on 193,000 hectares. Over 600,000 tons of soybeans, valued at over US$142 million, are imported annually to meet local demands. *Phakopsora pachyrhizi* (Syd.), the causal organism of soybean rust, is thought to have been wind-borne from Asia to Central Africa (Kawuki et al., 2003). The pathogen moved south and by 1997 caused yield losses of 60-80% in commercial crops in Zimbabwe, before arriving in South Africa in February 2001. It rapidly spread to the Vryheid, Piet Retief, Karkloof, Greytown and Winterton/Bergville farming areas of KwaZulu-Natal (KZN) and Mpumalanga. Rust is now believed to be endemic and a major threat to sustainable soybean production. A National Soybean Rust Task Team including the Department of Agriculture and Environmental Affairs (DAEA), Protein Research Foundation, Agricultural Research Council (ARC), seed and chemical companies and several universities was established to develop a collaborative research programme to control the disease. The aim of this paper is to highlight current research on soybean rust in South Africa.

Identification

Molecular identification confirmed that the South African race of soybean rust is *Phakopsora pachyrhizi,* and not the less virulent new world species, *P. meibomiae* (Pretorius et al., 2001).

Overwintering debris trials

P. pachyrhizi has a host range of 95 species in 42 genera in the Fabaceae, making it difficult to control inoculum (Keogh, 1978). Alternate hosts have yet to be identified in South Africa. As rust fungi are obligate parasites, it is assumed that uredospores are unable to survive on soybean debris during winter. However, long germ tubes were observed from uredospores on debris, and trials were undertaken to ascertain whether these uredospores were viable. Approximately two kilograms of debris were collected from soybean fields at Cedara, near Pietermaritzburg, in June 2001. Half the sample was stored at room temperature (approx. 20°C) and the other half refrigerated at 4°C for six months. A suspension of debris was plated onto water agar and incubated at 25°C for 72hrs. Light microscopy showed that no new germ tubes were visible and germ tubes from the previous

season did not increase in length, on cold-stored or room-stored debris, confirming that *P. pachyrhizi* is unable to overwinter on non-living plant material.

Germination trials

A high uredospore suspension (2.5 X 10^5 spores per ml) in distilled water, from uredospores stored at -70°C for 4 months, was inoculated onto water agar and incubated at 5, 11, 16, 20, 22, 27 and 29°C and 100% humidity (RH). Plates were left in the dark (24hrs) to favour germination (Melching et al., 1989). Germination was considered to have taken place if germ tubes were as long, or longer, than the length of the uredospore. Maximum germination occurred between 15-25°C, with an optimum at 20°C. At higher and lower temperatures, percentage germination decreased rapidly. This range is similar to that reported by Marchetti et al., (1976). Germination trials will continue in 2003/04 using fresh uredospores from the field to ascertain more accurate germination percentages at different temperatures.

Electron microscopy

Electron microscopy confirmed that local isolates of *P. pachyrhizi* penetrate the host leaf directly through the cuticle as reported by Koch et al., (1983), as opposed to the more conventional stomatal penetration employed by many rust fungi in the uredial stage. Under optimal environmental conditions (i.e., 25°C and 100% RH), germination commenced 6 hours post inoculation (hpi). Penetration of the epidermal cell wall occurred 11hpi. Electron microscopy studies in 2003 showed the presence of a hyperparasite. Under incubation very few spores were observed which could be due to the presence of the hyperparasite. Pothidee et al. (1980) identified a hyperparasite, as *Tuberculina* sp. on *P. pachyrhizi* when plants were incubated under similar conditions. However, sporulation of *Tuberculina* sp. decreased when it was subcultured and poor germination was observed from conidia produced under both natural and artificial conditions.

Epidemiology studies

A tolerant (LS677) and susceptible soybean cultivar (LS5995) were inoculated in a dew chamber with a conidial suspension (2.5 X 10^5 spores per ml) and incubated at various combinations of temperature (15, 20 and 25°C), leaf wetness duration (8, 16, 24 hrs), RH (65, 85 and 100%) and leaf age (old, medium and young). Disease symptoms were rated weekly until physiological maturity. Although disease severity differed between cultivars, data to date suggest that disease severity was greatest at 25°C

and 100%RH but there was very little difference between 16 and 24 hrs. leaf wetness and leaf age. Several combinations of these environmental parameters need to be completed during the 2003/04 season before any conclusions can be drawn from this research. Results will be incorporated into a model for predicting the onset of soybean rust at the beginning of summer as well as to time fungicide applications based on weather conditions, rather than on the calendar-base system currently used.

Cultivar trials

South African cultivars were screened in Zimbabwe from 1998 and were all found to be susceptible to *P. pachyrhizi*. Six lines from Zimbabwe tested locally showed resistance to the pathogen in 2002/03 trials. Fifteen rust resistant lines from the Asian Vegetable Research and Development Centre in Taiwan were evaluated in pot trials. All were susceptible to the South African race of *P. pachyrhizi*. A further 27 lines from a commercial seed company were tested in pot trials during 2002/03 and yielded levels of tolerance while one was resistant.

Effect of planting date and row spacing on disease onset, development and severity

Field trials were planted at Piet Retief and Cedara and included three varieties i.e. PAN494 (short), LS666 (medium) and SCS1 (long) and two row spacings, ie., 45 and 90cm rows. Trials were replicated three times during 2001/02 and four times during 2002/03 at each locality. Planting of replicates was spaced from late October to early January. At commencement of flowering, one block of each row spacing was sprayed with flusilazole/carbendazim (800ml ha^{-1}) at 21-day intervals. Disease development was monitored at weekly intervals. Since both rust and leaf drop (LD) are responsible for decreased photosynthetic area of plants, an integrated rating of disease severity was used, i.e., affected leaf area (ALA) = LD (%) + rust (%) on remaining leaves. Thus ALA = LD + ((100-LD) * (rust/100)). The assumption was made that leaves discoloured by rust are no longer photosynthetic. At maturity, trials were harvested and grain yield, 1000 kernel mass and protein and oil content were determined.

During 2001/02, rust development was closely related to host growth stage and this was confirmed during 2002/03. Although differences in rust development in the three maturity groups were recorded on a time scale, these differences were not apparent on a physiological scale and indicate that the disease is largely host-physiology driven under field conditions. Weather effects were also noted over localities and planting dates, as

indicated by area under disease progress curve (AUDPC), within genotypes. The relative effect of weather variables is still under analyses but leaf wetness appears to be a major variable affecting disease onset. Rust during 2002/03 was delayed at Cedara until mid-March when leaf wetness exceeded 10hrs per day. This may explain why rust is limited to the cooler, high humidity areas of the lowveld and coastal belt.

The effect of planting date was more pronounced in the shorter than the later maturing cultivars. At Cedara during 2002/03, AUDPC increased from 728.7 to 2253.6 in PAN494, from 1454.1 to 1665.3 in LS666 and from 1759.4 to 1974.2 in SCS1 over the four planting dates. This was attributed to reduced temperatures as the season progressed and increased hours of leaf wetness. This effect was, however, not sufficient as a control strategy to reduce disease severity to levels that would reduce significant yield losses.

In unsprayed plots during 2001/02, disease was up to 16 % more severe in 45cm rows than 90cm rows, although this effect was maturity group dependent. During 2002/03 results were variable but the drier conditions were more effective in reducing disease in the 90cm rows because of aeration. Despite these differences, it was apparent that row spacing as a practice to reduce disease to below damage thresholds, was ineffective. In contrast, rust was up to 40 % more severe in sprayed 45cm rows than in 90cm sprayed rows. This difference was probably related to penetration of the canopy by fungicides, which was reduced in the narrower rows.

Timing of fungicide sprays was critical during both seasons. Delayed spraying at Cedara until growth stage R5 in PAN494 during 2001/02 resulted in minimal reduction in disease severity (4.1 % based on AUDPC) compared with a 63.4 % reduction when sprays were applied prior to R2. During both seasons, fungicide sprays applied during early flowering reduced disease by 43 to 64 % at Piet Retief and by up to 80 % at Cedara, with concomitant yield effects.

Despite differences in disease severity with planting date, yield differences between early (October or early November) and later plantings (mid-December) outweigh any benefits that early planting has on disease severity. At Piet Retief, mean yields in sprayed plots over all varieties and row widths were 2.6 and 1.5t ha^{-1} in October and December plantings, respectively, and 2.0 and 1.3t ha^{-1} at Cedara. Similarly, during 2002/03, yield in the later plantings was up to 31.8% lower in later plantings compared to earlier plantings.

The extent of yield differences between sprayed and unsprayed plots was dependent on maturity group. This was apparent during both seasons. At first planting during 2001/02 at Piet Retief, yield differences between sprayed and unsprayed plots were 0.1t ha^{-1} in PAN494, 0.6t ha^{-1} in LS666 and 1.6t ha^{-1} in SCS1. This tendency was recorded in all plantings at both Cedara and Piet Retief during 2001/02 and 2002/03. It was apparent that the shorter maturity group may be able to escape the effects of disease more readily than the longer growers. Provisional analyses suggest that kernel size accounts for approximately 34.8% of yield losses while the remainder is due to pod/grain losses. Changes in protein and oil content due to the presence of rust were minimal.

Fungicide control

Extensive fungicide trials have been conducted by the DAEA at Cedara, KZN. Timing of sprays was critical and late spraying, after symptom development, did not significantly reduce disease severity or associated losses. A number of effective fungicides, mainly triazoles, have been registered for rust control. Fungicide efficacy and persistence, dosage rates, timing, curative effects will be discussed under "Chemical control of soybean rust, *Phakopsora pachyrhizi*, in South Africa" by du Preez (these proceedings).

Germplasm bank

Potential sources of resistant soybean breeding lines and cultivars for utilization in local breeding programmes is collected and maintained by the ARC-Grains Crop Institute. The soybean germplasm bank consists of 2162 true breeding lines and populations. All base collection entries were made by institutions in the USA, Taiwan, Zimbabwe, Canada, Brazil and Argentina.

Soybean breeding

The aim of this programme is to create segregating material so that maximum diversity is created for the selection of superior agronomic qualities. The breeding of cultivars resistant to soybean rust has been added to the list of breeding objectives.

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Soybean rust in Paraguay, evolution in the past three years

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Introduction

The soybean rust caused by *Phakopsora pachyrhizi* Sydow, is considered very destructive and in those places where it occurred there were important economic losses. Currently it is spread to many parts of the world.

In Paraguay soybean rust was first detected on commercial fields in March 2001, in the District of Pirapó, State of Itapua. Based on samples sent to the Foreign Diseases Weeds Sciences Research Unit, USA, the occurrence of the "Asian" rust was confirmed in the American Continent. On most infected cultivars severe defoliation was observed reducing the cycle by 25-30 days. The cultivar most affected was BR 16, with yield reduction estimated in more than 60 %. Fungicide experiments carried out showed good control of the disease, especially those of the chemical groups of triazols and strobilurins, and less with the benzimidazoles. Preliminary evaluation of resistance among commercial varieties, practically all showed susceptibility to the pathogen. The disease has spread to the main soybean regions in Paraguay.

Field and laboratory identification

Leaf symptoms are small lesions or brownish-gray dots with angular margin on the upper leaf surface. On the lower leaf surface the lesions are light- to dark-brown, with a small light-gray elevation in the center where uredia and intense hyaline urediniospores are observed. On highly susceptible cultivars the sporulation can occur on both leaf surfaces which can be seen by tilting the leaf in a position to have the sun light coming from one side so as to make a shadow of the uredia or by looking through a hand lens with 20x magnification.

Places where rust is present

In 2000/01 crop season, an extensive soybean area was affected in Paraguay, but it was not noticed by the farmers due to lack of knowledge of the symptoms which are easily mistaken by other diseases such as

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brown spot (*Septoria glycines*). Nevertheless, some farmers had noticed that soybean maturation was much faster than normal. The places where the disease was recorded went from Capitán Miranda, La Paz, Colonias Unidas, Pirapó, Capitán Meza, Maria Auxiliadora, Naranjito, Iruña, Naranjal, Santa Rita and Colonia Yguazú. In all these places the disease was found mainly on cultivars MG/BR 46 Conquista, COODETEC 205 and on volunteer plants that survived the Winter.

In 2001/02, the first detection was also at Pirapó, where cultivar BRS 133, growing close to kudzu (*Pueraria lobata*), used for cattle grazing, was severely infected. Rust was also detected in other counties such as La Paz, Bella Vista, Pirapey, Maria Auxiliadora, Naranjito, Santa Rita, Colonia Yguazú, Cruce Itakyry y Troncal 3. During this crop season rust severity was comparatively lower than in 2000/01 due to drought but on later plantings disease severity was much greater, especially on cultivar MG/BR 46 Conquista. On some farms yield reduction was more than 50%.

In 2002/03, rust showed up on 30-35 days old plants but did not evolve on early planted soybeans due to high temperatures but was very severe on late plantings.

Effect of the disease

The effect of rust could be seen directly in the field. Infected plants defoliated rapidly even when plants remained green; the grains were smaller with drastic reduction in seed weight and looked like lentils without completing maturation. Yields were significantly reduced with more than 1,100 kg/ha. between fungicide treated and untreated plots (Table 1).

Evaluation of genetic resistance

In order to determine the level of resistance to rust 49 commercial cultivars grown in Paraguay were tested in the greenhouse and under field conditions. In the greenhouse 1m row of each cultivar was sown spaced at 35cm; at 25-days after germination the plants were uniformly inoculated with a spore suspension containing 150,000 spores/mL + Tween 20, using a hand sprayer; after inoculation the plants were covered with a plastic sheet for 24hr to provide high humidity for the germination of the spores.

The field tests were set up at Naranjal (Alto Paraná), and at CRIA´s Tomas R. Pereira Experiment Station (Itapúa). Two 3 m. rows of each cultivar

were sown, spaced at 60cm between rows and divided in blocks 1.5m apart; the whole set of 49 cultivars was sown at four different dates (October, November, December and January) and rust development depended on natural infection. For the disease rating for both greenhouse and field tests a scale of rust severity based on percent leaf area infected (% lai) was used, taking the most infected leaf on each plant at growth stage R6 (full pod) and divided into five categories of reaction: R = resistant ; MR = moderately resistant; MS = moderately susceptible; S = susceptible, and HS = highly susceptible.

The results of both greenhouse and field tests (Table 1) showed that, except for cultivar FT 2001, which was moderately resistant, all cultivars were rated as moderately susceptible to highly susceptible (Table 2).

Table 1. Efficacy of fungicides for rust control in 2001/02. CRIA, Itapua, Paraguay, 2002.

Fungicide	Dosage c.p. L/ha	a.i. g/L	Yield		
			kg/ha	kg/ha difference	% difference
Check	---	---	1,940 b *		
Trifloxystrobin + Ciproconazole	0.40	187.5 + 80	3,072 a	1132 **	58 **
Carbendazim	0.50	500	2,288 b	348	17
Difenoconazole + Propiconazole	0.15	250	2,737 a	797	41
Piraclostrobin + Carbendazim	0.20	250 + 500	2,774 a	834	42
Azoxystrobin	0.20	250	2,720 a	780	40
LSD(kg.)			362.6		
CV (%)			7.7		

* Numbers with same letters did not differ at 5% probability. ** Yield difference from check.

Table 2. Reaction of commercial cultivars to rust under greenhouse and field conditions during 2001/02 crop season. CRIA, Itapúa, Paraguay. 2002.

Cultivar	Greenhouse	Field
1. UNIALA	AS *	S *
2. CRIA 2 DON RUFO	S	S
3. CRIA 3 PUA´E	MS	MR
4. AURORA	AS	AS
5. CD-202	S	S
6. NK SPRING 5.3	S	MS – S
7. NK COKER 8.1	S	MS – S
8. NK COKER 8.1 SC	S	MS – S
9. NK COKER 6738 SC	S	MS – S
10. ANDREA 66	S	MS – S
11. FT JATOBA	S	S
12. EMBRAPA 4	S	MS – S
13. EMBRAPA 48	S	MS
14. EMBRAPA 58	S	MS
15. OCEPAR 16	S	MS – S
16. MG/BR 46 CONQUISTA	AS	AS
17. BR 16	AS	S
18. BRS 132	S	MS – S
19. BRS 133	S	MS – S
20. BRS 134	S	MS
21. BRS 137	MS	MS
22. BRS 154	S	MS
23. BRS 155	S	MS – S
24. BRS 156	S	MS – S
25. BRS 183	S	S
26. BRS 184	S	MS – S
27. FT 2001	MR	MR – R
28. FT 2008	S	MS
29. CD 201	S	MS – S
30. CD 205	S	MS – S
31. CD 206	S	MS – S
32. CD 207	S	MS
33. CD 208	S	MS – S
34. CD 209	S	MS
35. CD 210	S	MS
36. NK CAMPEONA 6.4	S	S
37. MSOY 7101	S	S
38. MSOY 7701	S	MS
39. EUREKA 5.1	AS	S
40. ST 932056	S	S
41. FT ESTRELA	S	S
42. CORDELL	MS	S
43. BEDFORD	MS	MS – S
44. BRS MG RENASCENÇA	S	MS – S
45. BRS MT PINTADO	S	S
46. EPPS	S	S
47. BRS MG LIDERANÇA	S	S
48. GORDON	S	S
49. X 056	S	S

*** Reaction to rust:** R = resistant ; MR = moderately resistant; MS = moderately susceptible; S = susceptible, and HS = highly susceptible.

Soybean rust in Taiwan

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Abstract

Soybean rust was once a serious disease on soybean crops in Taiwan. The disease was most severe during spring and fall seasons and could reduce yields by 18 to 91%. Studies in Taiwan show that: 1) high humidity is needed to initiate infection; 2) variations in environmental conditions cause year-to-year, season-to-season, and location-to-location variations in disease severity; 3) inoculum concentrations to initiate infection do not reflect differences in severity of the disease; 4) resistant/tolerant cultivars suffer much less yield losses compared to susceptible cultivars; 5) genetic resistance is not durable in the field; and 6) resistant cultivars are not necessarily higher yielding. Due to the decline in soybean area, judicious use of fungicides, and the sowing of resistant/tolerant cultivars, soybean rust disease has almost disappeared on the island.

Introduction

Soybean (*Glycine max* L.) was introduced to Taiwan from Mainland China at the end of 15th century as a fodder crop. Around 1948, soybean became a grain crop and production levels increased dramatically. Production levels rose from 12,500 tons grown on 20,300 ha in 1950 to 52,700 tons grown on 59,600 ha in 1960 (Table 1). Thereafter, the area planted to grain soybean steadily declined despite the development of improved cultivars that contributed to increases in yields from 600 kg/ha in 1948 to 2000 kg/ha in 1987 (Cheng and Shanmugaunsdaram, 1992). Taiwanese farmers cannot compete in today's global soybean markets and the area now planted to grain soybean on the island has become negligible.

Soybean rust caused by *Phakopsora pachyrhizi* Sydow and downy mildew caused by *Pseudopernospora manshurica* were listed at one time as the most important soybean diseases in Taiwan (Cheng and Chan, 1965). Soybean rust was first reported in Japan in 1902 (Kiritani and Inoue, 1960). In 1906 it was reported in India and in 1914 it was recorded in Papua New Guinea, Philippines, and Taiwan. The fungus was first described from the infected leaf of yam bean, *Pachyrhizus erosus* in 1913 in Taiwan (Kiritani

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and Inoue, 1960). Soybean rust has been observed in the local wild *G. soja*, *G. tabacina* (Labill.) Benth, and *G. tomentella* (syn. *G. tomentosa*) populations (Yeh, 1981; Hartman, et al. 1992; Kiritani and Inoue, 1960).

In Taiwan, three soybean crops are grown per year: spring (Feb./March to May/June), summer (June/July to Aug./Sept.), and autumn (Aug./Sept. to Oct./Dec.). Soybean rust disease is most serious during the spring and autumn seasons (AVRDC 1977; Bromfield, 1984). The spring growing season is characterized by daylengths increasing from 10 to 13 hr and temperatures rising from 20 to 32°C from sowing to harvest. Much of the spring season is dry but relative humidity is often over 80%. At the time of harvest, the rainy season begins and sometimes the rains affect seed quality. In contrast, the autumn growing season is characterized with daylengths decreasing from 12 hr 30 min to 10 hr 30 min and temperatures dropping from 32 to 24°C. Under the above environmental conditions, the uredinia profusely sporulate and release spores (Yeh, 1981). High temperatures reduce the severity of soybean rust in the summer.

At AVRDC, teliospores were observed in soybean fields from November through March with maximum production in December to February when the field temperature was less than 20°C for more than 15 hr daily and the mean temperature did not exceed 25°C for at least 15 days (Yeh, 1981; Yeh et al., 1982). Artificial inoculation of spores in March under dry weather conditions failed to initiate infection (Yeh, 1981). In a related study, Yeh (1981) showed that yield differences were insignificant among plots inoculated with 2.5×10^4, 5×10^4, and 10×10^4 spores/ml and naturally infected plots.

Yield losses due to soybean rust have been estimated at 20–30% (Liu, 1966), 70–80% in individual fields (Hsu and Wu, 1968), 18–68% (AVRDC, 1976), 20–63% (Yeh et al., 1981; Yeh, 1983), and 48–91% and 58–90% for spring and fall crops, respectively (Tschanz and Tsai, 1983).

To control soybean rust disease, Yeh (1983) recommended farmers to begin spraying fungicides when the first lesion is observed and continue spraying at 7–14 day intervals until close to plant maturity. Recommended products (active ingredients) included Dithane M-45 and mancozeb (mancozeb), Bayleton (triadimefon), Plantvax (oxycarboxin), and Saprol (triforine).

Another strategy used to minimize rust disease has been the use of resistant cultivars. In 1965, the entire U.S. germplasm collection was screened for resistance to soybean rust in the field with natural infection by the Taiwan Agricultural Research Institute (TARI). They identified PI 200492, PI

200490, and PI 200451 to be resistant to soybean rust and used them to develop rust resistant cultivars (Cheng and Chan, 1965) (see related paper "Breeding for soybean rust resistance in Taiwan and AVRDC" in the proceedings for additional information). Shin (1986) reported 22% yield loss due to rust in tolerant cultivars compared to 69% in susceptible cultivars. Similar results were found by AVRDC (1976). Despite the noteworthy accomplishments of plant breeders, the genetic resistance has not been stable or durable in the field, and the resistant lines are not necessarily the most productive.

Today most of the soybean grown in Taiwan is vegetable soybean. Vegetable soybeans are harvested after pods have filled their seeds (R6.5 growth stage), but leaves are still green. Since 1995 rust infection has been very minor and usually one fungicide spray will control the disease. In recent years it is even difficult to find rust in the soybean fields.

In summary, studies in Taiwan show that: 1) high humidity is needed to initiate infection; 2) variations in environmental conditions cause year-to-year, season-to-season, and location-to-location variations in disease severity; 3) inoculum concentrations to initiate infection do not reflect the differences in severity of the disease; 4) resistant/tolerant cultivars suffer much less yield losses compared to susceptible cultivars; 5) genetic resistance is not durable in the field; and 6) resistant cultivars are not necessarily higher yielding.

Table 1. Grain soybean area, production, and yield in Taiwan, 1950–2000.

Year	Area ('000 ha)	Production ('000 t)	Yield (t/ha)
1950	20.3	12.5	0.6
1960	59.6	52.7	0.9
1970	42.8	65.2	1.5
1980	15.3	25.9	1.7
1990	4.2	8.1	1.9
2000	0.2	0.3	1.5

Table 2. Vegetable soybean area, production, and yield in Taiwan, 1950–2000.

Year	Area ('000 ha)	Production ('000 t)	Yield (t/ha)
1980	0	0	0
1985	6.7	38.6	5.8
1990	10.4	59.2	5.7
1995	9.2	74.4	8.1
2000	9.0	74.3	8.3

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Transgenic crops for insect resistance

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Abstract

Pest insects cause significant yield losses to agricultural crops each year. To deal with such a detrimental impact, farmers rely primarily on applications of insecticides. Unfortunately chemical control may have negative effects on the environment. In addition, the efficacy of insecticides is often affected by weather and cultural conditions. The recent remarkable progresses in insecticidal toxin genes and crop transformation methods have made intrinsic insecticidal crops available to farmers as an alternative to fight insect pests more effectively and economically. Many insecticidal protein toxins have been identified and molecularly characterized. Some were tested for *in planta* expression in crop plants. Only transgenic crops with *Cry* genes isolated from *Bacillus thuringiensis* (Bt) have been commercialized to date. These transgenic crops target Lepidopteran and Coleopteran insects. Cry proteins are highly efficacious against certain target insect species, and *Cry* genes are usually straightforward for transformation and gene expression in crop plants. Thus far, the predominant commercialized transgenic crops with insect resistance traits are still limited to corn and cotton. Those transgenic crops are cultivated on several million hectares around the world, mostly in North America. Insecticidal genes and the generation of transgenic crops for insect resistance are reviewed and discussed.

Introduction

Transgenic crops for insect control provide an attractive farm practice strategy for farmers to guard against insect damage. This new technology has significant advantages over the traditional chemical pesticide applications in efficiency, safety and cost savings. With transgenic insect resistant crops farmers reduce the risk of exposure to chemicals and save greatly in labor and chemical costs. These transgenic crops provide built-in insecticidal active ingredients that are always present to deal with insect attacks, even for those insects that are difficult to reach by chemical applications. Reduction of insecticide applications decreases the impact on the environment from the chemicals. In addition to these advantages, transgenic crops are flexible to weather conditions and varying farm operations.

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Transgenic insecticidal crops need at least three components: insecticidal genes, efficient transformation methods and optimization protocols for gene expression *in planta*. Academic institutions and agriculture biotechnology industries have given tremendous effort to develop new technologies to meet these needs. So far many insect-resistance genes have been identified and cloned from microbes, plants, animals and other origins by large-scale screening programs. New methods for crop transformation, promoters and protocols for gene optimization are available. Maturation of those technologies have made it possible to generate transgenic insecticidal crops to dramatically extend the scope of resistance genes available to plant breeders.

The first transgenic insecticidal plant experiment was published by Hilder *et al.* in 1987. To date, approximately 40 different insecticidal genes have been incorporated into crops (Schuler *et al.*, 1998). Over the past few years, impressive progress has been made in expressing insecticidal genes *in planta* and creating insect resistant plants through new plant transformation methods. A major milestone of this process occurred in 1995-1996 when Cry3Aa potato for Colorado potato beetle control, Cry1Ac cotton for Lepidopteran insect control and Cry1Ab maize for European corn borer control were introduced into the marketplace (Peferoen, 1997). Since then, many different transgenic crops have been produced and commercialized.

Source of insecticidal genes

Most of the insecticidal genes known so far were identified from microbes, especially bacteria. Unlike eukaryotic organisms, bacterial genes have no introns, and the protein toxins are usually not processed. On the contrary, insecticidal genes from fungi, plants and animals are usually more complicated to characterize and clone due to delicate gene structures and post-translational modifications.

Prokaryotic sources

Many microbial strains that have insecticidal activities have been discovered in the past few decades. The active ingredients found in microbial cultures and extracts contain toxins in many forms, i.e., single proteins, protein complexes, macromolecules and small molecules. Single protein toxins are usually preferred because it is relatively straightforward to characterize, clone, transform and express them. The other toxin forms are usually not pursued due to their complexity. Prokaryotic genes usually require a process called codon optimization for good expression in certain crops. For example, the maize genome is quite GC-rich, and therefore AT-rich genes usually do not express well in corn. To boost expression in maize, adjustments to

increase the GC content of a transgene is usually required. So far all the microbial genes that have been expressed in plants and have shown good insecticidal activities were from bacteria (Schuler *et al.*, 1998).

Among the bacteria that produce proteinaceous insecticidal toxins, *Bacillus thuringiensis* (Bt) is the most studied species. Several strains of this bacterium have been developed into different formulas for spray-on application to control Lepidopteran, Dipteran and Coleopteran insects. Although these formulas are still used in organic farming and gardening practices, they are not widely popular due to high production cost and UV-instability. The insecticidal activities of Bt mainly come from crystalline inclusions, which usually contain one or more proteins called delta-endotoxins, or Cry proteins, produced during the sporulation stage. The crystals dissolve in the insect guts and the protoxins are proteolytically activated to release the toxin core. The activated toxin binds to a specific receptor on gut epithelial cells and then inserts into the cell membrane. Pore formation on cell membranes results in cell lysis and eventually kills the insects (Van Rie, 2000). Many Bt delta endotoxin genes have been characterized and documented. A systematical nomenclature system has been well developed to classify these genes and proteins by the *Bacillus thuringiensis* delta-endotoxin nomenclature committee (Crickmore *et al.*, 1998 and 2002). It is important to point out that not all the Cry proteins are insecticidal. Those that are toxic to insects are known for their narrow spectra. Most of them are limited to certain species in an Order. For example, Cry1Ab is active against many, but not all, species of Lepidoptera. Only a few Cry genes show toxicity across different Orders, for example, Cry2Aa is toxic to Diptera and Lepidoptera. Most of the Cry genes have been cloned, sequenced and characterized. Some have been transformed and expressed in different crop plants. Those include Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ba, Cry1Ca, Cry1H, Cry2Aa, Cry3Aa, Cry6A, Cry9C (summarized in Schuler et al., 1998), Cry1Fa (Babcock *et al.*, 2001), Cry2Ab (Greenplate *et al.*, 2003), Cry3Bb (EPA Publication, 2003), and a binary Cry34Ab/Cry35Ab (Moellenbeck *et al.*, 2001). Currently only a few Bt delta-endotoxin genes have been commercialized and are still on the market. They are Cry1Ab, Cry1Ac, Cry1Fa, Cry2Ab, and Cry3Bb. Cry1Ab and Cry1Fa were introduced into corn hybrids for corn Lepidopteran insect control (Koziel *et al.*, 1993; Armstrong *et al.*, 1995; Babcock *et al.*, 2001). Cry1Ac and Cry2Ab proteins are potent for control of tobacco budworm and cotton bollworm. These genes have been transformed into cotton varieties for commercialization (Perlak *et al.*, 1990, Greenplate *et al.*, 2003). Cry3Bb transgenic maize represents the first corn rootworm-resistant product, which was commercialized in 2003 (EPA Publication, 2003). In general, Cry genes do not cause significant phytotoxicity.

In addition to insecticidal Cry proteins, some Bt and other *Bacillus* species produce and secrete a group of soluble proteins called Vegetative Insecticidal Proteins (Vip) during the vegetative growth phase. A few Vip genes have been cloned and characterized. Vip genes are very specific. Among the Vip genes found so far, the binary toxin Vip1/Vip2 is active against corn rootworms and the Vip3s are active against Lepidoptera (Warren, 1997). These toxins damage the insect midgut (Yu, *et al.* 1997). Vip3A has a mode of action that is different from those of Cry1A proteins (Lee *et al.*, 2003). This gene has been transformed into different plants and has shown good efficacy against Lepidopteran insects. Other than Cry and Vip insecticidal proteins, Bt also produces many diverse, and not yet characterized insecticidal small molecules. Genes and pathways that synthesize those molecules have not yet been well documented.

Cholesterol oxidase is a novel type of insecticidal protein produced by bacteria that shows activity against cotton boll weevil (*Anthonomus grandis*). The gene was cloned from *Streptomycete* sp. It was suggested that oxidization of cholesterol may disrupt the membrane composition of gut cells and cause a direct negative impact on cell biology because cholesterol is an important component of cell membranes. Indeed, histological studies showed that cholesterol oxidase lysed the midgut epithelium of boll weevil (Purcell *et al*, 1993). Cytosolic and chloroplast-targeted versions of the cholesterol oxidase *choM* gene were transformed into tobacco. This gene functioned *in planta*, but unfortunately it turned out to be phytotoxic. Cytosolic events showed severe developmental symptoms, yet the chloroplast-targeted version appeared normal (Corbin *et al.*, 2001). Another bacterial gene cloned and tested in plants was isopentenyl transferase of *Agrobacterium tumefaciens*. This protein is a key enzyme of the cytokinin biosynthesis pathway. Transgenic tobacco and tomato showed some degrees of insecticidal activity against tobacco hornworm (*Manduca sexta*) and peach aphid (*Myzus persicae*). Unfortunately these transgenic events also showed significant negative phenotypes, such as underdeveloped roots and a decreased number of chloroplasts (Smigocki *et al.*, 2000).

The bacterium *Photorhabdus luminescens* is known for its insecticidal properties against a broad spectrum of insects. Its genome has recently been sequenced (ffrench-Constant *et al.* 2003). The *tc* genes attracted the most attention among the insecticidal genes identified (Waterfield *et al.*, 2001). The insecticidal Tc proteins have broad spectra against insects in different Orders, but they are quite complicated and very large. So far no transgenic cases involving the *P. luminescens* toxins have been published.

Eukaryotic sources

Plants produce many types of insecticidal proteins such as digestive enzyme inhibitors, chitinases, lectins, peroxidases and others, to fight against phytophagous insects. Despite the diversity, there is no plant insecticidal gene with levels of insect control comparable to the Bt toxins (Schuler *et al.* 1998). Thus far, no transgenic crop expressing plant insecticidal proteins has been commercialized.

Among the plant-derived insecticidal genes, digestive protease inhibitors were studied most extensively. Protease inhibitors considered for a transgenic approach are single polypeptides or proteins that can efficiently inhibit specific types of proteases including serine, aspartic, cysteine, and metallo proteases. Inhibition of digestive proteases may cause growth retardation and mortality of insects. At least 14 protease inhibitor genes have been expressed in different crops and have been well summarized in Schuler *et al.* (1998). Cowpea trypsin inhibitor attracted much attention because it showed potent insecticidal activity against Lepidopteran insects. Yet, the field performance of cowpea trypsin inhibitor was not as good as Bt crops (Hoffmann *et al.*, 1992). Transgenic plants with protease inhibitor genes usually have major performance problems of inconsistency and lack of endurance. In addition, most insects have more than one type of digestive protease, and those enzymes are usually part of a gene family. Insects eventually find ways to compensate for the protease deficiency caused by inhibitors (Jongsma *et al.*, 1995). In addition, over expression of protease inhibitors *in planta* may have phytotoxicity concerns and may not be suitable for human and livestock consumption. Other plant self-defense genes such as an a-amylase inhibitor of common bean have been transformed to other legumes to control *Callosobruchus* and *Bruchus* weevils (Ishimoto *et al.*, 1996). However, this approach does not control all the storage weevils. For example, a-amylase inhibitor is ineffective against bean weevil (*Acanthoscelides obtectus*) or the Mexican bean weevil (*Zabrotes subfasciatus*) (Ishimoto and Chrispeels, 1996).

Lectins, which are carbohydrate-binding proteins, represent a unique type of insecticidal proteins. Some members of the plant lectin family are insecticidal against Homoptera, Coleoptera, Lepidoptera, and Diptera. Unfortunately the application of these genes for transgenics is hindered because most of them are also toxic to mammalians. GNA lectin isolated from snowdrop (*Galanthus nivalis*) is less toxic to mammalians when compared with other lectins. Because of this property, GNA has been transferred to many crops such as tomato, tobacco, potato and rice for control of aphids, potato leafhopper and rice brown planthoppers (Habibi *et al.*, 1993; Down *et al.*, 1996; Rao, *et al.*, 1998). GNA is not really

insecticidal. It is a feeding deterrent and causes a reduction of fecundity. Field trials of GNA crops did not give good performance because they could not stop propagation of insect pests, and were not considered for commercial use.

Insecticidal proteins from animals that can be delivered orally are rare. There are two good transgenic examples for discussion, equistatin potato and avidin corn. Equistatin is a cysteine protease inhibitor that was isolated from the sea anemone *Actinia equina* (Lenarcic *et al.*, 1997). This protease inhibitor is active against many different insects, and has been successfully expressed in potato for control of Colorado potato beetle. Unfortunately equistatin did not accumulate in potato tissues due to degradation *in planta* by arginine/lysine-specific and legumain-type Asn-specific cysteine proteases (Outchkourov *et al.* 2003). Protein stability is still a major obstacle in bringing this idea to market. The other case is avidin maize. Avidin is an egg white glycoprotein that sequesters the vitamin biotin. The insect toxicity of avidin is caused by a biotin deficiency. Expression of this gene in maize kernel showed promising results in controlling storage pest insects (Kramer *et al.*, 2000).

Conclusion

Creating transgenic insect-resistant crops is a powerful technology that offers farmers better options to efficiently and economically control insect damage. With successful cases like Bt corn and cotton, many farmers have adopted this new technology to improve yields and save on labor and chemical expenses. While these products are promising, transgenic insect resistant crops still have a long way to go. In spite of the proficient performance of transgenic crops in the fields and their acceptance by farmers, this technology has not been used in a wide variety of crops all over the world. So far only a few crops have justified the very expensive investments required to develop a transgenic product. Intensive regulatory requirements and promotion activities needed to demonstrate to the general public that transgenic products are safe for consumption and are friendly to the environment add to their large investment needs. Currently acceptance of genetically modified products in Europe is still a major barrier to promoting this technology (Saeglitz and Barsch, 2003). Since the launch of Cry1Ab corn in 1996, insect resistance to insecticidal traits has become a major concern. Farming practices, such as using refuge, have been instituted to delay this possibility. Fortunately, after 8 years, there is still no sign of resistance of European corn borer to Cry1Ab (Fox, 2003). Even so, insect resistance to insecticidal traits is still a potential threat and may have a huge impact on the future development of transgenic insecticidal crops. Alternatively, ideas such as combining multiple genes with different modes of

action to delay the development of insect resistance and increase the spectrum have been addressed (Peferoen, 1997). It was shown that transgenic plants expressing tow Bt toxins did delay the development of insect resistance (Zhao *et al.*, 2003; Gould, 2003). Echoing this concept, new gene discovery for insect toxins with different modes of action is important for the future of transgenic insecticidal crops. Even though only a few Cry crops have been commercialized, the number of novel insect resistance genes discovered is still growing. Up to now, 272 holotypes of Cry genes have been documented. The most recently named Bt Cry genes were Cry43Aa and Cry43Ba (Crickmore et al., 2002). Other than proteinaceous toxins, unexplored areas such as secondary metabolites may provide another intriguing area for transgenic insect control (Schuler *et al.*, 1998). Similar to all other insect control methods, transgenic insect resistant crops have their limitations. For example, the new biotech crops can not totally replace insecticides due to the specificity of the transgenes. The current traits do not control all secondary insect pests, and thus farmers still have to rely on some insecticidal applications. New genes for secondary pests may eventually be available for transgenic options. However due to the high costs needed to develop a new transgenic crop, traits for secondary pest insect control may never be commercialized if the market value does not justify the costs. Therefore combinations of transgenic traits and insecticides to cover all insect pests will be the trend to deal with the complicated insect problems.

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Biotechnological potential of genetically modified plants for nematode resistance

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Abstract

Plant-parasitic nematodes represent an economically important group of pests worldwide that can devastate a wide range of crop plants, causing annually billions of dollars in yield losses. In soybean, the most important sedentary endoparasites are the root-knot (*Meloidogyne* spp.) and cyst (*Heterodera glycines*) nematodes, in which the usual culture traits are inefficient to their control. Efforts have been focused to elucidate intrinsically characteristics of their life cycle with aim to develop new biotechnological methodologies to engineer nematode resistant plants. Different strategies are being applied for pest resistant transgenic plants including, natural resistance proteins, Bt toxins, lectins, proteinase inhibitors, interference RNA, proteinase prodomain peptide, among others. Propeptides act in a way similar to proteinase inhibitors and represent a new perspective in the control of the sedentary endoparasites. Recent data demonstrated that cysteine proteinase propeptide of *H.* glycines inhibits its cognate enzyme and the proteolytic activity of *M. incognita* females, suggesting be usefulness as tool for inhibition of cysteine proteinases of nematode in biological systems.

Introduction

Plant nematodes have evolved a diverse parasitism mechanisms and feeding relationships with their host plants in order to obtain nutrients that are necessary for its development and reproduction (Davis et al., 2000). The nematodes *Meloidogyne* spp., *Heterodera glycines*, *Rotylenchulus reniformis* and *Pratylenchus* spp. accounting for extensive worldwide soybean losses. However, the most important are the sedentary endoparasitic root-knot *Meloidogyne* spp. and cyst *H. glycines* nematodes (Atkison et al., 1993). Their life cycle consist in distinct developmental stages: (i) the egg, (ii) four juvenile stages and (iii) sexually dimorphic adult stage, that establishes the complex feeding site (Operman et al., 1994; Opperman and Bird, 1998). The infective juvenile (J2) form of *H. glycines* penetrates the host plant root and migrates to an area in the proximities of the vascular cylinder, where it establishes a complex feeding site, called syncytium (Jones, 1981). Differently from soybean cyst nematodes, the juvenile of root-knot nematode

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moves intercellularly after penetrating the root, migrating down in the plant cortex towards the root tip and to vascular cylinder migrating up in the root (Wyss et al., 1992). They establish a permanent feeding site (giant cell) in the differentiation zone of the root by inducing nuclear division without cytokinesis in host cells. The plant cells around the feeding site divide and swell, causing the formation of galls or root knots (Williamson and Russei, 1996). The nematodes ingest the cytoplasm of the plant cells through their stylets and, after three molts, develop into pear-shaped, egg-laying females. Both giant cells and syncytium serve as sources of plant resources to the parasitic nematode (Williamson and Gleason, 2003).

Certainly, a good strategy to control phytonematodes is preventing their entrance and dissemination in the field due its complex erradication. Current methods of cultural traits are mainly based on resistant cultivars, crop rotation and nematicides (Dong and Opperman, 1997). Although chemicals available in the market can partially control the nematodes, they are often highly toxic nematicides, with associated environmental risks and are not economic (Opperman and Bird, 1998). Resistant cultivars have been obtained predominantly by classical genetic improvement, but usually present partial resistance, besides the presence of undesirable agronomic characteristics. The transfer into plant host species of genes encoding nematicides proteins by using transgenic methodologies is an attractive and practical method to control plant parasitic nematodes, with advantages that no other changes to agronomic traits are introduced and toxicological and environmental risks associated with chemical control are reduced.

Many strategies using genes encoding proteins with activity towards nematodes have been used to engineer plants, which include proteinase inhibitors, proteinases proregions, Bt toxins and natural resistance genes (*Mi* and *Hs1*$^{pro\text{-}1}$). Here we describe several effectors and strategies with potential use in the development of nematode resistant transgenic plants and emphasize the use of proteinase prodomain peptides as usefulness biotechnological tool.

Effectors in transgenic plants nematode resistance

During the past years several important publications have significantly increased the knowledge about plant defense proteins by using in vitro bioassays and nematode resistant transgenic plants (Table 1). Afterwards, these resistant effectors will be discussed.

Table 1. Genes for nematodes resistance.

Gene resistance	Plant	Nematode	Reference
Mi1.2	Tomato	*M. incognita*	Ganal and Transkley, 1996
		M. javanica	Milligan et al., 1998
		M. arenaria	
Hs1^{pro-1}	Sugar beet	Beet cyst nematode	Cai et al., 1997
Hs2	Sugar beet	*H. schachtii*	Heller et al., 1996
Gpa2	Potato	*G. pallida*	van der Vossen et al., 2000
rgh1	Soybean	*H. glycines*	Webb et al., 1995
Rgh4	Soybean	*H. glycines*	Lewers et al., 2001
Cowpea trypsin inhibitor gene	Potato	*G. pallida*	Atkinson, 1993
Oc-I?D86 gene	*A. thaliana*	*H. schachtii*	Urwin et al., 1997
		M. incognita	

Resistance genes (R genes) have been used in resistance breeding programs for decades, with variable success degrees. One of the nematode resistance genes cloned was *Hs1^{pro-1}*, a gene from a wild sugar beet that confers resistance against *Heterodera schachtii* (Cai et al., 1997). The *Mi* gene from a wild tomato relative (*Lycopersicon peruvianum*) confers resistance to three of the most damaging of root-knot nematodes (Milligan et al., 1998) while *Gpa2* gene from potato confers resistance to cyst nematode, *Globodera pallida* (van der Vossen et al., 2000). Two other genes, *rhg1* and *Rhg4,* from soybean confer resistance towards *H. glycines* (Webb et al., 1995; Lewers et al., 2001).

The *Mi* is the best-studied gene for nematode resistance. A wild tomato genomic map showed a 52 kb region that contains three regions with similarity to R genes, where two of them, *Mi1.1* and *Mi1.2*, represent potentially functional genes. The *Mi1.2* confers nematode resistance in transgenic plants while any resistance were found in transgenic plants contained *Mi1.1*. The protein encoded by the *Mi1.2* contains a nucleotide-binding site and leucine-rich repeat region, protein motifs that are commonly found in numerous plant resistance genes (Milligan et al., 1998). The introduction of *Mi1.2* gene into cultivated tomato was responsible for the hypersensitive reaction of tomato roots after infection with *M. incognita*, *M. javanica* and *M. arenaria*, resulting in the death of juveniles (J2) of the infective stage (Ganal and Tanksley, 1996; Milligan et al., 1998).

The two resistance genes from wild sugar beet were isolated based on its chromossomal position and identified by genetic complementation. The *Hs1^{pro-1}* cDNA introduced in susceptible sugar beet roots under the control of the CaMV35S promoter showed resistance to beet cyst nematode (Cai et al., 1997) while the *Hs2* gene confers resistance to the nematode *H. schachtii* (Heller et al., 1996). Molecular analysis of *Gpa2* gene from potato

resulted in the identification of an R gene cluster of two active homologous genes; one corresponds to the *Rx1* gene, which confers resistance to potato virus X and other that corresponds to the *Gpa2* gene confers resistance to the potato cyst nematode *G. pallida*. The isolation of the *rgh1* and the *Rhg4* allele from soybean, located on chromosome G and LG A2, respectively, generated one possibility for nematode control in soybean, since these genes have been described as candidate for resistance factor against *H. glycines* (Webb et al., 1995; Lewers et al., 2001).

Proteinase inhibitors are other class of proteins with potential in the control of plant parasitic nematodes. Four main classes of proteinases (cysteine, serine, asparthyl and metallo) are known occur in organisms. The potential of proteinase inhibitors as antinematode effectors was intensive explored for both serine and cysteine proteinase inhibitors. The expression of cowpea trypsin inhibitor, a serine proteinase inhibitor, in transgenic potato influenced the sexual fate and predominance of the much smaller and less damaging males in *G. pallida* (Atkinson, 1993). However, no reduction occurred in the fecundity of the same plant nematode (Atkinson, 1993). On the other hand, this proteinase inhibitor reduced the fecundity of females of *M. incognita* without influencing their sexual fate (Urwin et al., 1998). Histochemical assays performed on *H. glycines* indicate a high serine proteinase activity that could be inhibited by the cowpea trypsin inhibitor in the nematode intestine (Lilley et al., 1997). Several studies focusing on the use of proteinase inhibitors in transgenic plants as tomato, potato and *Arabidopsis thaliana* as mean of controlling both cyst and root-knot nematodes have been described (Urwin et al., 1995, 1997, 1998). The cysteine proteinase inhibitors, oryzacystatin I (Oc-I) and II from rice, were submitted to several mutation programs, and the mutant Oc-I"D86, obtained by delection of an aspartate residue at position 86, resulted in an increased inhibitor activity towards cysteine proteinase from *Caenorhabditis elegans* (Urwin et al., 1995). When expressed in tomato hairy roots, both Oc-I and Oc-I"D86 had a detrimental effect on growth and development of *G. pallida*. This effect was significantly greater on Oc-I"D86-expressing roots leading to a reduction in size of *G. pallida* females to a level at which fecundity is profoundly affected (Urwin et al., 1995). The Oc-I"D86 expressed as a transgene in *A. thaliana* showed a major effect on the size and fecundity of females for both *H. schachtii* and *M. incognita*. No females of either species achieved the minimum size required for egg production (Urwin et al., 1997).

The proteinases when synthesized are inactive proenzymes, which require processing to convert to their mature active forms. Their activation occurs through proteolytic cleavage of the N-terminal prodomain fragment of the enzyme, called proregion. Several essential roles are attributed to the proteinase proregions as intracellular trafficking; correct folding of the enzyme

during maturation, and inhibition of the mature enzyme (Tao et al., 1994). The inibition of proteinases by their respective proregion peptides is most of time a specific process (Cygler and Mort, 1997; Podobnik et al., 1997). The proregion of human cathepsin L as well as the proregion of rat cathepsin B has been shown to be a potent inhibitor of their cognate enzyme (Carmona et al., 1996; Chen et al., 1996). However, proregion of papaya proteinase IV inhibits the colorado potato beetle digestive cysteine proteinases as well other papaya proteinases, including papain, suggesting that it could represent a general inhibitor of papain-like cysteine proteinases (Visal et al., 1998). Studies exploring the relationship between proteinase-proregions can develop a new generation of specific peptides inhibitors to be used as strategy for the control of plant sedentary nematodes.

Other toxic compounds with interesting properties to be used in the development of nematode resistant transgenic plants are Bt toxins, produced by *Bacillus thuringiensis* (Bt). They are widely used as natural insecticide in agriculture; and several important insect resistant transgenic crops containing Bt genes are now commercialized (Jouanin et al., 199; Carlini and Grossi de Sá, 2002; James, 2002; Pray et al, 2002; Toenniessen et al., 2003). The Cry5 and Cry6 toxins family have been described with nematicidal toxicity (Schnepf et al., 1998) and the toxicity for *C. elegans* nematode were demonstrated for the crystal proteins: Cry5B, Cry6B, Cry14A and Cry21A (Wei et al., 2003). Recently, Khyami-Horani and associates (2003) have isolated a novel strain of *B. thuringiensis* J112, active towards juvenile forms of root-knot nematodes *M. javanica* and *M. incognita* in which the crystal produced by this strains contains proteins encoded by *cry1*, *cry3A*, *cry4*, *cry5* and *cyt2a* genes.

The use of RNA interference (RNAi) is a new promising strategy in the development of transgenic plants. According to Tijsterman and associates (2004), RNAi in the *C. elegans* nematode occurs systematically, in which double-stranded RNA (dsRNA) provided in the diet can be absorbed by the gut lumen and distributed throughout the body, resulting in the fragmentation and inactivation of the trigger RNAm. RNAi has been intensive used in gene function studies in *C. elegans* (Timmons et al, 2003; Tijsterman et al., 2004). In *H. glycines*, using as target cysteine proteinases, Urwin and associates (2002) have found a shift from the normal female/male ratio of 3:1 to 1:1 in 14 days after the infection while dsRNA for a protein with homology to C-type lectins reduced the number of parasites by 41%.

Our team has focused our research on the characterization and gene cloning of bioactive molecules involved in plant defense by using different biochemical and molecular strategies. Efforts were concentrate in natural compounds as, amylase and proteinase inhibitors, Bt toxins, lectins and

resistance proteins (Grossi-de-Sá et al., 1997; Chrispeels and Grossi-de-Sá, 1998; Paes et al., 2000; Franco et al., 2000; Iulek et al., 2000; Campos et al., 2002; Franco et al., 2003; Melo et al., 2003). More recently, by application of techniques based on DNA shuffing and Phage display, recombinatorial library of a large array of mutants for a-amylase and proteinase inhibitors and Bt genes were generate in order to select improved chimeric molecules, more active and highly selective to the pests target.

Based on studies which indicate that peptides derived from the propeptide domain of various proteinase zymogens can inhibit their corresponding enzymes (Fusek et al, 1991; Roche et al., 1999; Lesage et al., 2001), a cDNA encoding a cysteine proteinase, called HGCP-Iv, isolated from a *H. glycines* J2 cDNAs library has been expressed and used to assess the ability of its propeptide to act as an proteinase inhibitor of the active mature form. HGCP-Iv produced in *E. coli* degrades the synthetic peptide benzyloxycarbonyl-Phe-Arg-7-amido-4-methylcoumarin and is inhibited by E-64, a substrate and a synthetic inhibitor commonly used for functional characterization of cysteine proteinases, respectively. The recombinant HGCP-Iv propeptide expressed in *E. coli* showed *in vitro* high inhibitory activity towards its cognate enzyme and to the proteinases activity of *M. incognita* females (fig.1), suggesting be usefulness as a tool for the inhibition of digestive cysteine proteinases of nematode and to engineer plants with detrimental effect to the nematodes.

Since the isolation of the first gene involved in nematode resistance plant, more effective molecules with potential to nematode control have been searched in nature and some molecules are being modified by genetic engineering in order to obtain new effectors with enhanced activity and specificity to inhibit the parasitism. Biotechnology tools have been successfully used to create and enhance new nematicide genes as potential candidates to generate transgenic plants.

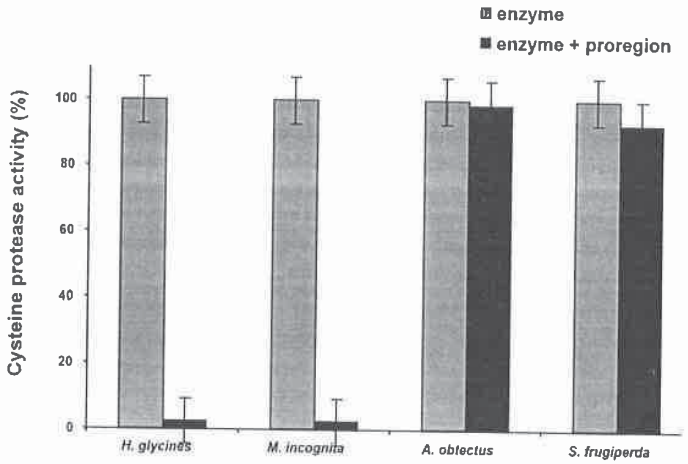


Figure 1. Effect of the cysteine proteinase propeptide towards phytonematodes and insect pests. The recombinant HGCP-Iv propeptide was overexpressed in *E. coli* and the inhibitory activity against cysteine proteinase from *H. glycines*, *M. incognita*, *Acanthoscelides obtectus* and *Spodoptera frugiperda* was determined prewarming 10 ng HGCP-Iv/assay with HGCP-Iv propeptide for one hour at 37°C, prior the addition of 10 mM of the substrate (Z-CBZ-Phe-Arg-7-amidomethylcoumarin). The fluorescence was measured in a DyNA Quant 500 fluorescence reader (Pharmacia-Biotech).

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Genetic engineering of soybean for improved nutritive value

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Current research seeks to improve the quantity and quality of soybean (*Glycine max* (L.) Merr.) seed-storage proteins, a vital nutritional resource for humans and livestock. Although recurrent selection and breeding have produced varieties with improved protein content, efforts to enhance the essential amino acids, methionine and cysteine, have remained uninspiring. Genetic engineering technology has been widely utilized to improve the nutritional quality of crop plants. Protein quality improvement by genetic engineering methods includes introduction of either heterologous or synthetic protein, elevation of endogenous protein, and alteration of abundant endogenous proteins. This presentation will summarize the successes and obstacles involved in improving protein quality of soybeans. Enhancing the quality of soybean protein will obviate synthetic methionine supplementation of animal feeds, a process that costs in excess of 100 million U.S. dollars annually.

Although soybean provides 70% of the protein meal utilized by humans and livestock worldwide, quality is compromised by a low concentration of the essential amino acids cysteine and methionine. The nutritional quality of soybean protein has been predicated on evidence from studies using rat as a model for human dietary amino acid requirements (Young, 1991). In 1991, a method of evaluating protein quality was developed which considered not only amino acid content, but also digestibility of the protein in a particular species. The protein digestibility corrected amino acid score (PDCAAS) indicated that soybean protein was equivalent to casein and egg white (Food and Agricultural Organization-World Health Organization, 1991). Since the inception of PDCAAS, however, experiments have shown that the quality of protein, from sources containing antinutritional factors, such as trypsin inhibitor in soybean, is overestimated (Sarwar, 1997). In the United States, poultry, swine, beef, and dairy feeds account for 50, 25, 13, and 6% of the domestic soybean meal usage, respectively. (American soybean association www.soystats.com/2003). As monogastrics, growing swine and poultry have a higher dietary requirement for methionine than is provided by soybean meal, necessitating in costly

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supplementation of feeds with synthetic amino acids. Because soybean is the predominate protein meal used for animal feeds, development of high methionine varieties would benefit the animal industry.

Although traditional breeding and recurrent selection have produced soybeans with increased protein content (Brim and Burton, 1979; Burton et al., 1982; Wilcox and Shibles, 2001) the quality of protein stored by these plants was not enhanced. Methionine levels have remained stable regardless of total protein content of the improved varieties (Burton et al.,1982; Wilcox and Shibles, 2001). Induced mutagenesis has resulted in soybeans containing a 20% increase in methionine content, but it is not known if this modest increase is sufficient to meet the nutritional needs of the livestock (Imsande, 2001). Experiments utililizing genetic engineering techniques have produced soybeans that exhibit enhanced levels of sulfur-containing amino acids. Manipulation of soybean protein quality thus far involves four basic approaches. In the first approach, heterologous proteins rich in methionine are introduced into soybeans. Proteins that are exceptionally rich in methionine have been identified in Brazil nut (*Bertholletia excelsa* H.B.K) (Altenbach et al., 1987), sunflower (*Helianthus annuus* L.) (Kortt et al., 1991) and corn (*Zea mays* L.) (Kirihara et al., 1988; Chui and Falco, 1995). When Brazil nut albumin was expressed in soybean, methionine content was increased between 15 and 40 percent in different transgenic lines (Townsend and Thomas, 1994). Similarly, methionine and cysteine contents in soybean were increased between 12-20% and 15-35% respectively, due to the presence of the introduced 15 kD d-zein. (Dinkins et al., 2001). Recently, we have introduced an 11 kD d-zein into soybean. The gene encoding the 11 kD d-zein was oriented downstream from the a' subunit of b-conglycinin promoter. When the construct was expressed in soybean, a unique distribution with respect to protein storage was noted (Kim and Krishnan, 2003). Native soybean storage proteins accumulate in protein storage vacuoles while the exogenous 11 kD d-zein was contained in novel protein bodies that had attached ribosomes on the surfaces. Although the 11 kD d-zein is comprised of 22% methionine, the accumulation of this protein in transgenic soybean did not increase the overall content of this sulfur amino acid.

The second approach taken to improve methionine content of soybean is modification of abundantly expressed endogenous proteins. Glycinin is an abundant seed storage protein that is encoded by a multi-gene family. Within one group of these proteins there are regions of diverse amino acid sequences denoted as hypervariable regions (HVRs). Natural variability in primary structure of these proteins at the HVR suggests modification at these sites would be sustainable (Nielsen et al., 1990). However, when

modifications were made and the protein expressed, accumulation did not occur, ostensibly due to proteolytic degradation (Nielsen et al., 1995). Further work elucidated sites that were capable of entertaining modification and subsequently allowed the altered protein to be processed and stored (Utsumi et al., 1994). Another method being investigated in attempts to improve the quality of soybean protein is elevation of expression of endogenous sulfur-rich proteins. Soybeans contain water-soluble albumin proteins, which comprise a small fraction of the total protein content. Since methionine accounts for approximately 10% of the amino acids of these proteins, elevating their expression levels may result in increased sulfur amino acid content. The last avenue being explored is the use of synthetic proteins, which are rich in essential amino acids. Synthetic proteins have been successfully expressed in rice (*Oryza sativa* L.) and sweet potato (*Ipomoea batatas* [L.] Lam) resulting improvement of protein quality (Zhang et al., 2002, Potrykus, 2003).

Genetic engineering technology has expedited improvements in soybean protein quality. Unfortunately, concomitant with the expression of exogenous methionine-rich protein, a decline in the content of endogenous sulfur rich proteins was observed (Streit et al., 2001). This suggests that the sulfur amino acid supply was insufficient to meet demand imposed due to the introduction of methionine-rich proteins. Regardless of the genetic alteration method used to enhance methionine content, an adequate supply of the amino acid is essential. Flux through pathways encompassing sulfate uptake, translocation, and assimilation is presumably limiting accumulation of methionine and cysteine. Genetic engineering of enzymes resulting in altered sensitivity toward control mechanisms responsible for sulfate assimilation has the potential of increasing methionine and cysteine.

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Soil fertility management for soybean in Argentina

F. O. García[1]

Abstract

Fertilizer use in soybean has been historically low in Argentina, and adequate nutrient management could contribute to future increases in grain yield in most regions. This paper summarizes the results of investigations and experimentations on soybean fertilization management carried out in the last years. Most of the soils under soybean production have N, P and/or S deficiencies. Seed inoculation with *Bradyrhizobium* bacteria is a generalized practice. Soybean yield response to P fertilization is related to soil Bray 1 P content and current critical levels are between 10 and 14 mg/kg Bray P. Sulfur recommendations are based on previous field history and management since responses are not strongly related to soil sulfate-S concentrations at planting. Recent experiments have not shown generalized responses to nutrients other than N, P or S. Research has shown high residual effects of P and S applications in wheat for the next double cropped soybean crop. Significant interactions among deficient nutrients applied through fertilization (P and S) or supplied by biological fixation (N) are observed in double cropped and full season soybeans.

Introduction

Soybean planted area in Argentina has sharply increased in the last decade, from 4,966,600 ha in 1990 to 12,630,000 ha in 2002, a 154% increase. This expansion mainly occurred in the Pampas region (Fig. 1). However, important increases in soybean area are now apparent in the northwestern (NOA) and north central and northeastern (Chaqueña-NEA) regions. Soybean area in these northern areas accounted for only 7% of the total planted area in 1990 and reached 16% in 2001.

Average national yields for soybean have increased from 2187 kg/ha in 1990 to 2771 kg/ha in 2002/03, an annual rate of increase of 49 kg/ha. Most of this increase is explained by the introduction of glyphosate resistant varieties in the mid 90's that significantly improved weed control. However, higher yielding varieties and better crop and soil management practices also contributed to these grain yield gains.

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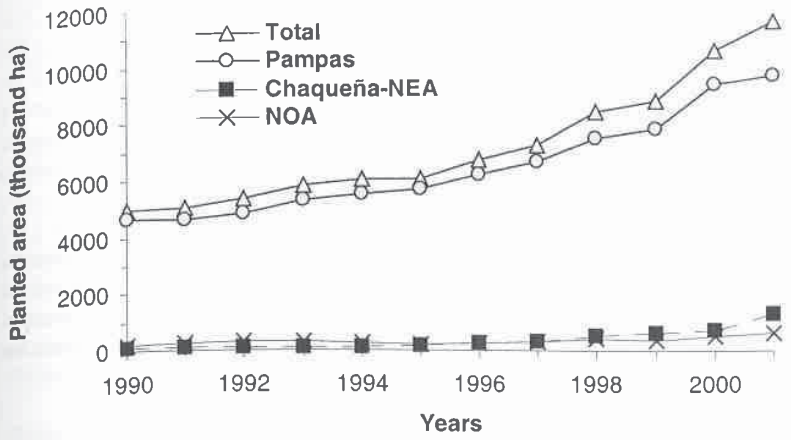


Figure 1. Area planted to soybean in Argentina (Total), the Pampas region (includes provinces of Buenos Aires, Santa Fe, Córdoba, La Pampa, Entre Ríos and San Luis), the north central and northeastern regions ("Chaqueña-NEA", includes provinces of Chaco, Santiago del Estero, Formosa, Corrientes and Misiones), and the northwestern region ("NOA", includes provinces of Tucumán, Salta, Catamarca and Jujuy). Adapted from data of SAGPyA.

Native soil fertility of the Pampas and the northwestern and north central regions of Argentina are characterized by high soil organic matter content, high base saturation, moderately acid to neutral pH, and high K contents. Soil P availability is inherently low in some areas (e.g., southeastern Buenos Aires, Entre Ríos, and eastern Tucuman) but is medium to high in the rest of the region. The northeastern region is dominated by ultisols and oxisols of low soil fertility (i.e., low pH, low base saturation, and low P and K availability).

Intensive annual crop production in the last 20 years has decreased soil Bray P contents in several areas of the Pampas region (García, 2001). Intensification of agricultural production has also resulted in lower soil organic matter levels, and thus, soil N and S availability for crops. No-tillage has drastically expanded in the last decade allowing for better water use efficiency and higher and more stable grain yields of soybean, corn, wheat, sunflower, and other crops (García et al., 2000). Soybean area under no-tillage is estimated at 74% for the 2001/02 season (AAPRESID, 2003).

Fertilizer use in soybean has been historically low in Argentina, and adequate nutrient management could contribute to future increases in grain yield in

most regions. Fertilized area of soybean was estimated at less than 4% until the late 90´s but has increased to 20-25% in the recent past. Surveys for the 2003/04 season indicate that % fertilized area has the potential to reach 50-60%. The great expansion of soybean and past under fertilization of the crop has resulted in negative nutrient balances (i.e., differences between nutrient application and removal by grain), and as a consequence, decreased soil fertility in several areas of the country.

The objective of this paper is to summarize the results of investigations and experimentations on soybean fertilization management carried out in Argentina in the last years. Most of the soils under soybean production have N, P and/or S deficiencies. In some areas, micronutrient deficiencies (boron, zinc) are observed.

Nitrogen

Seed inoculation with *Bradyrhizobium* bacteria is a generalized practice for most soybean farmers in the country. Biological N fixation supplies 30-70% of the total N need depending on the soil N availability and growing conditions (González, 1996). Past seed inoculation experiments conducted in fields previously sown to inoculated soybean found yield responses of 235 to 823 kg/ha (Perticari et al., 1998), and 150 to 527 kg/ha (Devani et al., 2003).

Direct N fertilization at planting has not shown grain yield responses (Bodrero et al., 1984; Scheiner et al., 1999; Devani et al. 2003). However, application of small amounts of N fertilizer (less than 30 kg N/ha) has resulted in yield increases in double cropped soybean after harvesting high yielding wheat crops (> 5,000 kg/ha) in southeastern Buenos Aires (Pablo Calviño, comm. pers.).

Applications of N at reproductive stages (R1-R3) have shown variable results according to soil N availability, crop growth and development, and yield potential (Scheiner et al., 1999) but this is not a common practice for farmers.

Phosphorus

Soybean yield response to P fertilization is related to soil Bray 1 P content and current critical levels are between 10 and 14 mg/kg Bray P. Melgar et al. (1995) compiled information from 65 trials and found a 70% probability that a response of 300 kg/ha or greater could be achieved in soils with Bray P levels lower than 9 ppm, and a 40% probability of achieving a 200 kg/ha yield response in soils with 9 to 14 mg/kg Bray P. An extensive

network of fertilization experiments carried out by Project INTA Fertilizar and other institutions in the 2000/01 and 2001/02 seasons have confirmed a critical level of 12 mg/kg Bray P. Below this level profitable yield responses to P fertilization are highly probable (Fig. 2) (Díaz Zorita et al., 2002).

Table 1 shows recommendations of P fertilization for southeastern Buenos Aires according to soil Bray P content and yield goal (Echeverria and Garcia, 1998). These recommendations have proved to be useful in other areas of the Pampas region. Main P fertilizer sources are diammonium phosphate (DAP) and monoammonium phosphate (MAP) applied at planting with the seed or banded below and to the side. Preliminary results of current research show that broadcast P application 40-50 days prior to planting could result in similar P use efficiency to banded P applications under no-tillage systems (Bianchini et al., 2003).

Sulfur

Sulfur deficiencies are observed in soybeans and several other crops (e.g., corn, wheat, alfalfa) in the Pampas region, mainly in south central Santa Fe, southeastern Cordoba, central and western Buenos Aires, and the northwestern region (i.e., Tucumán and Catamarca provinces).

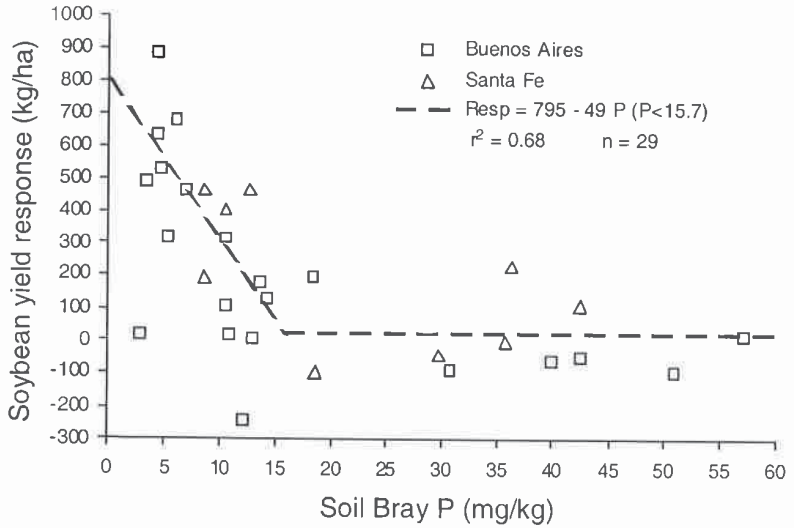


Figure 2. Soybean yield responses to P fertilization (20 kg/ha P at planting), as a function of soil Bray P content for 31 experiments in the provinces of Buenos Aires and Santa Fe. Network of Project INTA Fertilizar, 2000/01 and 2001/02 (Díaz Zorita et al., 2002).

Table 1. Recommendations of P fertilization for soybeans according to soil Bray P content and yield goal (Echeverría and Garcia, 1998).

Yield goal	Bray P content (mg/kg)				
	Less than 4	4-6	6-8	8-11	11-16
ton/ha	kg P/ha				
2	19	14	12	10	-
2.5	21	17	15	13	-
3	24	20	17	16	11
3.5	27	22	20	18	14
4	29	25	23	21	16
4.5	32	28	25	24	19

Responses to S in south central Santa Fe are related to the presence of degraded soils with low organic matter content as a result of soil erosion and a long cropping history (especially under continuous soybean or continuous wheat/soybean) (Martínez and Cordone, 1998). Martinez and Cordone (2000) reported increases of 626 kg/ha with S fertilization for full-season soybeans as average of six field experiments in south central Santa Fe province. Residual effects of S applications to wheat on double cropped soybeans were observed in several experiments (Díaz Zorita et al., 2002, Salvagiotti et al., 2003).

Sulfur recommendations are based on previous field history and management since responses are not strongly related to soil sulfate-S concentrations at planting (Martínez and Cordone, 2000; Díaz Zorita et al., 2002). Current research is looking at other soil S availability indicators such as short-term incubations and other soil sulfate-S extractants.

Recommended S rates vary from 10 to 15 kg/ha S for full season soybean and from 15 to 20 kg/ha S for double cropped wheat/soybean. Sulfur sources mainly include sulfate forms such as ammonium sulfate, calcium sulfate, magnesium sulfate, sulfate of potash magnesia, and others.

Other nutrients

Recent experiments have not shown generalized responses to nutrients other than N, P or S (Fig. 3). Responses to molybdenum (Mo) and cobalt (Co) were observed as seed treatments or foliar applications at early stages (Melgar et al., 2001). Boron (B) responses have also been observed in some areas of the Pampas (H. Fontanetto, pers. comm.).

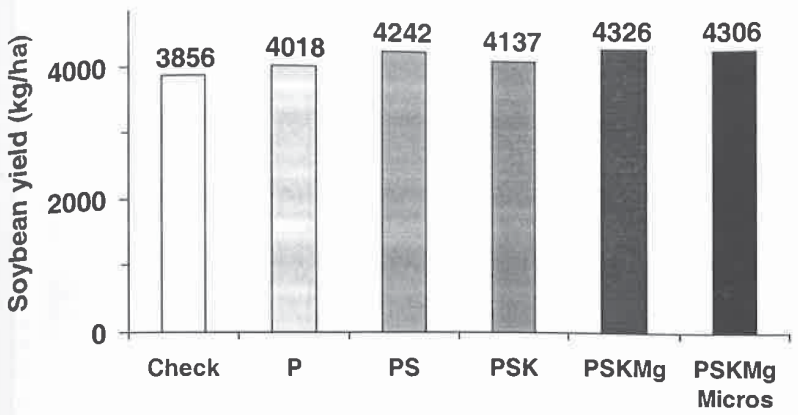


Figure 3. Full-season soybean yields with different fertilization treatments. Average of 10 field trials of the network AAPRESID-INTA-INPOFOS. 2001/02.

Fertilization management of double cropped wheat/soybeans and nutrient interactions

Fertilization of double cropped wheat/soybeans may be carried out at wheat drilling in most of the soybean regions of Argentina. Research has shown high residual effects of P and S applications in wheat for the next soybean crop (Table 2) (Cordone and Martínez, 2002; Díaz Zorita et al., 2002; Fontanetto et al., 2003; Salvagiotti et al., 2003). Thus, P and S could be applied for both crops at the time of wheat fertilization reducing operational costs and time at planting of double cropped soybean.

Table 2. Double cropped soybean yields with N, P, and S fertilization treatments applied in the previous wheat crop. Data from (1) Network of CREA South of Santa Fe and (2) H. Fontanetto and H. Vivas (EEA INTA Rafaela, pers. comm.).

Treatment	SW Santa Fe-SE Cordoba (1)	Central Santa Fe (2)
	kg/ha	
Check	3343	2153
NS	3615	2689
NP	3408	2502
NPS	3681	3036

Significant interactions among deficient nutrients applied through fertilization (P and S) or supplied by biological fixation (N) are observed in double cropped (Table 2) and full season soybeans (Fig. 4).

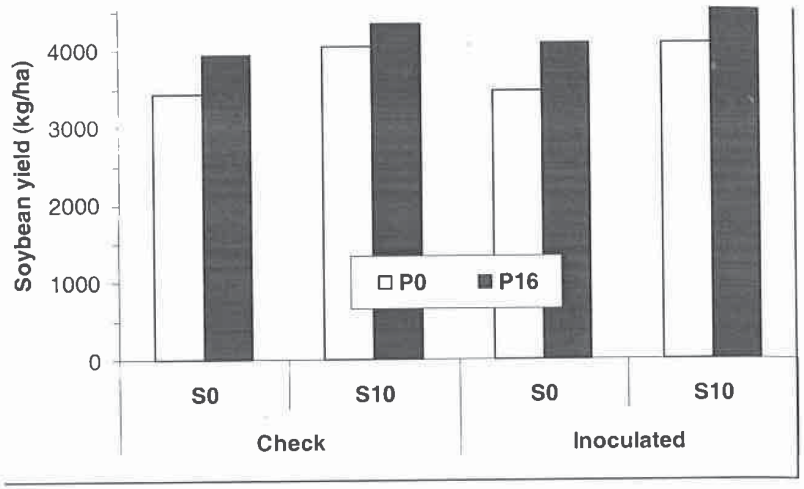


Figure 4. Full-season soybean yields with two rates of S (0 and 10 kg/ha) and two rates of P (0 and 16 kg/ha) with or without seed inoculation. Averages of two field trials at east central Santa Fe, 2002/03. Data from H. Fontanetto and H. Vivas (EEA INTA Rafaela).

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Soil fertility management for soybean in India

O.P. Joshi[1]

Abstract

Soybean has established itself as a premier oilseed crop of India. Presently it is estimated to cover more than 6.5 m ha, which is likely to produce more than 7 mt. In spite the varieties developed have potentials to yield 3.5 to 4.0 t/ha, progressive farmers harvesting 2.5 to 3.0 t/ha, and the productivity of Frontline Demonstrations conducted all over the country is 1.8 t/ha, the national productivity remains only 1.0 t/ha. Constraint analysis brought out that the inadequate and unbalanced nutrition provided to the crops evokes the "Law of minimum" as discovered by Sprengel-Liebig in the beginning of 19th Century. India shall have to resort to the balanced nutrition through the integrated nutrient management to maintain soil fertility and harness sustained yield levels / production of soybean.

Introduction

Soybean is one of the important oilseed crops of India. At present, it occupies nearly 6.5 million hectares and poised to produce above 7 million tons. Although, Indians knew this crop since ages (Tiwari *et al.* 1999), its commercial cultivation commenced only in seventies. The stride in productivity from 450 kg/ha in 1970 to above 1 ton/ha has been achieved. It has been a matter of concern that in spite of availability of improved varieties with high yield potentials (3.5 to 4.0 t/ha), crop harvested by progressive farmers (2000 to 2500 kg/ha), and yield levels achieved in demonstrations under real farm conditions (average 1800 kg/ha), the national productivity still remains around 1000 kg/ha. Constraint analysis done in the past (Anonymous, 1981 and 2000; Jain, 1986; Sharma *et al.* 1996: Tiwari, 2001) has indicated that the lack of soil fertility management by provision of balanced nutrition is one of the main reasons of low productivity of soybean in India.

The soils of soybean-growing regions

Madhya Pradesh, Maharashtra and Rajasthan are the major soybean growing states in that order. Madhya Pradesh contributes to the extent of 73% with respect to area and 65% to production (2000-01). Madhya Pradesh and Maharashtra together grow soybean largely on Vertisols and associated

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soils and constitute an area of 88%. Addition of Rajasthan makes this figure to be 98%. Soils of major soybean growing states fall within the three agro-ecological regions (AER 5, 6 and 10) as established by The National Bureau of Soil Survey and Land Use Planning, Nagpur (Table 1). The crop has wide adaptability and is grown from north to south and east to west regions of the country.

3. Present practices and analysis of soil fertility management

Soybean is one of the energy rich crops, but it does not receive adequate nutrition on account of poor fertility management practices. The farmers gradually forget the age-old practice of addition/recycling organic resources, which not only supplements nutrition but also provides sustainability. The fertilization is not only on lower side, but also skewed (N:P:K consumption ratio is 6.7:2.5:1 against ideal 4:2:1) providing sub optimal and unbalanced nutrition. Balanced fertilization (4.2:2:1) in Maharashtra since last couple of years (Anonymous 2003), has led to higher yields of all the crops including soybean (State average of 1.4 t/ha). Planting of seed mixed with fertilizer is in vogue. Top dressing of urea/DAP at later crop growth stages is resorted to. Use of bio-fertilizers in the major soybean command area remarkable but viability of culture used is questionable.

Table 1. The predominant soybean-growing agro-ecological regions

Agro-ecological region	Description
5	Swell-shrink soils are the dominant group in the region with loamy to clayey Vertic Ustochrepts and clayey Chromusterts. These soils are predominantly high in available K, but medium/medium to high in exchangeable K. Although high in available K, but K saturation is quite low. These are slightly to moderately alkaline, calcareous as well as non-calcareous and low to medium in organic matter.
6	Swell-shrink soils are the dominant group in the region with loamy to clayey Ustorthents, Chromusterts and Ustropepts. These soils are predominantly high in available K, but medium/medium to high in exchangeable K. Soils are neutral to moderately alkaline, calcareous and medium to high in organic matter.
10	Medium to deep black soils are interspersed to patches of red soils. The dominant groups are Ustoorthants, Ustochrepts, Chromusterts and Haplustalfs. These soils are predominantly high in available K, but medium/medium to high in exchangeable K. Black soils are calcareous, slightly to moderately alkaline and low to high in organic matter.

Based on the agronomic experimentation in different agro-climatic regions, the All India Coordinated Research Project on Soybean has recommended

application of nitrogen (N), phosphorus (P), potassium (K) and sulphur (S) at the levels of 20.0, 26.0-34.6, 16.6 -33.2 and 20 kg/ha, respectively with a provision of tailoring these levels based on soil test values. The crop uptake of these nutrients in order reported was 125-190, 19-43, 101-120 and 9-22 kg/ha (Nambiar and Ghosh, 1984 and Aulakh, *et al.* 1983), respectively. Recommendation of N application @ 20 kg/ha is well justified as the soybean crop has been reported to fix atmospheric nitrogen to the extent of 50 to 300 kg/ha (Keyser and Fudi Li, 1992), which is further supplemented by biomass recycling and non-symbiotic nitrogen fixation. Soybean, on an average, is capable of recycling about 1 ton leaf litter per hectare (Joshi, O.P., NRC for Soybean - unpublished). Recommendation of phosphorus and sulphur also corroborates the respective nutrient uptake. There appears to be a wide gap between the recommendations of potassium vis-à-vis uptake. To maintain the soil fertility leading to sustainable yield levels, it is imperative to standardize and readjust the recommendation for potassium. The revision of K levels are further needed in view of lower fertilizer use efficiency of K (20-40%) than N (above 40%) for soybean and corn (Carpenter, 1975). The use of fertilizer adjustments equations (Anonymous, 1980-81) to achieve targeted yields may help in maintaining soil fertility and productivity.

Soil fertility management

Organic manuring and bio-fertilization

Organic manures and composts have been used as a means of maintaining and increasing soil fertility all along the history of farming. The value for soil organic carbon (SOC) stock for upper 30 cm soil layer in India is only 21 Pg (1 Pg = 10^{15} gm). India's share in overall SOC stock of world is only 3%, though it covers 11.9% of total geographical area (Singh and Biswas, 2000). To keep our soils sustainable, fertile and healthy, it is obligatory on our part to ensure its restoration by appropriate agricultural activities. The supplementary and complementary use of organic manures and bio-fertilizers improves soil physical, chemical and biological properties (Ramesh, A., NRC for Soybean – Unpublished), the fertilizer use efficiency, mitigates short supply of micronutrients (Joshi *et al.* 2000), stimulates the proliferation of diverse group of soil microorganisms and play an important role in the maintenance of soil fertility (Ramesh, A., NRC for Soybean – Unpublished).

The use of biofertilizers like *Bradyrhizobium*, phosphorus solubilising microorganisms (*Bacillus megaterium*, *B. circulans*, *B. subtilis*, *Pseudomonas striata* etc., fungi: *Aspergillus awamori*, *Pencillium digitatum*, *Trichoderma* etc) and Plant Growth Promoting Rhizobacteria (PGPR) (*Actinoplanes*, *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Pseudomonas*, *Erwinia*,

Rhozobium Bradyrhizobium etc.) have been useful to maintaining soil fertility and sustainability of land/production. Co-inoculation of PGPR *viz Pseudomonas* and *B. japonicum* increased soybean plant root and shoot weight, grain yield, plant vigour, nodulation and N-fixation.

Balanced Fertilization

Organic inputs cannot be considered as the alternative to mineral fertilizers. The two cannot be projected as mutually exclusive. To meet the total nutrient needs in intensive cropping systems, an integrated supply of nutrients from fertilizers and organic manures seems to be inevitable to cope up the mineral nutrition stress faced by the crops in India. We shall have to keep in mind the "Law of minimum" as discovered by Sprengel-Liebig (Ploeg van der, W. Bohm and M.B. Kirkham, 1999) in the beginning of 19th Century to provide balanced nutrition to soybean.

Response to application of fertilizer nutrients in dry lands was reported across the locations in the country (Katyal and Reddy, 1997 and Katyal *et al.* 1999). There has been a wide gap between nutrients removed and replenished. Nutrients (N, P and K) removed by all dry land crops were to the tune of 7.4 m t. Assuming that dry lands receive 10% of the total nutrient use in the country, which comes to about 1.4 m t, there still remains a net negative balance of about 6 m t. This gap is going to widen further in future, if adequate care is not taken to replenish the soil fertility using fertilizers or /and organic resources. India shall have to increase the use of fertilizers (it is only 85 kg /ha at present) to maintain fertility and enhance the productivity to meet the demand of food.

Integrated plant nutrient management system

In the year to come, for a system to be sustainable, it becomes imperative for the management of organic matter and the rational use of organic inputs. Even if we utilize the whole of our organic resources presently available for agricultural use, it would be able to meet only one third of the nutrient required for the present level of food grain production (Singh and Biswas, 2000). To meet the total nutrient needs in intensive cropping systems, an integrated balanced supply of nutrients from fertilizers and organic manures following integrated nutrient management (INM) seem to be a need of the time. This has been amply demonstrated in the field studies and long-term fertilizer experiments conducted in the country so far (Joshi, 2002).

Moreover, all the possible care is needed to be taken to minimize the nutrient drain (Mani, 1975) caused by the prolific growth of weeds in the soybean crop, which is normally cultivated in monsoon season.

Further trends in fertility management

To be more efficient in use of nutrients, it is necessary to work out the mining capacities of soybean varieties. It has been reported that of the five varieties tried, PK 472 and PK 416 were better extractor of native soil P whereas Pb 1, JS 80-21 and JS 335 efficient in utilizing the applied P (Dravid *et al.* 2000). Soybean along with groundnut and pigeon pea were better feeder of native soil P as compared to other rainy season legumes namely black gram, green gram and cowpea at 30 days after sowing (Joshi *et al.* 1977). Young seedlings of soybean do not use much potassium, but the rate of uptake climbs to a peak during the period of rapid vegetative growth. The nutrient balance and ratio of nutrients to break the plateau in yields of the crops is to be looked into (Ahmad and Abdin, 2000) to refine the process of nutrient management. The potassium in vegetative parts is transferred to seed during pod fill process. The soybean seed contains nearly 60% of the total K in plant (Hoeft *et al.* 2000). Information on the nutrient feeding pattern at varying growth stages also comes handy for fertility management.

Conclusions/basic issues to be addressed

- Genetic improvement of crop varieties with high yielding trait. The traits of better ability to mine native soil nutrients, particularly P and K, with promiscuity, better rooting pattern, super nodulation etc.
- Creating awareness among the farmers on INM.
- Maintenance/enhancement of soil organic carbon stocks.
- Efficient use of bioagents/biofertilizers in nutrient recycling and nutrient dynamics.
- Enhancement in fertilizer use efficiency.
- Ascertaining the nutrient requirement at different growth stages.
- Utilization of varietal differences in mining nutrients from soil.
- Appropriate replenishment of nutrients removed by the crops.
- Working out the nutrient requirement under various cropping systems. The exercise is to be done after identifying the crop rotation for larger period utilizing the diversification in crops/varieties.
- Balanced use of fertilizers.
- Enablement and empowerment of farmers.
- Participatory approach, the utilization of targeted yield approach utilizing the equations developed under STCR programme.

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Soil fertility management for soybean in Brazil

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Abstract

Most of the soils in Brazil are highly weathered, with the mineralogy of the clay fraction dominated by kaolinite and hydrous oxides of iron and aluminum. As a result, these are soils of variable charges and this affects the relations of exchangeable cations with soils and also of anions that can be retained by ligand exchange, such as phosphate and sulfate. Soil acidity and phosphorus deficiency are the main soil fertility problems in soybean cropping. Nitrogen is not recommended in fertilization because of biological N_2 fixation. Phosphorus is the plant nutrient most deficient in soil and therefore the most required in fertilization for cultivation of new areas of the cerrado regions. Potassium is a nutrient highly demanded by soybean, especially in some sandy and medium texture soils that present important leaching losses. Sulfur deficiency occurs especially in the cerrado region and is usually corrected by the use of single superphosphate or phosphogypsum. Calcium and magnesium deficiencies are not important because of the widespread use of dolimitic limestone. Deficiencies of all micronutrients except chlorine and iron may occur. Low soil fertility can be corrected by liming and fertilization to promote high soybean yields.

Introduction

Soybean is an unique crop in the sense that it is the only major agricultural crop of the world that does not require fertilization with nitrogen, the nutrient that is in the largest demand for other crops. In Brazil, where nitrogen fertilization is not recommended for soybean, inoculation with *Bradyrhizobium japonicum* is essential for adequate nitrogen fixation. Soil acidity and deficiencies of almost all nutrients are important limiting factors. Thus the improvement of soil fertility of nutrient-deficient soils of the tropics and subtropics of Brazil for soybean is a very important issue for obtaining high yields, as discussed in this article.

Soils

Most of the soils in Brazil belong to the orders of Oxisols, Ultisols, Alfisols and Inceptisols. They are moderately-to-highly-weathered, presenting surface properties of the clay fraction dominated by organic matter, kaolinite

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and aluminum and iron hydrous oxides. As a result, these are soils of variable charges and this affects the relations of exchangeable cations with soils and also of anions that can be retained by ligand exchange, mainly phosphates and sulfate. Most calcium, magnesium and potassium are in exchangeable forms and soil contents are generally low. Large areas in Brazil present soils with extremely low soil fertility, including the Cerrado region, most of the Amazon region and also the soils originally with natural grassland of the South. Although chemically poor, such soils have, generally, very good physical properties and rainfall is abundant and well distributed for the requirements of soybean, in most places even allowing a second crop in the growing season.

Thus, to become productive for soybean, chemical constrains of the soils must be removed, with liming and phosphate fertilization as the major factors to be taken care of. Other elements must also be added, including potassium, sulfur, boron, zinc, manganese, copper and molybdenum.

Phosphorus

Soil acidity and phosphorus deficiency are the most important soil fertility problems in Brazil. Soil acidity is the subject of another paper in this Symposium (Rosolem et al., 2004). For this reason the subject will not be discussed here, except for the influence of soil acidity on nutrient availability for plants.

In Table 1, the results of an experiment on the effect of liming and phosphate fertilization are presented, indicating the effect of both factors in increasing yields of soybean. In this experiment an important interaction effect was observed.

Table 1. Soybean production obtained with increasing rates of limestone and phosphorus on an allic dusky red Latosol from Chapecó, state of Santa Catarina.

Limestone, t ha^{-1}	Soybean production for rates of P_2O_5, in kg ha^{-1}		
	0	80	160
0	1,800	2,750	3,160
7	1,980	3,180	3,960
14	2,420	3,620	3,900

Fonte: Kochhann et al. (1982).

However, in the great majority of cerrado soils, there is a larger response to phosphorus application than to lime, because the soils are more weathered and less electronegative. An example of such soil, with a severe phosphorus deficiency, is the case of an Oxisol in the Northeast of Brazil, presented in

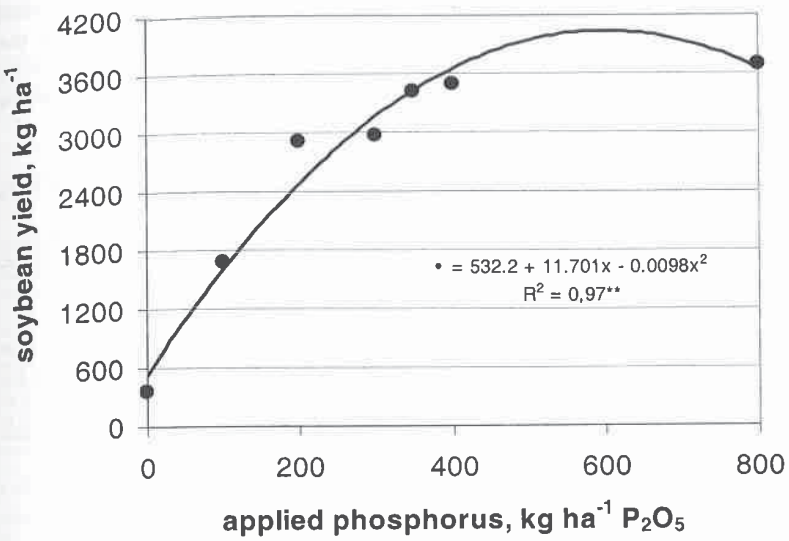


Figure 1. Effect of phosphate on the yield of soybean variety BRS Sambaíba on a red yelow Latosol with 28% clay from Piauí state. (Klepker et al. 2003).

Phosphorus availability in soil for crops is largely determined by solubility relations, which are affected by soil reaction. Thus the effect of the soil reaction on phosphorus availability for plants is determined largely by the solubility product of the various phosphates existing in soils. Theoretically, this is shown by solubility isotherms, such as those presented by Olsen and Khasawneh (1980). Aluminum phosphates such as variscite, and iron phosphates such as strengite, have their solubility decreased with the pH increase. On the other hand, all calcium phosphates become more soluble at lower pH values. Thus, liming favors the solubility of iron and aluminum phosphates and reduces the solubility of calcium phosphates. This remark explains also the well known effect of the pH on P availability for crops, with the highest availability in a range around pH 5.5 to 6.8 (Tisdale et al., 1985), values that correspond approximately pH 5.0 to 6.2 in 0.01 mol L^{-1} calcium chloride. In this range, the conditions of maximum P solubility occur for all phosphates present in the solid phase in equilibrium with the soil solution.

In practice, the consequences of phosphate solubility considerations can be made only on a qualitative basis, because of the complexity of soil with a large variety of phosphorus compounds in the solid phase and of chemical

species in the soil solution. But there are some experimental results available for soybean in Brazil, as shown in Table 2, that allow some conclusions. The data show that the increase of soil pH due to liming is in line with yield increase and, also, with the increase in leaf P. Thus, the pH increase of the soil favors a higher availability of P for the soybean crop, as shown by resin-P, in Table 2. In these experiments all treatments received the same amounts of fertilizer phosphate.

Table 2. Effect of liming on soil pH, soybean yields, leaf P, and soil P as determined by different extraction methods.

Location	Limestone,	pH in	Yield	Leaf P	Soil P, in mg dm^{-3} for extraction method			
	kg ha^{-1}	$CaCl_2$	kg ha^{-1}	mg kg^{-1}	Mehlich 1	Bray 1	Olsen	Resin
Mococa	0	4.3 a	1,274 a	1.85 c	6 a	15 a	10 a	13 c
(Ultisol)	2	4.8 d	2,020 b	2.06 bc	7 a	16 a	11 a	16 c
	4	5.5 c	2,308 bc	2.44 ab	5 a	13 a	7 a	17 bc
	6	6.1 b	2,649 cd	2.26 a	7 a	17 a	8 a	22 ab
	8	6.4 a	2,372 d	2.55 a	7 a	15 a	8 a	27 a
Ribeirão Preto	0	4.5 d	1,734 a	2.35 b	9 a	20 a	18 a	16 c
(Oxisol)	3	4.9 c	2,246 b	2.69 ab	8 a	22 a	15 ab	19 bc
	6	6.1 b	2,483 cb	2.88 a	8 a	20 a	13 ab	23 b
	9	6.6 a	2,622 c	2.85 a	10 a	24 a	12 b	34 a

However, the comparison of the values of soil P with the different extractors do not point to the same direction, showing thus a lack of sensibility of some of the soil testing methods in detecting the variation of labile or available P promoted by liming. The results obtained by Mehlich 1, a method in general considered more adequate to extract P bound in calcium compounds (Kamprath and Watson, 1980), were not affected by liming and the results were lower than for the other methods. The results with Bray 1, a method that should determine especially aluminum bound phosphates, were also not affected. For the Olsen method, with a higher pH that should release Ca and Al phosphates by hydrolysis, the results decreased for the increase in soil pH, thus determining values that were opposite to the P availability for soybeans. This unusual effect was also observed in experiments with beans and sunflower (Raij and Quaggio, 1990). On the other hand, resin extractable P, indicating an increase in the results of soil analysis, was according to plant response and theory. This observation has an important practical consequence in determining the fertilization needs of phosphorus using soil analysis.

Other macronutrients

Since soybean does not require nitrogen fertilization besides P, the other nutrient that is most necessary for the crop in fertilization is potassium. For each ton of soybean, the crop removes about 23 kg of K_2O in harvests. Since most highly weathered soils of Brazil do not have much plant available K in non-exchangeable forms, as is the case in soils of temperate regions, adequate K fertilization is necessary for crop growth. Exchangeable K is an adequate index of availability of the element for crops.

The potassium removal by the 41.9 million tons crop harvested in 2002 represented about one million tons K_2O. This is around one third of the 2.9 million tons K_2O used in 2001 in Brazilian agriculture (ANDA, 2003).

Sulfur deficiency is important in several Brazilian states, but especially in the cerrado region of central Brazil. The correction is easily made using several S sources available, especially single superphosphate and phosphogypsum. Sulfate is easily leached out the surface soil layer, but accumulates in the subsoil were it is strongly adsorbed by ligand exchange. This feature renders soil testing for S based on samples of the surface layer insufficient for a proper diagnosis. Sulfur deficiency, if not corrected, increases with time of cropping and reduces soybean yields considerably, as was shown by Vilela et al. (1995) for a Cerrado soil from the Brasilia region.

Calcium and magnesium deficiencies, on the other hand, are rare because of the widespread use of dolimitic limestone.

Micronutrients

The cerrado region in central Brazil is where the cultivated area of soybeans is rapidly increasing. An extensive survey was made concerning soil fertility in soil samples, including micronutrients, indicating that deficiencies of micronutrients are widespread in the area (Lopes, 1983). Rosolem et al. (2001) in a general review on micronutrients in Brazilian agriculture (Ferreira et al., 2001) confirmed that deficiencies of boron, copper, manganese, molybdenum and zinc occur in several parts of Brazil.

Galrão (1991) conducted research on the response of soybean to micronutrients on an Oxisol of the Brasília region. Significant responses were obtained for boron, copper and zinc, but not for manganese and molybdenum.

During the last few years, manganese deficiency has attracted attention, maybe because it is strongly affected by liming and the deficiency is more difficult to correct using soil applications of the nutrient. In general, the deficiencies are lime-induced or at least aggravated by liming.

In other experiments, the influence of soil acidity, represented by the base saturation of the soils, had little influence on the effects of Zn, Mn, B and Cu for soybean. The major effect on yields was due to copper application, followed by Zn, B and Mn (Oliveira Junior, 2003). The yield response of soybean to Cu application is illustrated in Figure 2. Although significant increases were obtained only with the lower rates, the slope of the curve indicate that the correction of Cu deficiency might require larger inputs of the nutrient in this clayey soil.

Soil analysis

In Brazil, two systems of soil analysis are in use for soybean and other crops (Raij et al., 1994; Cantarella et al., 1998). There are important differences between the two systems. The first is based on extraction of P by Mehlich 1, the determination of pH in water, and the calculation of lime requirement based on exchangeable Al, Ca and Mg to reduce aluminum and increase exchangeable Ca and Mg. This set of methods was introduced in Brasil by North Carolina State University in a joint project with the Brazilian Ministry of Agriculture. The determination of metallic micronutrients in soils using the Melich 1 extractant was introduced later in soil testing laboratories. The second system was developed by the Instituto Agronômico and the main features are the extraction of P from soil using an ion exchange resin, the determination of pH in 0.01 mol L^{-1} $CaCl_2$, and the calculation of liming requirements by the increase of the base saturation of the cation exchange capacity at pH 7 to 50 or 60%. This method has similarities to the method of the SMP-Shoemaker McLean and Pratt (1961) buffer solution, used in the states of Rio Grande do Sul and Santa Catarina, which considers liming up to a given pH value. Later, the determination of metallic micronutrients with a DTPA solution was introduced.

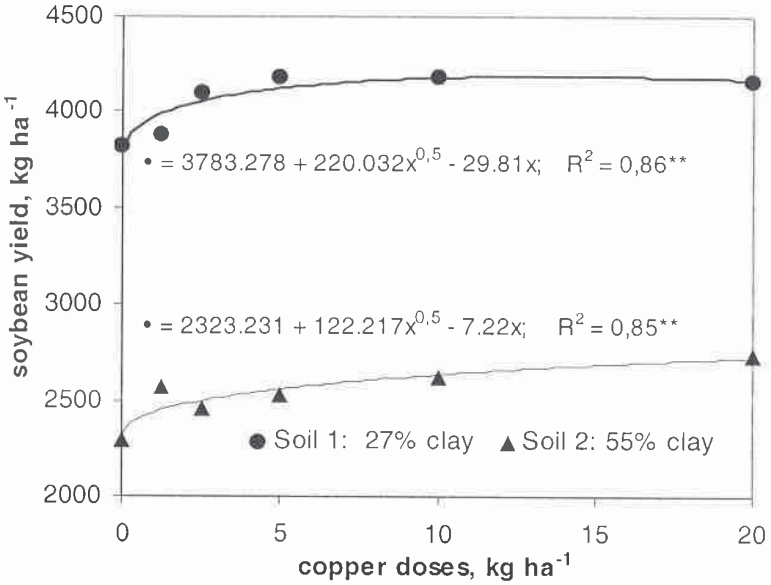


Figure 2. Productivity of soybean (Cv. BRS Sambaíba) as affected by copper applications on two Oxisols of the region of Balsas, state of Maranhão (Oliveira Junior, 2003).

The first system has some problems. The P determination with an acid extractor presents, in general, a much lower predictability of the availability of P in soils than the resin method. Furthermore, the Mehlich 1 extractor tends to overestimate P in soils that have received reactive rock phosphates under conditions in which they dissolve slowly, such as surface applications in minimum tillage systems. For liming, the so called aluminum criteria in the way it is used, adding up the amounts of limestone required to either neutralize aluminum or increase calcium and magnesium, does not allow the prediction of the soil pH that will be reached and can result in overliming in sandy soils. In the case of micronutrients, different sources of varied solubility are used in Brazilian agriculture. Abreu et al. (1996) have shown that manganese sources of low solubility, that are not much available for soybean, nevertheless present higher results in the Mehlich 1 extractor, as compared with DTPA, which in such cases is more reliable to predict the plant availability of Mn in soils that received application of different Mn fertilizers. Unpublished results with Zn show the same tendency.

Conclusion

Brazil has extensive areas of soils of low fertility that can be used to grow soybean. These soils have good physical properties, but present, in general, very low soil fertility and frequently, deficiencies of various macro and micronutrients may occur if proper action is not undertaken. This includes proper management of lime and fertilization with macro and micronutrients, as well as soil and plant testing to confirm requirements. In this manner, maximum and optimum yields will be achieved.

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Soybean nutrient management in the United States

H. F. Reetz, Jr.[1]

Soybeans have over the past 60 years become a major crop in the United States, currently grown on about 72 million acres (29 million hectares). Figure 1 shows the distribution of soybean acreage among the major soybean-producing states.

In most cases soybeans are grown in rotation with other crops, predominantly corn. Since corn is usually the primary focus of nutrient management planning, the nutrient management program is usually designed to meet the needs of the corn crop, and the soybeans feed on the nutrients remaining. As soybeans have increased in total acreage, it has become more important to directly address the nutrient requirement for the soybean crop.

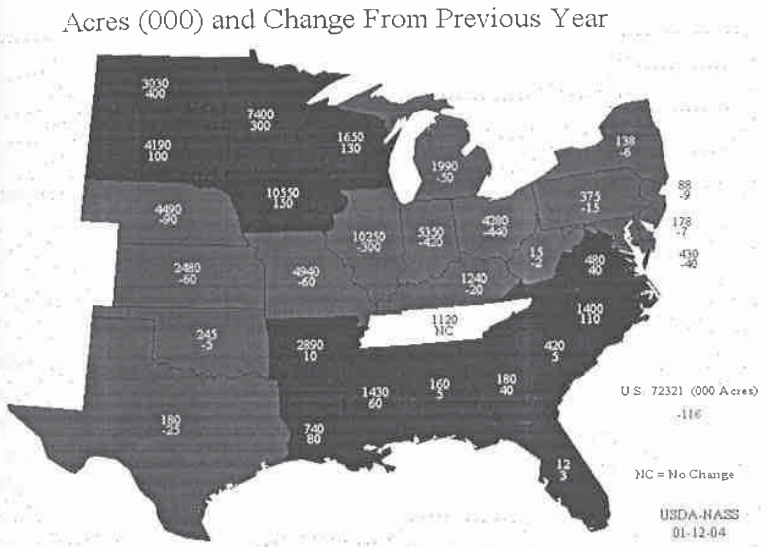


Figure 1. Distribution of Soybean Acreage in the United States.

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Total soybean production is highest in the Midwest states, roughly the same area as the Corn Belt. This area has the best soils and best climate for soybean production, so the concentrated acreage and higher yields makes this the top soybean production area as well. Figure 2 shows the relative yields for different counties in the US. The darker colored areas represent counties that produce the highest soybean yields.

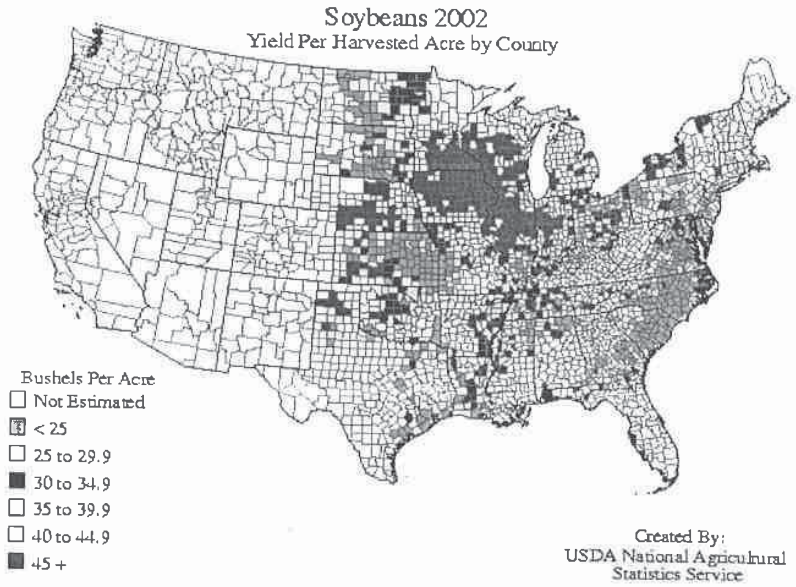


Figure 2. Distribution of Soybean yields in the United States.

Fertilizer use and fertility management

When the soil cannot supply the plant nutrient requirements, fertilizers and/or manure can be added to supplement the nutrient supply. Soil tests are the best tool to determine the need for supplemental nutrients. Nutrient management is not, however a simple "add/subtract" process. Uptake of nutrients added to soils is not always an efficient process. Under good conditions, the recovery in the year of application ranges from 5 to 20 percent for phosphorus and 30 to 60 percent for potassium. However, additional nutrients are recovered in future years. So a long-term nutrient management plan can use soil tests and crop removal as a basis for estimating needs. Depending on the soil clay minerals present, and chemical reactions that occur, some of the nutrients applied are fixed into the minerals and chemical compounds into unavailable forms, and are only slowly

available to the growing crops. Knowing the detailed characteristics of the soil is an important part of nutrient management.

Likely nutrient deficiencies

1. Soybeans acquire most of their nitrogen from atmospheric nitrogen fixed symbiotic *Rhizobium* bacteria in the nodules on the roots. Where soybeans have not been grown previously, inoculation is needed to supply the desired bacteria. Liming of acidic soils is usually beneficial. By making conditions favorable for N-fixation, the need for N fertilization is reduced or eliminated.
2. Continuous cropping depletes phosphorus and potassium levels, and in most soils the rate of mineralization release of nutrients is not adequate for optimum yields. Fertilizers and/or manure applications are needed to meet the needs of growing crops. Depending on soil pH, lime also may be needed.
3. Applications of some micro-nutrients may be necessary on some soils where deficiencies exist. Elements most commonly needed are S, Fe, B, Mn, or Zn.

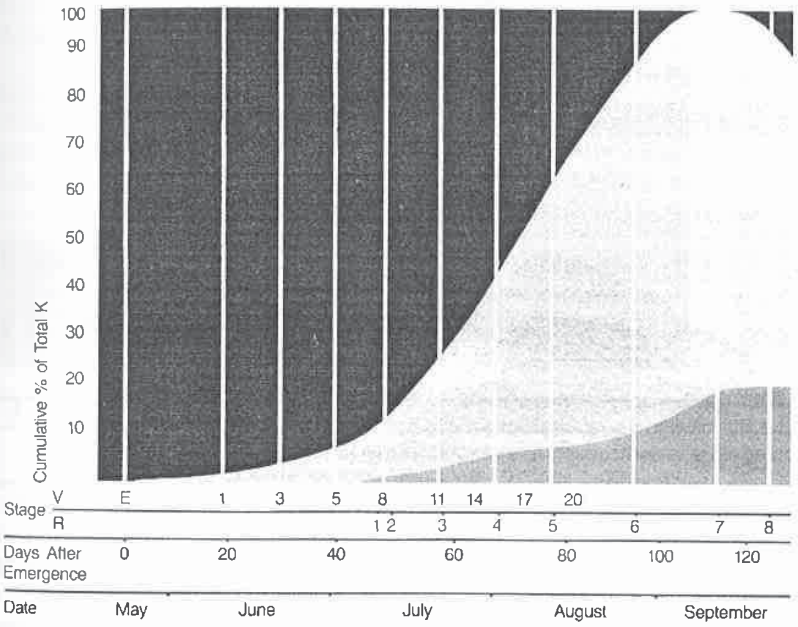


Figure 3. Rate of uptake of potassium (K) by soybeans throughout the growing season. Source: Iowa State University, "How a Soybean Plant Develops".

Nutrient requirements and uptake by plants

Farmers and their advisers need to have a basic understanding of the growth of the crop and its nutrient requirements to develop a successful plan to supply the nutritional needs of the crop.

Total uptake is important. Nutrients must be available in sufficient amounts to supply the needs of the growing crop at all *times* during the growing season. Any shortages, at any time, may restrict development of the plant or the reproductive (grain yield) capacity. At critical times, shortages can severely limit yield potential, regardless of what happens during the rest of the season. A successful nutrient management plan must supply adequate amounts of all nutrients at all time during the crop's growing season. For soybeans, the potassium requirements follow the patterns shown in Figure 3.

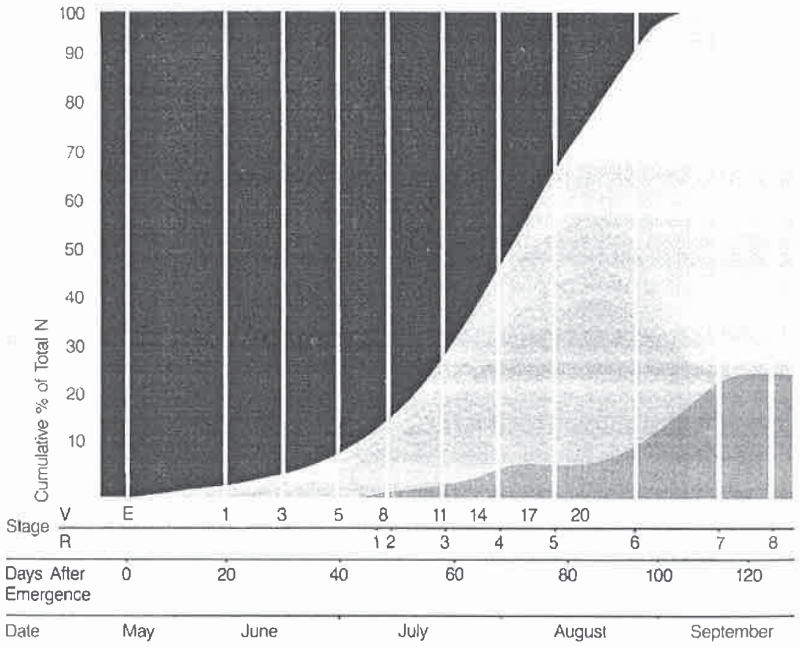


Figure 4. Rate of uptake of nitrogen (N) by soybeans throughout the growing season. Most of the N is obtained from the atmosphere by *Rhizobium* bacteria nodules on the roots. Source: Iowa State University, "How a Soybean Plant Develops".

The crop removal rate for each nutrient is also important. Each unit of grain (bushel, kg, etc.), removes a certain amount of nutrients. Standard removal numbers have been established by research, but these amounts can vary widely depending on genetic differences and environmental conditions, and may vary with yield level as well. Having grain samples analyzed may be the best way to get a reliable estimate of crop removal. Table 1. provides an example of the nutrient uptake and removal rates for soybeans.

Table 1. Soybean nutrient uptake with 50 bu/acre seed yield, 5000 lb/acre stover yield.

Nutrient	Removed in Seed	Remaining in Stover (pounds per acre)	Total Uptake
N	188	127	315
P_2O_5	44	30	74
K_2O	66	76	142
S	5	15	20
Zn	0.05	0.3	0.35

Source: Franzen, D. and J. Gerwing. 1997. Effectiveness of Using Low Rates of Plant Nutrients. North Central Regional Research Publication 341.

Each crop has different nutrient removal rates. Soybeans remove much more potassium per bushel, for example, that does corn. As soybean acreage has increased over the past 20 years, and soybeans have appeared in the rotation more frequently, there has been a shift in nutrients removed by the crops. Yields have also steadily increased, so that total production and total removal are dramatically increased. Figure 5. shows the trend in U.S. soybean yields over the past 30 years.

Since virtually all soybeans are sold off the farm, and a large percentage are exported, the total nutrient removal by soybean grain is always removed from the farm, creating a net loss of nutrient resources. During this period, fertilizer applications in the U. S. have not changed sufficiently to replace the removal rates. As a result, soil test K levels, for example, have been shown to be declining.

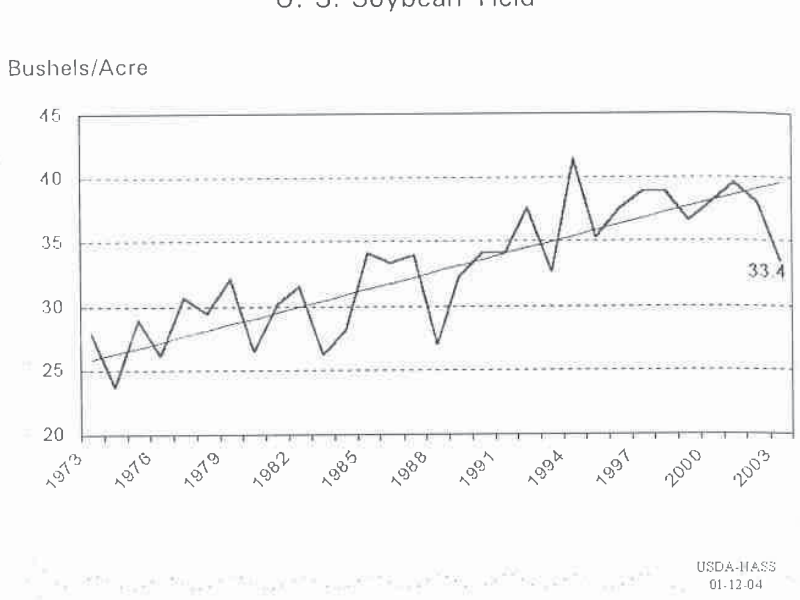


Figure 5. Average US Soybean yields, 1973-2003. Source: USDA - National Agricultural Statistics Service.

Sustaining productivity

A 2001 Summary of Soil Tests in North America shows that for the heavy soybean production areas of the Midwest U. S., 60 to 70% of the soil samples analyzed were testing medium or below in potassium. That means that they would likely show an economic response to additional potassium applications. A map of potassium soil K tests indicating percentage of samples expected to respond to K fertilizer (Figure 6.) clearly shows that K applications are not meeting crop removal needs. The result is a decrease in soil tests, which leads to reduced crop yields and inefficient use of other nutrients.

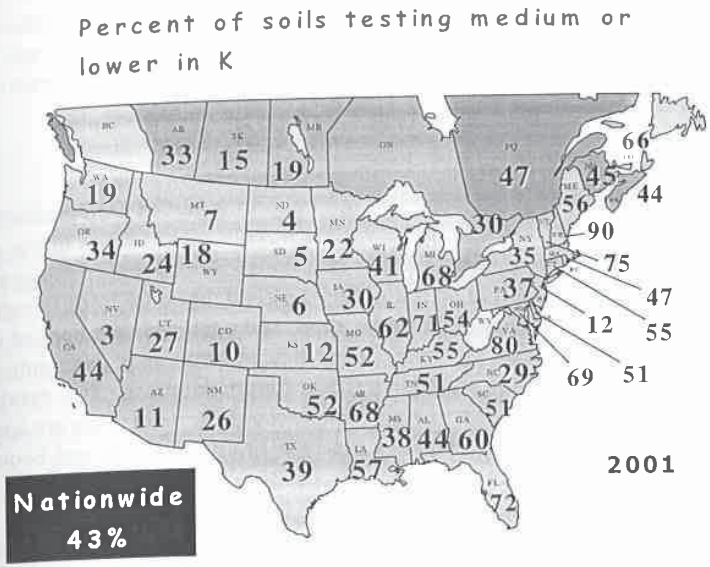


Figure 6. Results of Soil Test Summary of approximately 2 million samples. Source: Potash & Phosphate Institute.

Similar deficits in phosphorus were also found in this survey of soil testing results. Continued productivity, or sustainability, requires that the nutrients removed from the soil be replaced systematically. Soil testing is an important management tool to help guide nutrient applications to be sure soil levels are adequate to supply the needs of the growing crop. If initial soil tests are low, additional applications maybe needed to build soil tests to the optimum levels for crop production. The "maintenance + buildup" concept is used in much of the corn/soybean growing area. It takes care of both initial limitations and crop removal.

Soil tests in the areas of intensive grain have been declining in recent years, because fertilizer additions have not been sufficient to replace the total nutrient removal rates by all crops in the rotation. In the Midwest, a corn-soybean rotation is the most common cropping system. Most commonly, farmers have planned to fertilize for both crops in the rotation, usually applying the fertilizer before the corn crop, and in amounts necessary to supply the needs of both crops. In recent years, soybeans have been in the rotation more frequently—commonly every other year instead of once in 3 or 4 years—but fertilizer applications have not been increased to account for the increased total removal. In many cases, the crop yields have also

been higher than projected, so nutrient removals have been greater than the levels used in planning applications. The result is a decline in soil test values. Adjustments must be made to future plans to replace the nutrients removed.

Technology adoption

Nutrient management is a complex, but important part of crop production. Many US farmers have adopted a variety of new technologies in the past few years to help them fine-tune their decisions. Site-specific management is now being implemented on many farms. Yield monitors track variability in yields within the fields. Fertilizers, seed, and pesticides are applied with variable-rate application systems. All of this information is geographically referenced with GPS and stored in a GIS software database. This database becomes more valuable as a management resource as more years are added. After 5 or more years, it becomes possible to study trends and begin to analyze relationships among different records.

As crop profit margins get smaller, the need for intensive, site-specific management increases. Farmers who monitor yields, soil tests, and profitability will have the best chance to stay competitive with their neighbors...either on the next farm or on the other side of the world. Proper nutrient management is a key part of that process, and one of the easiest to manage.

Soybean breeding for rust resistance in Thailand and extent of rust resistant cultivars used

M. Nuntapunt[1]; S. Srisombun[2] and J. Chunwongse[3]

Abstract

Soybean is one of the important economic crops in Thailand. Soybean rust (*Phakopsora pachyrhizi* Syd.) which caused economic losses is considered as one of the major diseases. Breeding for soybean rust resistance has been one of the main objectives of soybean breeding programs. Since 1970, conventional breeding, mutation techniques and DNA marker assisted selection for rust resistance have been carried out for soybean breeding program in the kingdom. The tolerant varieties, SJ4, SJ5, Chiang Mai 60 (CM60) and Doi Kham are recommended to grow in soybean producing areas. In the part of Plant Pathology, the update research results indicated that there were eight races of rust in the North of the country. The new promising resistant lines, CM60-10kr-71 and MJ 9519-5 resisted to those eight races. (Reddish Brown Type), while MJ 9518-5 resisted to only seven races and susceptible to the race SM-44-1 (Tan Type). At the present time, the molecular biology has been used to facilitate the breeding program. It was found that two markers (Satt 12 and Satt 472) were associated with soybean rust resistance. Marker assisted selection will be used to select soybean rust resistant lines.

Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the most important economic field crops of Thailand. According to its valuable food, Thai people use soybean for human food and animal feed industries. With 20 % of oil and 40 % of protein from seed are beneficial uses. In addition, isoflavone which can be extracted from seed reduces cholesterol, heart diseases and gives anti-cancer action.

During 1988-1992, the average annual soybean growing areas was about 400,000 ha and the total production was 530,000 tons. Ten years later soybean growing area decreased, due to low productivity and the other competitive crops. At the present there are only 230,000 ha. Its yield is

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1.43 t/ha which increased from 0.99 t/ha in 1978. However, the annual production is not enough for country demand. Thailand imports soybean grain mainly from the USA and Brazil about 60-70 % and soybean cake from Argentina and India about 50-60 % of the total imports.

The main soybean growing areas are in the lower northern part of the country such as Sukothai, Kampangphet and Tak Provinces. In the North, soybean was grown in the upper land in the rainy season and lower land in the dry season after harvesting rice. The most favourite varieties are SJ 5, Chiang Mai 60 , SJ 4 Sukothai 1 , Sukothai 2 and Chiang Mai 2.

There are many factors which affect to the yield reduction. Soybean disease is one of those factors, especially soybean rust, caused by *Phakopsora pachyrhizi* Syd. Soybean yield losses became up to 45-60 % in cultivar SJ 5 (Nuntapunt et al. 1992A) particularly when grown in the northern area of the country.

Soybean breeding program for rust resistance

Conventional Breeding, Mutation Techniques and DNA Marker Assisted Selection are carried out for Soybean Rust Resistant Breeding Program in Thailand.

Conventional breeding

During 1970-1976, soybean varietal improvement for rust resistance was conducted at Chiang Mai Field Crops Research Center formerly known as Maejo Experiment Station. As a result, two tolerant varieties of soybean, SJ 4 (ACADIAN x TAINUNG 4) and SJ 5 (TAINUNG 4 x SJ 2) were released in 1976 and 1980, respectively. At that time the selection criteria of soybean breeding program were as follows:

a. High yield
b. Good plant types
c. Good grain qualities
d. Good germination
e. Resistant to rust (IWGSR SYSTEM)
f. Not seed shattering
g. High oil contents

Subsequently, SJ.4 and SJ.5 were not tolerant to rust probably due to the environment and the races of rust had been changed. Then the conventional breeding of some new soybean groups were conducted. The advanced soybean lines which were selected from screening techniques for rust resistance under field conditions were 7508-50-10, 7507-38-4,7507-66-7,7508-17-3 and 7608-25-4. Since 1980, the new techniques for evaluation from Dr. Arnold T.Tschanz of AVRDC were introduced to select rust tolerant varieties, e.g. the percentage of rust severities (Horsfall and Barratt, 1945),

defoliation (%), growth stage (Fehr and Cavines, 1977), days to maturity (R8), % yield loss, 100-seed weight and comparison of seed size in relation to pod cavity. The result of the new evaluation techniques, the line 7508-50-10 was determined to be the soybean rust tolerant line (Table 1). It was released as Chiang Mai 60 in 1987.

Table 1. Comparison of rates of rust development, total rust affected foliage yield and yield loss from three cultivars grown under fungicide and non-fungicide protected conditions during severe soybean rust epidemics at Chiang Mai Field Crops Research Centre in the wet season 1983.

			Yield (t/ha)		
Cultivar	Rate of Rust Development[a]	Rust Affected Foliage[b] (%)	Fungicide Protected	Non-Fungicide Protected	% Yield Loss
7508-50-10	1.8	75	2.3	2.1	7
SJ 5	1.7	72	3.3	2.0	39
TK5	2.5	92	1.8	1.0	44

a = Regression coefficient of the correlation between total rust affected foliage (rust affected foliage + defoliation) and days after planting
b = Estimated total rust affected foliage (rust affected foliage + defoliation) when 70% the soybean life cycle is completed

Later, soybean rust epidemiology was studied. The factors of rust epidemics were abundant inoculum, cultivars and favourable environments such as abundant and frequent precipitation, quite cool temperature (22-27 °C), and relative humidity was above 75 % (Nuntapunt et al. 1986).

In Thailand, screening for rust resistance or tolerance commenced at F6 generation and for rate- reducing rust resistance and tolerance at late generations. Soybean seeds were sown in the second week of August under the upper north environment of the kingdom (Nuntapunt et al. 1983). The rust development started after flowering and the rapid rust development with high rust severities were shown in the later stages of ontogenetic development. It was found that the correlation between rust severity and growth stage were in a straight line (linear regression) (Nuntapunt et al. 1982). The criteria used for the selection were as follows :

a. Resistant to downy mildew, bacterial pustule and virus diseases
b. RB type lesion or RB and Tan type in the same leaf
c. Less severe rust infection

d. Moderately severe rust infection, but still green leaves and full-pod formation at R6 growth stage
e. Good plant type, no stem lodging, no seed shattering and uniform in maturity

The highly rust resistant lines were reselected 2-3 times. Later, they were evaluated for rate-reducing rust resistance and tolerance under non-fungicide and fungicide spraying with triadimefon 25 WP at 25 and 40 days after emergence (Nuntapunt et al. 1992A). The resistant or tolerant lines were selected with the following objectives:

a. RB type lesion or RB and Tan type in the same leaf
b. Slow rust development
c. Low rust affected foliage (%)
d. High yield under non fungicide treatment
e. Less yield loss
f. Good seed qualities

Some rust tolerant lines, 8517-3-4, 8523-11-2 and 8520-102-7-1 were included in the soybean standard yield trials. They gave 30-40 % higher yield than SJ.5 and Chiang Mai 60 in some locations. They had been used as germplam for rust resistance to make new crosses.

Mutation techniques

Soybean breeding program for rust resistance by using mutation techniques were carried out by Dr. Sumint Smuthkupt from Kasetsart University in 1979. Ten cultivars were irradiated at 150 and 300 Gray. The line 81-1-038 was selected and released as Doi Kham. This variety was similar to SJ.4, because the line 81-1-038 was derived and selected from the irradiated SJ.4 plant at 150 Gray. A mutant soybean variety 'Doi Kham' grows very well in the northeast of Thailand, due to show lower rust infection and higher yield than of SJ.4 It might be due to the effect of the environment which was not favourable for rust epidemics.

In the part of Thai Department of Agriculture (THDA), mutation techniques were used for anthracnose resistance program, but the anthracnose resistant line was not obtained. Thus, the M4 seeds were screened for rust resistance at the Royal Pangda Experiment Station in 1989. (Nuntapunt et al. 1992B). Under natural inoculation, the screening techniques were carried out with the following objectives as previously mentioned. A number of 134 plants

were selected including SJ.2, SJ.5, Chiang Mai 60 and 7608-25-4. In the rainy season (1990), plant to row (134 plants) were grown and there were only 28 rows were selected. They were resistant to downy mildew (immune) and gave less severe of rust infection. The reselection was done again during 1991-1992. It appeared that there were only 4 resistant lines. In 1993, they were evaluated for rate-reducing rust resistance and tolerance under non-fungicide and fungicide treatments. The results showed that CM60-10 kr-71 was only a resistant line to rust. The lesions were RB (Reddish Brown type) and also resistant to downy mildew and bacterial pustule diseases. This line gave slower rust development than SJ.5 and Chiang Mai 60. The total rust affected foliage leaves were very low (1-6 %) (Table 2). The yielding reduction was about 0-17%, compared with SJ.5 and Chiang Mai 60 about 37-43 % and 12-41 % respectively (Table 3).

Table 2. Comparison of lesion, rate of rust development and estimated total rust affected foliage on

Cultivar	Lesion	Rate of Rust Development[a]	Rust Affected Foliage (%)[b]
CM 60-10kr-71	RB (RB,T) [c]	0.10 (0.12) [c]	1 (6) [c]
SJ 5	T (T)	0.14 (0.14)	89 (53)
CM 60	T (T)	0.25 (0.23)	89 (67)

a = Regression coefficient of the correlation between total rust affected foliage (rust affected foliage + defoliation) and relative life time (DAP , Days to R_8x100)
b = Estimated total rust affected foliage (rust affected foliage + defoliation) when 70 % the soybean life cycle is completed
c = Data in parenthesis are for the wet season in 1996

Table 3. Comparison of yield and yield loss from three cultivars grown under fungicide and non- fungicide protected conditions during severe soybean rust epidemics at Chiang Mai Field Crops Research Centre in the wet season 1995-1996.

	Yield (t/ha)		
Cultivar	Fungicide Protected	Non-Fungicide Protected	% Yield Loss
CM 60-10kr-71	2.6 ab (3.0 a) [a]	3.1 a (2.5 a) [a]	0 (17) [a]
SJ 5	2.7 a (2.4 a)	1.7 b (1.4 b)	37 (43)
CM 60	1.8 b (2.8 a)	1.6 b (1.7 ab)	12 (41)

a = Data in parenthesis are for the wet season in 1996

At present, the result of conventional breeding and mutation techniques gave the resistant lines: CM 60-10 kr-71, MJ 9518-5 (KUSL 20004xCM 60-10 kr-71) and MJ 9519-5 (KUSL 20004x8517-3-4). These lines are being tested for yield and yield losses due to rust infections prior to be released to the farmers.

DNA markers assisted selection for rust resistance

At present, the technological advancement in molecular biology particularly regarding marker assisted selection (MAS) has been widely used to improve the efficiency of traits selection in many crops including soybeans. Accordingly, Field Crops Research Institute (FCRI) has a purpose to develop its research project. The project was initiated between FCRI and Office of Research and Development of Biotechnology and Nuclear Technique, THDA under supervision of the staff from Kasetsart University and National Centre for Genetic Engineering and Biotechnology, National Science and Technology Development Agency. Soybean variety improvement for rust resistance using MAS was considered to be a pilot project as the soybean is an economic crop and soybean rust is a major disease severely reducing grain yield in a large scale of main soybean producing areas of the country. The activities proposed to achieve objectives are as follows:

a. Collection and Evaluation of Soybean Varieties Resistant/Susceptible to Rust Disease
b. Genetic Diversity of Soybean Varieties
c. Collection of Soybean Rust
d. Genetic Diversity of Soybean Rust
e. Survey of Genetic Diversity of Parents Used for Crossing
f. Hybridization and Advanced Progeny Populations
g. Identification of Markers in F2 Populations
h. Soybean Rust Resistant in F3 Populations

According to the results from Identification of Markers in F2 Population and Soybean Rust Resistant in F3 Populations, it was found that two markers (Satt 12 and Satt 472) were associated with soybean rust resistance. Marker assisted selection will be used to select soybean rust resistant lines.

In the rainy season 2003, F5 generation was grown at the Royal Pangda Experiment Station, Samoeng, Chiang Mai. Single plant selection will be selected for rust resistance with RB type and superior agronomic traits.

In the part of Plant Pathology, fifteen isolates of soybean rust were collected. It was found that there were eight races of rust in the northern part of Thailand, six races collected from Samoeng district and two races from Prao district, Chiang Mai province. The other isolates collected from Sansai district

and Mae-Tang district, Chiang Mai province were the same as the two races collected from Samoeng district (Table 4). The results from Collection of Soybean Rust showed that the new promising resistant lines, CM60-10kr-71 and MJ 9519-5 gave RB lesions type against the above eight races while MJ 9518-5 was susceptible to the race SM-44-1 but resisted to the rest of those races.

Table 4. The eight races of soybean rust in the northern part of Thailand during 2001-2003.

Cultivar/Location	Year	Race
CM 3 (Pr) A	2001	Pr-44-1
CM 4 (Pr) A	2001	Pr-44-2
SJ 4 (SM) A	2001	SM-44-1
ST 2 (SM)	2001	SM-44-2
VSB#75 (SM)	2001	SM-44-2
CM60 (SM) B	2001	SM-44-3
[a] Eva #5 (SM)	2001	SM-44-3
[b] G 2800 (SM)	2001	SM-44-4
CM60-10kr-71 (SM)	2001	SM-44-5
CM60 (SM) E	2001	SM-44-6
SJ5 (SM)	2001	SM-44-6
ST2 (SS) C	2001	SM-44-3
SJ1 (SS)	2001	SM-44-3
SJ5 (MT)	2002[c]	SM-44-3
CM1 (SS)	2003[c]	SM-44-6

a = CM 9238-54-1 (ST) b = Wild type c = dry season

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Chemical control of soybean rust (*Phakopsora pachyrhizi* Syd.) in South Africa

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Since the first report of soybean rust (SBR) in South Africa in 2001, nine fungicides, mainly triazoles from the sterol biosynthesis inhibitor group, have received emergency registrations for the control of this disease. Due to a dearth of published data for the control of SBR using new systemic fungicides, trials were designed to determine the efficacy, residual period, dosage rate and time and frequency of application of fungicide sprays necessary for effective control under South African conditions. Trials were planted at Cedara, KwaZulu-Natal province. The 2001/02 season was characterized by early-season wet and mild conditions, followed by a mid-season drought. Disease incidence reached 30% in unsprayed plots. The 2002/03 season was characterized by dry conditions throughout the growing season. Disease incidence averaged 10% in unsprayed plots. The low disease incidence in the 2002/03 season resulted in mainly non-significant results among treatments in the fungicide efficacy trials. In the 2001/02 season, significant differences occurred between fungicide sprayed and unsprayed treatments, but differences among triazole fungicides were not significant. Although there were no significant yield differences between triazole and strobilurin fungicides, disease incidence was consistently higher in the strobilurin-treated plots. Dosage rates did not affect disease severity under current disease potentials and the trials will be repeated under irrigation in 2003/04. All fungicides tested provided between 13 and 19 days effective control of SBR, supporting the recommended 21-day spray interval. Results showed that time of first application was more important for the strobilurin than for the triazole fungicides. The strobilurin treatments applied subsequent to the appearance of rust resulted in yield reductions of 22% when compared with applications that were made prior to the onset of disease. Yield was not significantly different for the triazole at different treatment times (just before flowering, during flowering, at first signs of disease and at the R5 growth stage). Under current conditions there were no significant yield differences—irrespective of whether one, two or three sprays of fungicide were applied. However, sprayed plots yielded better than the unsprayed control plots.

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Introduction

Soybean rust (SBR) was first recorded in February 2001 in KwaZulu-Natal and confirmed as *Phakopsora pachyrhizi* (Pretorius, Kloppers & Frederick, 2001). Chemical control of SBR was first reported during the 1960s, with the use of protectant fungicides such as Bordeaux mixture, copper, lime-sulphur and Dithane variants. In the 1970s systemic fungicides were tested in the form of Plantvax (oxycarboxin) and Benlate (benomyl) (Sinclair & Hartman, 1996). Additional products have become available to control SBR with the development of new systemic fungicides in recent years. Nine fungicides have received emergency registrations in South Africa for SBR control (Nel, 2003, pers. comm.)[1]. Little information has been published on the use of triazole and strobilurin fungicides to control SBR. Trials were therefore conducted to test the efficacy, persistence, dosage rate, timing and frequency of ten systemic fungicides under South African conditions.

Methodology

Trials were conducted at Cedara (29° 32'S, 30° 17'E), which has an altitude of 1070 m and long-term average summer season rainfall of 552 mm. A medium season cultivar, LS666, was planted at a row spacing of 45 cm and plant populations of 300 000 to 350 000 seeds per hectare during the 2001/02 and 2002/03 seasons. Trials were replicated three times. Plots comprised four 5.0 m rows. The central two rows of each plot were sprayed with the fungicides. Spray solutions were applied with a CO_2-pressured back-pack sprayer and full cover sprays of 160 l.ha^{-1} at 2 bar pressure were applied to each data row.

Disease severity assessments were made from just prior to flowering until the crop was physiologically mature. This data was used to calculate the area under disease progress curve (AUDPC). Grain yield, one hundred seed mass and protein and oil contents were determined at maturity.

The 2001/02 growing season was characterised by mild temperatures and a midsummer drought during the months of February and March. The weather conditions until flowering were mild, with normal rainfall. It was only in April, during pod-fill, that the drought receded. The 2002/03 season was relatively dry, with below average rainfall. Environmental conditions were not particularly conducive to disease development and these results are therefore not discussed.

Fungicide efficacy

Fungicide treatments were mainly triazole chemicals. Some triazole/benzimidazole and triazole/strobilurin premixed combinations were also

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evaluated, as well as a single strobilurin fungicide (Table 1). Fungicide applications were made at the early flowering stage and at 21-day intervals thereafter, until the plants were physiologically mature.
All fungicide treatments reduced disease (AUDPC) and final disease severity with concomitant significantly higher grain yields than the unsprayed control. Although disease was more severe in mancozeb and azoxystrobin treatments than with triazoles, yields were not significantly different. There were no statistical differences among fungicides with respect to yield.

Table 1. Fungicides evaluated at Cedara in the 2001/02 and 2002/03 seasons

Active ingredient	Formulation and ai content (g.l^{-1})	Manufacturer	Rate applied (ml.ha^{-1})
Azoxystrobin*	SC, 250	Du Pont	300
Triadimenol	EC, 250	Bayer	500
Flusilazole	EW, 250	Du Pont	400
Triforine	EC, 190	BASF	1500
Mancozeb*	WP, 800 **	Algro-Chem	2000***
Epoxiconazole/Pyraclostrobin*	SC, 182.5	BASF	850
Tebuconazole	EW, 250	Bayer	750
Flutriafol	SC, 125	FMC Chemicals	1000
Flusilazole/Carbendazim	SC, 250 / 125	Du Pont	400
Carbendazim/Flusilazole	SC, 250 / 125	Du Pont	800****
Difenoconazole	EC, 250	Syngenta	325

*Not registered in South Africa for SBR control.
**g.kg^{-1}
***g.ha^{-1}
****The registered rate for this product has been reduced to 600 ml.ha^{-1}, but at the time of these trials was still 800 ml.ha^{-1}.

Dosage rate

Three rates were evaluated for each of the fungicides listed in Table 1, except for mancozeb, which was not included. The full dosage rate listed in Table 1, together with half and double rates were compared. Spraying commenced at the early flowering stage and was repeated at 21-day intervals until the crop reached physiological maturity.

Phytotoxicity was detected in two of the fungicides sprayed. Tebuconazole showed necrotic scorching of leaves, whilst epoxiconazole/pyraclostrobin displayed a malformed cupping of leaves with slight stunting. The low spray volume (160 l.ha^{-1}) may have contributed to the manifestation of these symptoms. Future trials will be conducted with higher spray volumes of between 300 and 500 litres per hectare to determine whether symptoms continue to manifest themselves.

Disease pressure was low and results were inconsistent with no statistical differences between the means of final disease, disease severity and yield, for the different fungicide rates. These trials will be repeated in the 2003/04 season, under irrigation, to improve conditions for infection.

Fungicide persistence

Eleven fungicides (Table 1) were evaluated to determine their persistence and hence the number of effective days of disease control. Disease severity assessments were made twice a week, from ten days after initial flowering until the first two significant disease severity ratings had been made. Thereafter the trial was sprayed once only. Assessments were made daily for approximately three weeks, until the disease showed rapid signs of progress, at which stage the ratings were conducted twice a week until the plants were physiologically mature.

The effective length of control ranged from 10 days for triforine to 19 days for flusilazole/carbendazim. Triazole fungicides (16 days) generally had longer persistence than strobilurin fungicides (14 days). The curative application of the fungicides in this trial may have contributed to the shorter length of control of the strobilurin fungicides. When sprayed preventatively, it has been suggested that the effective length of control with stobilurin fungicides may be as long as 45 days (Jarvie, 2002, pers. comm.)[2]. Mancozeb, which was applied with a sticker, gave 14 days of control. This enhanced protection is thought to be due to a combination of the use of the sticker and dry weather conditions, which not only prevented the product from washing off but also did not favour disease development.

Fungicide timing and frequency

Fungicides were applied at four stages: before flowering (V9 – V12 growth stage), during flowering (R1 growth stage), at the first sign of disease and at the R5 stage of growth, which is defined as commencement of pod fill (Fehr & Caviness, 1977). Initial applications commenced at the specified times and were sprayed thereafter with none, one or two follow-up sprays at 21-day intervals. Two fungicides, azoxystrobin and flusilazole/carbendazim, were tested for each combination of timing and frequency of application.

Significant differences in disease severity were associated with timing of application, regardless of the number and type of fungicide sprays. Spraying during flowering resulted in the least disease, followed by spraying before flowering, then at first signs of disease, while spraying at the R5 growth stage resulted in disease levels that were not significantly different from unsprayed treatments. Two distinct yield groups were recorded, *i.e.* the

R5 and unsprayed treatments, which yielded significantly less than the remaining treatments.

The number of sprays applied, regardless of timing and fungicide type, resulted in reduced disease. Two or three sprays were significantly better than one spray, which was better than the unsprayed control. The differences, however, were not expressed in terms of yield.

The type of fungicide used resulted in significantly different disease levels. Flusilazole/carbendazim had less disease than azoxystrobin sprayed plots, which, in turn, had less disease than unsprayed plots. A corresponding yield difference was also observed.

The time of application had no effect on yield with the flusilazole/carbendazim fungicide. There was, however, a significant difference in time of application for the azoxystrobin fungicide. Yields for treatments that were sprayed curatively (first sign of disease and R5) were significantly lower than preventatively sprayed treatments (before flowering and during flowering), and not statistically different from the unsprayed control. This has serious implications for the control strategy designed for the United States of America, since azoxystrobin, which is one of only three registered fungicides (Miles *et al.*, 2003), is ineffective in curing existing infections. Thus, in the first year that SBR occurs in the U.S.A., considerable yield losses can be expected. In subsequent years, however, when sprayed preventatively, SBR control could be satisfactory, provided resistance management strategies are adopted.

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Epidemiological approached and methods for modeling of soybean rust

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General approaches

The development of soybean rust in a soybean field is a dynamic process, having the nature of a population growth of any biological organisms. Population of soybean rust fungus develops when the soybean plant is susceptible and environmental conditions are suitable for the reproduction of the pathogen. Outbreaks or epidemics of soybean rust occur when conditions are highly favorable to the growth of the pathogen. Rust outbreaks are the sudden increase of rust pathogens in a soybean field within a short period of time, often a few weeks or a month.

Prediction of the likelehold of a soybean disease outbreak plays an important role in prevention of disease outbreaks. Timely and accurate predictions on the likelihold of soybean rust outbreaks enable soybean farmers to apply various preventative measures, such as spraying fungicides, to control the growth of rust pathogen population before it reaches damage levels while reduce unnecessary spray. A good prediction model calculates thresholds of disease outbreaks that are used by farmers in making decision in fungicide applications, in which fungicides are applied only necessary, reducing environmental damage. Predictions at regional or national level also provide useful information to assess potential risk of the rust, critical to make farming policies for governmental agencies.

Disease prediction model is a mathematical expression for the relationship between disease dynamics and a set of weather variables. It is the central tool for disease prediction. A disease prediction model often consists of mathematically structured parameters that capture the dynamic nature of a disease. Most of disease prediction models use a set of weather variables and previous disease measurement as inputs to predict future development of a disease with time lag of several days, weeks, or even months. In the regional level, disease outbreaks have been correlated to extreme climate events, such El Nino events. In People's Republic of China, annual prediction of the occurrence of wheat scab is made using Southern Oscillation Index, a measure of El Nino.

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Development of disease prediction models started as early as in 1950's and several disease prediction models using regression equation were developed. These systems provided disease warning to guide fungicide applications. Disease prediction models that time were less applicable because of amount of calculation beyond capability of farmer. In 1970's, the first computerized disease prediction model was developed. Current disease prediction models are computer-based to handle vast amount of data collection, analysis, and prediction.

Disease prediction models have been developed for soybean rust from data collected in China and Taiwan during 1990's. Three types of prediction models have been developed: 1) simulation models which require daily inputs of weather information for disease prediction; 2) neural network model which also requires daily weather input for disease forecasting; and 3) med-term regression models. Below two types of models are reviewed and the third one will be introduced in the presentation.

Simulation models

The model consists of a main program, an input and an initiation program, and a graphic and statistic output program. The main program has five subroutines with 10 state variables, four rate variables, five driving variables, and some constants. Plant growth regression model was used to calculate the growth of leaf area of a soybean population. The regression models were developed based on extensive data from sequential planting experiments 5 with $R2 = 0.89$ and 0.92 for two different cultivars. Equation of relative infection rate was developed after reanalyzing the data published by Marchetti et al. The relationship between physiological day after inoculation and percentage of uridium maturity or uridium senescent was exponential. Models of infection rate, latent period, and senescence were developed and relationship between relative infection rate and dew period and temperature was significant. Model of latent period explains up to 98.7% of the variation with F test $P < 0.0001$.

The simulation model well reflected the effects of environment on disease development as shown by the fact that the predicted trends followed the observed which varied from season to season. Accuracy of the model was demonstrated by the regression between predicted and observed. The prediction of onset stage was also showed by values of intercept which were close to zero with deviation less than 10% in most cases during the regular planting seasons. The progress of epidemics in the regular planting seasons was well predicted. The values of slope were up to 0.85 and r2 were greater than 0.9 in most cases. For the epidemics during the winter

(Day 0 30 and Day 300 to 365), the deviation between predicted and observed was high. The simulation model was intensively validated with data of total 69 sequential planting experiments during 1980 and 1981 in Taiwan. For validation with data not used for model development, the model well predicted disease development during the regular season when validated with data not used in model development. The predicted lines fit the observed ones well although the epidemic period of three growing seasons varied. However, the simulation model often underestimated the disease development after disease severity was up to 90%.

SOYRUST can be integrated as a subroutine of the plant growth model without changing the program structure of the main model because there is an interface for coupling foliar disease effects on plant growth in SOYGRO. Because soybean rust affects plant growth by reducing green leaf area, only leaf area in the growth model was considered affected directly. SOYRUST takes the variable of AREALF as an input. It calculates the disease every day and passes diseased leaf area (DISLA) as an output back to SOYGRO. In SOYGRO, DISLA has been built in as a variable determining plant photosynthesis for growth, plant growth under the disease effects can be calculated. At the end of simulation, the model outputs disease progress during the season and predicted yields with and without the disease.

Neural network models

The first soybean rust NN model was developed by Iowa State University using the data published by Yang, same data set that was used for SOYRUST. Neural network model can be used to predict the disease severity in the source region. As to the dispersal regions, a regression model provides a better prediction. The model uses four factors in prediction, average monthly rainfall, its variance, number of rain days, and variance of rain days in a growing season. In 1996, further efforts were made by in a collaborative project between Iowa State University and Oil Crop Research Institute, Chinese Academy of Agricultural Science. Goal of the project was to develop neural network models to assess the risk of the diseases. In the first step, field experiments were established in Chinese soybean production regions in regions. Multi-location and multi-season data were collected for rust disease progress, environmental variables affecting the disease development. Different disease forecasting models including a NN model were developed with historical data and new data. Values of observed disease severity at two test sites were consistent with the model predictions.

The historical data of the occurrence of soybean rust in China were analyzed in relation to our current neural network model. This data set was collected

over a relatively long period (since 1978) from several locations in China. Disease progresses of sequential planting date experiments (7 planting dates) were monitored and recorded more than 16 times over the growing season. These data were matched with environmental variables (temperature, moisture (Rh), rainfall, and dew). By analyzing of the epidemics data of soybean rust in China, it was found that the characteristics of soybean rust epidemics are different among different regions in China.

Regional models to predict disease occurrence have been developed using monthly weather variables as inputs. In certain regions in China these models provide good prediction. With the advance that climatologists have made in prediction of regional weather, regional models that make warning of a month ahead of disease occurrence would have greater application potentials then models relying on daily weather predictions as inputs.

Variation of *Phakopsora pachyrhizi* isolates on soybean

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Abstract

Phakopsora pachyrhizi Sydow, the causal fungus of soybean (*Glycine max* (L.) Merrill) rust, occurs in most soybean-growing areas of the world except continental North America. Initial studies on soybean rust isolates from the Western Hemisphere indicated that they were different than isolates from the Eastern Hemisphere. In 1992, the Eastern Hemisphere species, *P. pachyrhizi*, and the Western Hemisphere species, *P. meibomiae*, were established for the soybean rust fungi based on morphological differences. The first molecular differentiation of the two species was reported in 2002. A number of studies have reported the occurrence of race in *P. pachyrhizi* either on soybeans or on other hosts. In 1984, a set of four native Australian *Glycine* species were used to identify six different virulence combinations of *P. pachyrhizi*. Much of the research on differentiating isolates on soybean was completed in a containment facility at in the U.S. Genetic characterization on four plant introductions (PIs) indicated the occurrence of four independently inherited dominant genes. These genes are known to be effective to a limited number of isolates. There are many studies that need to be completed to determine if all isolates respond equally in terms of survival, urediniospore production, telia formation, and host range under different environments. Over the next few years, our understanding of pathogen diversity will increase as more concerted research efforts take place in different parts of the world.

Introduction

Soybean rust is caused by two fungal species, *Phakopsora meibomiae* and *P. pachyrhizi*. The Asian soybean rust pathogen, *P. pachyrhizi*, is the species of greater concern since it is the more aggressive species and has been identified in new geographical locations beyond Asia. *P. meibomiae*, the less virulent species, has only been found in the Western hemisphere, and it is not known to cause severe yield losses in soybean (Sinclair and Hartman

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1999). Soybean rust, caused by *P. pachyrhizi* is one of the major diseases of soybean in many Asian countries (Sinclair and Hartman 1999) and now in Brazil, since its recent introduction in 2001 (Yorinori et al. 2003). Significant yield losses due to rust have been reported in most soybean-producing countries throughout Asia, where the disease is endemic and may limit soybean production. North America is one of the last major soybean production areas without soybean rust.

On the African continent, the distribution of soybean rust was not well known before 1996, but since then a more expanded view of soybean rust in Africa has been reported indicating that it was found in 1996 in Uganda, Kenya, and Rwanda, in Zambia and Zimbabwe during 1998, Nigeria in 1999, Mozambique in 2000, and South Africa in 2001 (Levy 2003).

The first detection of *P. pachyrhizi* in South America was in Paraguay in the 2000-2001 growing season (Yorinori et al 2003). The disease was found on soybeans grown in the Parana River basin on the eastern border with Brazil in a limited number of fields. Argentina confirmed the occurrence of soybean rust in early 2002 (Rossi 2003). During the 2003 growing season the pathogen was found in most of the soybean growing regions of Brazil and came late in the season for the first report in Bolivia (Yorinori et al. 2003).

The objective of this paper is to provide an overview of what is known about the variation of *Phakopsora pachyrhizi* isolates. This will include a review of some of the studies that confirmed that two species infect soybean, and the variation observed in pathogenicity on soybean and other hosts. Because of the restrictions in length, this overview will highlight some of the refereed papers published and is not meant to be a review of all of the literature on this subject.

Studies on species differentiation

One of the earliest reports of soybean rust in the Western Hemisphere was when it was found on soybean and other legumes in Puerto Rico (Vakili and Bromfield 1976). Along with soybean, rust was found on *Centrosema pubescens* (butterfly pea), *Dolichos lablab* (hyacinth bean) *Phaseolus coccineus* (scarlet runner bean), *P. lunatus* (lima bean) and *P. vulgaris* (bean). It also was shown that the Puerto Rican cultures were less virulent than three strains from the Eastern Hemisphere on soybean cv. Wayne.

In 1980, Bonde and Brown (1980) examined isolates from Australia, India, the Philippines, Taiwan and Puerto Rico on cv. Wayne. Isolates were indistinguishable in their pre- and post-penetration, colonization phases,

and morphology of uredinia. The only observable difference was the appearance of germ pores, in which those of the Puerto Rican isolates were more easily seen suggesting that their spores may have thinner germ pore plugs. A more thorough examination of isolates was reported in 1988 when isozymes of *P. pachyrhizi* from the Eastern and Western Hemispheres were compared (Bonde et al. 1988). No differences in isoenzyme banding patterns were detected among any of the eleven isolates from the Eastern Hemisphere or among those from the Western Hemisphere; however, distinct isoenzyme polymorphisms were observed when comparing isolates from the two hemispheres.

Ono et al. (1992) established two species for the soybean rust fungi. This was based on morphological differences between their anamorphic and teleomorphic stages, and was based primarily on layering of telia and wall thickness of teliospores. *P. pachyrhizi* [uredinal anamorph *Malupa sojae* (syn.: *Uredo sojae*)] included the Eastern Hemisphere populations before its known spread and confirmation in Africa, Hawaii, and South America, and *P. meibomiae* [uredinal anamorph *Malupa vignae* (syn: *Uredo vignae*)] isolates from the Western Hemisphere, before the first report in Hawaii in 1994 (Killgore 1995).

The first molecular differentiation of the two species was reported by Frederick et al. (2002). They showed that the nucleotide sequence of the internal transcribed spacer region had only 80% sequence similarity between the two species. Based on these differences, four sets of polymerase chain reaction primers were designed specifically for *P. pachyrhizi* and two sets for *P. meibomiae*. Classical and real-time fluorescent polymerase chain reaction assays were used to identify and differentiate the two species from inoculated and field samples from South America.

Variation on hosts other than soybean

One of the earliest reports of *P. pachyrhizi* races is from Lin (1966) in Taiwan. Nine isolates were used to inoculate six soybean genotypes and five leguminous plants. No marked differences in pathogenicity of the isolates were observed on the soybean genotypes; however, the nine isolates were separated into six pathogenic groups differing mainly in their reactions types with and without sporulation or no infection on *Vigna unguiculata* subsp. *sesuquipedalis* (asparagus bean), *P. vulgaris* (kidney bean), and *Pachyrhizus erosus* (short-podded yam bean).

In 1984, Burdon and Speer (1984) established a set of differential *Glycine* hosts for the identification of *P. pachyrhizi* races. They used 257 accessions

of four native Australian species of *Glycine* (*G. canescens*, *G. clandestina*, *G. tabacina* and *G. tomentella*) and eight Australian isolates of *P. pachyrhizi*. Based on differences in infection responses, they distinguished six different virulence combinations. Additional research by Burdon (1987) found nine races *P. pachyrhizi* in two natural populations of *G. canescens*. Genetic analysis showed that the two host populations contained at least 10 and 12 resistance genes with some lines having up to three dominant genes. In another study by Burdon and Lenne (1989), 10 *P. pachyrhizi* isolates from *Kennedia rubicunda* were evaluated on two hosts, *G. canescens* and soybean, known to be susceptible to all tested *P. pachyrhizi* isolates from soybean. Three patterns of were detected with isolates being either avirulent or virulent on both host lines, or being avirulent on *G. canescens* and virulent on soybean. When five isolates from *K. rubicunda* were tested on 10 populations of *K. rubicunda*, significant interactions were detected between particular host population-pathogen isolate combinations.

Variation on soybean

Much of the research on differentiating isolates on soybean has been done in the containment facility at Fort Detrick, Frederick, Maryland, U.S. Melching et al. (1979) used four isolates to compare their ability to colonize and reproduce on the cv. Wayne. All isolates required a similar time from inoculation to lesion appearance (7 days) and initiation of urediniospore production (9 days). The Indian culture produced more lesions per unit leaf area per inoculum unit than the others. Urediniospores were collected daily 13 to 52 days after inoculation and the calculated total number of spores produced over the life of the lesion was 2028, 3768, 6268, and 6600 for the Australian, Indian, Indonesian, and the Taiwanese isolates, respectively.

Based on research by Bromfield et al. (1980), isolates from Australia, India, Puerto Rico and Taiwan differed in virulence and three infection types were observed. A susceptible reaction or tan (TAN) lesion with 2-5 uredia per lesion, a resistant reaction or a reddish-brown (RB) lesion with 0-2 uredia, and an immune reaction or Type 0 with no macroscopically visible evidence of rust. Isolate Australia-72-1 induced both TAN and RB on each of eight accessions. Taiwan-72-1 induced TAN on 13 accessions. The rates of lesion enlargement and increase in uredinia per lesion on the lower surface of leaves of Wayne were about equal for India-73-1 and Taiwan-72-1, but were lower for Australia-72-1 indicating that Australia-72-1 was less aggressive. Taiwan-72-1 consistently produced more uredinia per lesion at a given time on the upper surface of leaves of Wayne than did India-73-1.

Specific resistance to *P. pachyrhizi* has been described as four single dominant genes (*Rpp1*, *Rpp2*, *Rpp3*, and *Rpp4*). Hartwig (1995) summarized the finding of the reports on resistance. These four genes condition resistance to a limited set of rust isolates. The *Rpp1* was described as

having an immune reaction when inoculated with a few isolates, including India 73-1. Inoculation of most rust isolates on *Rpp1* or the other genes produces a resistant red-brown (RB) lesion with no or sparsely sporulating uredinia. There are numerous examples of single gene resistance not being durable. Only Bing Nan, the source of the *Rpp4* gene, has not been reported to be defeated in the field, although observations both in the field in Paraguay and greenhouse inoculation tests indicate that it is susceptible to at least some isolates (Miles et al, pers. comm.).

Based on the studies mentioned in this paper and many others in the literature, it appears that *P. pachyrhizi* possess multiple genes for virulence in most isolates and/or field populations are a complex mixture of many races with possible multiple virulent factors. In recent studies done at Fort Detrick, isolates of from southern Africa and South America were significantly more virulent on soybean cultivars containing the single resistance genes when compared to the Asian isolates from the 1970s (Frederick et al, pers. comm.). The most virulent isolate was collected from Zimbabwe in 2001.

Variation due to environment

There are only a few papers that have reported on the interaction of isolates to differing environmental conditions. Marchetti et al. (1976) compared the effects of temperature and dew period on germination and infection by urediniospores of four isolates. Spores germination and infection occurred over similar temperature ranges with the maximum infection of cv. Wayne soybean leaves occurring at 20-25° C with 10-12 hours dew and at 15-17.5° C with 16-18 hours dew. The minimum period for infection was 6 h at 20-25° C and 8-10 hour at 15-17.5° C. Infection did not occur above 27.5° C. Dufresne et al. (1987) studied the effects of temperature and light intensity on telia development by Puerto Rico and Taiwan isolates. The Taiwan isolate produced telia after 21 and 30 days and the Puerto Rico isolate produced telia after 34 and 35 days at 10 and 15° C, respectively. The Taiwan isolate produced larger lesions with more telia. Light intensities did not greatly alter the time it took for telia to form.

Conclusion

The literature indicates without a doubt that there are two species that cause soybean rust. The geographic range of *P. meibomiae* is restricted compared to that of *P. pachyrhizi*, which has been on the move in last few years and now occurs in all major soybean growing areas except North America. More research is needed to better define the geographical limitations of *P. meibomiae*. As more work is done in the taxonomic area using new molecular tools, it may not be surprising to find another rust species that may infect soybean as well. It is also clear from the literature

that *P. meibomiae* and *P. pachyrhizi* have effective pathogenicity genes for infecting many leguminous plants. *P. pachyrhizi* in particular also has many virulence genes, as isolates are known that overcome any dominant resistance genes in soybean. Additional research in this area will surely uncover many more resistance genes in soybean and other hosts, and many more races of *P. pachyrhizi*. There is less information about effects of environment on isolates. For example, it is not known if urediniospores from India survive better in high temperatures than those collected from Taiwan. Many more studies are needed to determine if all isolates respond equally in terms of survival, urediniospore production, telia formation, host range under different environments. Over the next few years, our understanding of the diversity of this pathogen will expand as more concerted research efforts develop throughout the world.

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Country report and rust control strategies in Brazil

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Abstract

Soybeans (*Glycine max*) are infected by two species of *Phakopsora* that cause rust: *P. meibomiae*, native to the American Continent occurs from Puerto Rico to south of Paraná State (Ponta Grossa); and the most feared, *P. pachyrhizi* ("Asian" rust), present in most traditional soybean producing countries and absent in the Americas until 1999/00. The only procedure to differentiate both species is by the DNA test. Since its first detection in Paraguay and in the state of Paraná, in 2001, the "Asian" rust has spread to all Paraguay, Bolivia, most of Brazil and parts of Argentina. In the two following years (2002 and 2003) it caused an estimated 4.011 million tons in grain losses and the equivalent to US$884.425 millions. The States most affected were: Mato Grosso, Bahia, Rio Grande do Sul, Goias and Minas Gerais. In 2003, rust was continuously present on Fall-Winter second soybeans and volunteer plants in Central-West and North Brazil. With 2003/04 crop season plantings extending from mid-September through early January, the rust had an early start with the first commercial fields showing symptoms by mid-December. The fungus *P. pachyrhizi* is adversely affected by temperatures above 30° C. Under favorable weather it infects the soybeans and develops extremely fast, considering the normal disease outbreak, demanding quick action for control. Under greenhouse conditions, first lesions are visible (looking through the leaf) within 48 hours and first uredia and urediniospores were produced in 6-7 days. Germination tests showed that at temperatures from 25° C to 27° C, most spores germinated within one hour. The main difficulties in controlling rust are: a. the lack of resistant cultivars; b. the pathogenic variability of the fungus (at least two races are present in Brazil); c. the difficulty in distinguishing the early stage of rust from other foliar diseases; d. the continuous production of inoculum on second soybeans and volunteer plants and the long sowing period in most States; e. the excess rain in the most vulnerable regions (Cerrados) making timely spraying difficult, and f. the limited availability of fungicides. The 11 fungicides recommended for rust control belong to the groups of triazols and strobilurins. In the 2003/04 crop season, the continuous production of inoculum on second Fall-Winter crops and the early rust outbreak on commercial fields (Dec. 18), may result in general incidence where rain distribution remains normal throughout the growing season.

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Introduction

Soybeans [*Glycine max* (L.) Merrill] are infected by two species of the genus *Phakopsora* that causes rust: *P. meibomiae* (Arthur) Arthur, which is native to the American Continent ("American" rust) and is present on wild and cultivated legumes from Puerto Rico to the south of State o Paraná (Ponta Grossa); and the most feared *P. pachyrhizi* Sydow & Syd, present on most traditional soybean producing countries in Asia, South of Africa and Australia, and was absent in the Americas until 1999/00 crop season.

The "American" rust (*P. meibomiae*), rarely causes losses, and occurs under conditions of mild temperatures (average below 25° C), high relative humidity and is mostly found at high altitudes (above 800m) in the Cerrados and in Southern Region. The "Asian" rust (*P. pachyrhizi*) is adapted to higher temperatures (up to 30° C) and can cause severe losses where there are frequent periods of leaf wetness beyond 10 hours. The only way to distinguish both species is by the DNA test. They may occur in the same field and the only possibility to suspect that both may be present is by the distinction of their lesion types: *P. meibomiae* will, in general, produce reddish-brown lesions (RB type lesions) and less profuse sporulation, whereas, *P. pachyrhizi*, will produce light-brown (TAN type lesions) and will produce abundant sporulation. Under adverse conditions (above normal temperatures and reduced relative humidity) following infection and on tolerant cultivars, both may produce RB type lesions.

Dynamics of the "Asian" rust

Following the first detection in Paraguay and South Brazil in 2001, the "Asian" rust is now present in all Paraguay, Bolívia, most of Brazil and parts of Argentina. Following is an account of the progress of the disease, the losses caused and the current situation (January 2004) in Brazil.

2000/01 crop season

The "Asian" rust was first detected in the American Continent on March 2001 at Pirapó (Itapúa), Paraguay (Wilfrido M. Paiva, CRIA, Capitan Miranda, Itapúa). Yield losses were as high as 60%. Later field surveys carried out from May 23-28, 2001, showed that rust was widely disseminated into Paraguay, west and north of Paraná, Brazil. No losses were recorded in 2001.

2001/02 crop season

In its second year since first detection, rust was found in the states of Rio Grande do Sul (RS), Paraná (PR), São Paulo (SP), Mato Grosso do Sul (MS), Goiás (GO), Minas Gerais (MG) and Mato Grosso (MG), extending to

about 60% of the soybean área in Brazil. The counties most affected were: Passo Fundo, Cruz Alta, Cruzaltinha (Ciríaco, RS); Ortigueira, Ponta Grossa, Guarapuava (PR); Chapadão do Sul, Costa Rica, São Gabriel D´Oeste (MS); Chapadão do Céu, Rio Verde, Jataí, Mineiros, Portelândia, Santa Rita do Araguaia (GO) and Alto Taquari, Alto Araguaia, Alto Garças (MT). Yield losses from 30% to 75% (3.300-3.600 kg/ha from previous season to 840-900 kg/ha), were reported from Chapadão do Sul (MT). In three of the most affected counties (Chapadão do Sul and Costa Rica -MS, and Chapadão do Céu - GO), with about 220.000 ha of soybeans, an estimated 30% of the total production was lost (ca. 59,281.4 tons or US$13.00 millions) (US$220,50/t). At Cruzaltinha (RS), yield difference between sprayed (3,015 kg/ha) and unsprayed (1,632 kg/ha) reached 46% (1.383 kg/ha). Estimated yield losses based on field surveys, farmers reports and comparisons with area and production reports from CONAB for the 2001/02 crop season (Jun/03), reached a total grain losses of 569,200t or US$125,513 millions. Farmers agronomists were totally unprepared to fight the rust and most sprayings were too late.

2002/03 crop season

In this season the occurrence of rust was totally different from the previous year. Where rust was severe in 2001/02, inspite of abundant rain, the high temperatures prevented the rust from reaching epidemic levels, except in RS and Santa Catarina (SC) (Papanduva), where late plantings were affected. In the state of SP, one rust focus was found in mid-January, at Itapeva, but it did not progress into the surrounding fields during the regular season. The disease was later found on second soybeans sown on February-March at Taquarituba, near Itapeva. In MT, GO and MS, where rust had been severe in the previous season, the low disease severity may have been due to the extensive use of fungicides and also to the lack of inoculum production in the southern region, where the high temperatures inhibited the fungus.

On the other hand, where rust had not been detected previously, the favorable weather and a new race of *P. pachyrhizi* caused severe losses. The states of Bahia (BA), GO, MG and MT, were severily hit. Although the first detection of rust was made on February 7, according to the severity of the disease in early affected fields, the disease should have been established in BA ("Roda Velha" and Bela Vista), and MT (Sorriso and Tapurah), at least, since early January of 2003. From these places the fungus must have disseminated rapidly throughout the entire soybean fields of BA, parts of GO and MG and most of MT.

Despite the intense campaign for alert and training courses on identification and control of rust, through field days, seminars, publications, radio and

TV interviews, newspapers, given in many parts of the country, carried out in 2002 and January-February 2003, most farmers and field consultants were not prepared to fight the rust.

Based on data gathered from field surveys on losses, cost of chemical control, obtained from field consultants, farmers reports, fungicide trials, comparisons between rust severity and age of plants affected and based on national soybean area and production provided by CONAB (Jun/03), the total grain losses due to rust was estimated at 3.442 million tons or US$758.868 millions (US$220.5/t). The cost of rust control (average of two sprays, including spraying and chemical cost on 80% of the soybean area), estimated at US$40,00/ha, reached the figure of US$592,00 millions. Therefore, the total cost of rust for the 2002/03 crop season reached US$1.351 billion.

2003/04 crop season

Being halfway through (Jan. 2004), this crop season had as background a severe rust epidemics caused by a new race of *P. pachyrhizi* and a continuous presence of inoculum on Fall-Winter soybeans in the Cerrados (BA, GO, MA, MG, MT, SP e TO). Despite of this condition the beginning of the season (Dec. 2003) was characterized by irregular rainfalls and temperatures higher than the previous season. This may, probably, have inhibited the outbreak of the rust which, otherwise, could have been very serious. In addition, the bad experiences with rust from previous year prompted the farmers to do early protective spraying. Fields of early maturing cultivars, sown from mid-September to early October, with or without central pivot irrigation (at Lucas do Rio Verde, Sorriso, Primavera do Leste, MT) and harvested from early to mid-January, in general, had two fungicide sprays. In other regions, untreated fields (Planura, MG; Guairá, Miguelópolis, Paranapanema, Itaí, Taquarituba e Itaberá, SP) or with faulty sprays (Bela Vista e Luis Eduardo Magalhães, BA), were affected by rust at different levels of severity and are serving as sources of inoculum for the neighboring fields.

In the South (Paraná state and Paraguay), the beginning of the season was characterized by mild temperatures and frequent rains that favored early development of rust. In Paraguay, the first rust lesions were detected on kudzu (*Pueraria lobata*) on October 29, at trace levels but, one month later (Nov. 28), the disease had progressed to epidemic levels on kudzu and had spread to the neighboring soybean fields where plants were only 25-30 days old. The month of December was rainy and stimulated rust development, demanding fungicide sprays at the vegetative stages. To this wet weather followed a dry and hot January stopping the progress of

rust. The rain resumed in Paraguay in the last week of January and this may restart the rust on soybeans. In the state of Paraná the first rust lesions started on December 18 (Londrina) and 23-26 (Rolandia, Mauá da Serra, Bela Vista do Paraíso), but, progress of the disease was halted in the north and west of the state by the drought and high temperatures during January. The first reports of rust on other states had the following chronology: in SP, the first report was on December 18 (Guaira and Miguelopolis); in MG, first lesions were found on December 9 (Conceição das Alagoas) and 18 (Planura); in GO, first lesions were found in early November at Goiânia (E.E. do CTPA), on December 9 at Rio Verde and Santa Helena, on December 11 at Campo Alegre, Montividiu and Santo Antonio da Barra, in December 17 at Mineiros, and on December 20 at Porteirão. Since then, new outbreaks have been reported almost daily from different states. By the end of January the rust had been reported from the states of RS, PR, SP, MS, GO, MG, BA, Tocantins (TO), Piauí (PI) and Maranhão (MA). Nevertheless, few are the reports of severe outbreaks and those are mostly restricted to Fall-Winter plantings under central pivot irrigation and where either unsprayed or sprayed late (Planura, Uberaba, MG; Guaira e Miguelópolis, SP). If periods of normal rains and favorable temperature follows, the potential damage by rust and/or the cost of chemical control may be greater than in 2002/03 crop season. There is also the risk of shortage of fungicides when most needed, mainly in those areas treated at early growth stage and that may require a second application. This situation is already occurring at Joviânia (GO), Uberlandia and Uberaba (MG). The effect of rust on the 2003/04 crop season will depend on the weather condition during the months of February and March and on the efficiency of the chemical control.

Longevity of the urediniospores - Longevity studies of urediniospores from greenhouse grown infected plants, done at Embrapa Soja, and collected using different methods, showed that viability of spores stored at 25° C - 27° C, remained viable for periods varying from 28 to 50 days.

Speed of urediniospore germination - Under normal laboratory conditions (temperatures of 25° C to 27° C), 80% of the urediniospores had germinated within one hour. Spores were considered germinated when germ tubes had extended to twice the widest diameter of the urediniospores.

Symptom development and sporulation - Under greenhouse conditions, with leaf wetness of more then 10 hours at night, and temperatures from 25° C to 28° C, first symptoms were visible, when leaf was looked through the leaf blade, 48 hours after exposing healthy plants to airborne spores from infected greenhouse grown plants. The first uredia were formed by the fifith

day and sporulation occurred by the sixth to seventh days following exposure to infection.

Genetic variability of *P. pachyrhizi*

The severe outbreak of rust in the Cerrados in 2003 and the fact that cultivars resistant to the 2002 isolates of *P. pachyrhizi* became susceptible to the 2003 isolates from the Cerrados is a clear indication of genetic variability of the fungus. A question that remains to be clarified is the occurrence of the new race in regions where rust had not been recorded in the previous year. Comparisons between reactions of tests performed at Embrapa Soja and Paraguay (by Ag. Eng. Wilfrido M. Paiva, E.E. do CRIA, Capitan Miranda), with isolates from 2002, with inoculations on germplasms that possess the four major genes for resistance to *P. pahcyrhizi*, Rpp1, Rpp2, Rpp3 e Rpp4, showed great similarity, with many resistant germplasms. But when these same germplasms were tested against isolates from the Cerrados and compared with previous results, they all became susceptible. On the other hand, when the results from the tests with the Cerrados isolates were compared with the tests done in the United States (Dr. Reid D. Frederick, FDWSR/ARS/USDA, Fort Detrick, Maryland) with an isolate from Zimbabwe, the isolate from the Cerrados showed great similarity with the isolate from Zimbabwe. The similarities between the isolates from the Cerrados and the one from Zimbabwe strengthen the suspicion that the new race may have come from Africa, through the wind, crossing the Atlantic Ocean.

Reaction of Brazilian cultivars

Among 452 cultivars tested against *P. pachyrhizi* isolates obtained in 2002 from naturally field infected plants from the Central-South to South Brazil, the following cultivars were resistant (R) to moderately resistant (MR): BRS-134 (R), BRSMS-Bacuri (R), Campos Gerais (MR), CS 201 (Esplendor)(R), FT-2 (MR), FT-3 (MR), FT-17 (Bandeirantes)(R), FT-2001 (R), KI-S 601 (MR) and OCEPAR 7 (Brilhante)(MR). Nevertheless, all these cultivars became susceptible to the race of *P. pachyrhizi* that occurred in the Cerrados in 2003. Currently the program on breeding for rust resistance is screening for cultivars and germplasms with reddish-brown (RB) reaction type, regardless of the disease severity. The selection is done under severe inoculum pressure in the greenhouse.

Control measures

The prolonged sowing period in different regions, from mid-September of 2003 (at Lucas do Rio Verde, Sorriso, Primavera do Leste, Campo Verde,

MT) to early January (BA), makes it difficult to recommend a standard procedure for rust control. Besides, at the early stage of lesion development rust is often mistaken or confused with other foliar diseases: brown spot (*Septoria glycines*), bacterial blight (*Pseudomonas savastanoi* pv. *glycinea*) and post-emergent herbicide injury. This has resulted in unnecessary or untimely use of fungicides. Based on currently available information and as general control measures, the following strategies are suggested:

- use, preferably, early cultivars and at the beginning of the recommended sowing time for each region (late sowings will suffer more damage due to build up of inoculum on early plantings); the objective is to escape the period of higher inoculum potential of the fungus;
- avoid dense sowing that makes spraying inside the plant canopy difficult;
- do field surveys for rust throughout the season; at the first sign and under favorable weather for rust, spraying may be necessary; the chemicals and dosages (g a.i./ha or L c.p./ha) recommended are: (Subcommittee of Plant Pathology, XXV RPSRCB, Uberaba, August, 11-13, 2003): **protectant**: fluquinconazole (62,5g i.a./ha) (Palisade 250 CE-0,25L p.c./ha); difenoconazole (50g)(Score 250 CE - 0,20L); azoxystrobin (50g)(Priori 250 - 0,20L) e trifloxystrobin + propiconazole (50g + 50g)(Stratego 125 + 125 - 0,4L); **protectant and curative**: tebuconazole (100g)(Folicur 200 CE - 0,5L, Orius 250 CE - 0,4L); epoxyconazole + pyraclostrobin (25g + 66,5g)(Opera - 0,5L); flutriafol (50g - 75g)(Impact 125 SC - 0,4L a 0,6L); tetraconazole (50g)(Domark 100 CE - 0,5L); trifloxystrobin + ciproconazole (56,25g + 15g)(Sphere 187,5 + 50 - 0,3L) and azoxystrobin + ciproconazole (60g +24g)(Priori Xtra 200 + 80 - 0,3L);
- the volume should be 150-250L/ha and 40L/ha, for ground and aerial applications, respectively;
- use cone or double fan nozzles and follow the technical directions for efficient leaf coverage:
- the moment of spraying, in relation to the plant growth stage must be determined case by case, and will depend upon the presence/severity of the disease and the weather condition.

Future prospect

The history of "Asian" rust in the American Continent is still very recent (three years) and much needs to be learned on its epidemiology and genetic variability. The expectation is that it may be a severe and permanent disease in regions with high rainfalls and at high altitudes (above 800m) in the Cerrados regions and in the South, where heavy dews and mild temperatures are more common during Summer. Along the years, due to variation in the weather conditions, there should be changes in disease severity or even

absence of rust. The continuous cropping of soybeans with or without irrigation will represent a continuous threat, having the soybean as its own alternate host in between two main crop seasons. In Paraguay and in the state of Parana (Palmeira) the presence of kudzu (*Pueraria lobata*), a highly efficient alternate host, increases the risk of early epidemic of rust in the region where it is present, making control more difficult.

The limited sources of genetic resistance to counteract the genetic variability of the pathogen and the continuous production of inoculum on second soybeans and volunteer plants, makes rust control through breeding for resistance a difficult task at short- to mid-term. Therefore, currently, the most efficient rust control is by the chemical approach. Nevertheless, its efficient and economical use depends upon many factors: a. on the ability to identify the disease in its earliest stage; b. continuous surveying of the field and mapping the disease distribution at national level; c. reduction/ shortening of the sowing period; d. reduction of the population density to the level that will allow proper coverage of the leaf surface with the fungicide; e. the correct choice of fungicide, in relation to the growth stage and the disease severity; f. the weather condition at time of spraying; g. the quality of the spray equipments used, either for ground or aerial applications; h. the spraying capacity to cover in due time the field that needs to be sprayed, especially under rainy weather, and i. the nutritional balance and/or the fertility levels of the soil.

The co-existence with the "Asian" rust, without significant losses or undue costs of chemical control, will demand a combination of several strategies, among which are crop rotation, avoidance of second soybean cropping and elimination of volunteer plants. In order to face such a devastating disease, several studies are underway looking for tolerant germplasms and the relative efficacy and residual effects of fungicides.

Despite the numerous talks and training courses provided in order to make farmers and field agronomists apt to identify the first symptoms of rust, most people still have difficulties in visualizing the early symptoms and signs. This indicates that further training and assistance must be provided in order to improve the skill to identify the rust symptoms at the earliest stage which is the key to successful and timely application of fungicide.

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Breeding for soybean rust resistance in Taiwan

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Abstract

AVRDC – The World Vegetable Center and several national agricultural research and educational institutions in Taiwan have conducted research to incorporate rust resistance in soybean. Most races of rust in Taiwan produce TAN-type profusely sporulating lesions and the predominant rust races are complex. Screening of germplasm initially resulted in three resistant lines, namely PI 200492, PI 200490 and PI 200451, and the former line was used to develop three improved rust-resistant cultivars, namely Tainung 3, Tainung 4 and Kaohsiung 3 in 1967, 1970 and 1971, respectively. Further screening of germplasm by AVRDC showed G 8586 (PI 230970), G 8587 (PI 230971), PI 459024, PI 459025 and *G. soja* (PI 339871) to be resistant. In subsequent years all the above germplasm were observed to be susceptible to rust apparently due to new race(s) of the pathogen. Two distinct reactions, namely, reddish brown (RB) hypensensitive reaction and TAN symptom with profuse sporulation, were observed in different host genotypes. Again due to the emergence of new races, mixed reactions occurred in RB-types in subsequent years. Although four independent dominant genes were identified to confer resistance in different genotypes, new races with multiple virulence factors for compatibility to the known host resistance factors resulted in susceptible reaction. Recognizing the ineffectiveness of single gene resistance, AVRDC conducted research on rate-reducing and partial resistance, and tolerance. A combination of genotypes combining the above three strategies can withstand rust and give higher yield. The resistant and tolerant materials appear promising in India, Africa, and Latin America. AVRDC's germplasm is available to any scientist who needs soybean rust resistant/tolerant materials.

Breeding for rust resistance in soybean by national agricultural research institutions

Soybean varietal improvement research in Taiwan has been going on for 50 years. In 1960 the Taiwan Agricultural Research Institute (TARI) obtained 3000 soybean accessions from the United States Regional Soybean

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Laboratory at Urbana, Illinois and they were evaluated in spring and summer seasons in 1961 for 15 different characters. Special attention was given to identifying sources of resistance to soybean rust disease caused by *Phakopsora pachyrhizi* Sydow. Of the 2907 accessions screened, 70 were selected for use in breeding and three of these accessions, PI 200492, PI 200490 and PI 200451, were identified as resistant to soybean rust.

TARI, National Taiwan University, National Chung Hsing University, and District Agricultural Improvement Stations (DAIS) in Kaohsiung, Tainan, Taichung, Hsinchu and Hualien, have conducted research to incorporate resistance to soybean rust in grain soybean. Thirty-seven cultivars with a wide range of reactions to rust have been released since 1956 (Cheng and Shanmugasundaram, 1992). Tainung 3 and Tainung 4 were the first cultivars with resistance to rust released in Taiwan, the source of resistance being PI 200492. The resistance of PI 200492 was also good in Australia and India. But in subsequent years the resistance broke down with the emergence of new physiologic races of the pathogen. Most races in Taiwan produce TAN-type (profuse sporulation normal) lesions. The data suggest that the predominant rust races are complex. Using 42 isolates on at least seven of the lines, the isolates were classified into nine races.

Kaohsiung 3 (with PI 200492 resistance) was released in 1971, but became susceptible a few years later to a new race. Kaohsiung Sel. 10 with narrow leaflets was found to be tolerant, i.e., the yield was not significantly reduced in spite of rust infection (Tschanz and Shanmugasundaram, 1986). Research continued until 1990, when varietal improvement of grain soybean shifted toward the improvement of vegetable soybean, a crop where Taiwan was more globally competitive. Soybean rust is not a major threat to vegetable soybean since vegetable soybean is harvested in the green stage. With one or two fungicide sprays, vegetable soybean farmers have been able to manage the disease. Currently the major emphasis in varietal improvement for vegetable soybean is focused on yield, quality improvement, and adaptation to different seasons and locations.

Breeding for rust resistance in soybean by AVRDC

AVRDC began its research on soybean in March 1973 and by the end of the year had 3064 soybean accessions. AVRDC's soybean germplasm collection has since grown to 14,200 accessions, one of the largest collections in the world. AVRDC immediately recognized that soybean rust was the most serious disease of soybean in Taiwan and Southeast Asia. In 1974, the germplasm and cultivars previously reported as resistant were evaluated at AVRDC, where they were found to be susceptible with the exception of slight resistance shown by Tainung 4.

An International Working Group on Soybean Rust (IWGSR) was organized in 1976 and a new soybean rust rating system was developed (Shanmugasundaram, 1977). A three digit system was used: the first digit indicating the leaf position (1, 2, and 3 indicating top, middle, and bottom, respectively), the second digit denoting lesion density (1, 2, 3, and 4, indicating none, light, medium, and heavy levels of lesions, respectively) and the third digit denoting nature of pustule development (1, 2, and 3, indicating none, non-sporulating, and sporulating pustules, respectively). A final classification for different ratings was established (Table 1).

Accessions G 8586 (PI 230970) and G 8587 (PI 230971) were identified to have resistance. Crosses were made to combine the resistance from G 8586 and G 8587 and the following selections from inbred generations were made for rust resistance: AGS 181, AGS 182, AGS 183, AGS 229, AGS 233, AGS 240, AGS 244, and AGS 247. In 1980 two symptom types, RB (reddish brown necrotic lesion with less sporulation) and TAN (profuse sporulation normal), were identified. G 8586 and G 8587 had RB lesions while susceptible genotypes had TAN lesions. This resistance was short lived. Both RB and TAN lesions occurred on G 8586 and G 8587. Apparently a new race of the fungus was able to attack the two previously resistant accessions. Later, PI 459024, PI 459025 and *G. soja* (PI 339871) were found to be resistant; however, their resistance was also overcome, apparently by a new race of the pathogen with higher virulence factors (Bromfield, 1981).

Four independent dominant genes, namely Rpp1 (PI 200492), Rpp2 (PI 230970), Rpp3 (PI 462312), and Rpp4 (PI 459025), have been identified (Hartwig and Bromfield, 1983; McLean and Byth, 1980; Hartwig, 1986). D 86-8286, a selection from a cross between Forrest × PI 230970 has been released as resistant to rust (Hartwig, 1988). Three other lines, namely Tainung 4, PI 459024, and PI 459025, as well as *G. soja* were reported to have additional specific genes for resistance (Hartwig and Bromfield, 1983; McLean and Byth, 1980). The rust isolate from Taiwan (80-2A) was found to have virulence factors that can cause rust in all known resistant sources with specific resistance including PI 200492, PI 230970, PI 339871, PI 459025 and PI 462312 (Bromfield, 1981). Since the pathogen contains unnecessary virulence genes, resistant gene deployment techniques such as rotating genes and pyramiding of known resistance genes will likely be ineffective. Therefore, AVRDC shifted its breeding strategy to use tolerance to rust, with degree of tolerance defined by the relative yielding ability of plants under stress from the pathogen.

In a breeding scheme to incorporate tolerance in the early generation, selection was made for simply inherited traits and undesirable genotypes

were discarded. Since soybean rust reduces the pod number and seed size, selection of pedigrees in F5 and subsequent generations focused on more number of pods per plant with fully filled seeds without abnormalities. Selection for agronomic traits was also included. Rate reducing resistance was also observed and selections were made. The selected inbred lines were evaluated with and without fungicide in different seasons and locations. Along with rate reducing resistance and partial resistance, a stress tolerance index (STI) was used in evaluating rust.

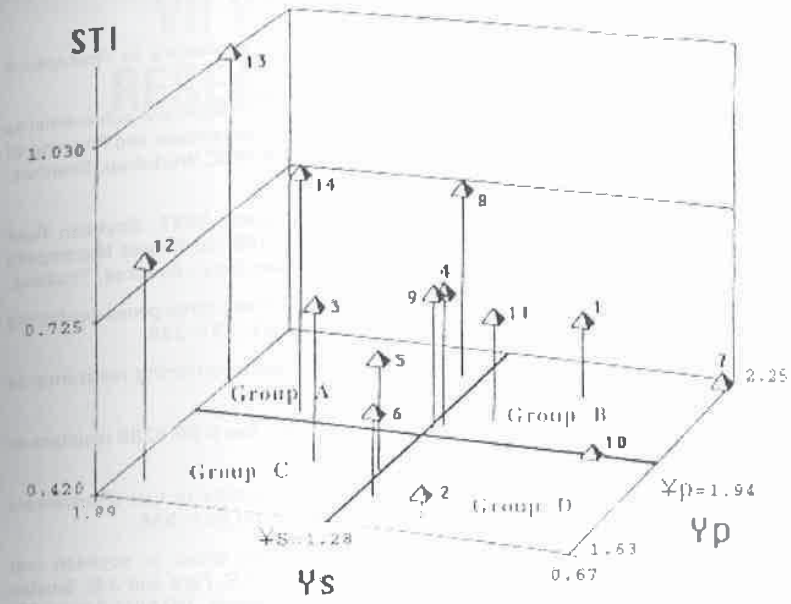
A three dimensional plot distinguished the test materials into four groups. The X-Y plane is divided into four segments by drawing intersecting lines at the midpoint of the X and Y axes. The Z axis indicates the level of stress tolerance index (STI) that a line has in any one of the four groups. STI is estimated as: $(Ys*Yp)/(\bar{Y}p)^2$, where Ys = yield in stress environment (X axis), Yp = potential yield (Y axis), and $\bar{Y}p$ = mean potential yield. The use of three-dimensional plots was effective in separating and enabling selection of rust-tolerant, high yielding selections (Hartman et al., 1997). As shown in Fig. 1, Group A genotype (GC 87019-4-1B-4, AGS 129, and SRE-D-14B) expressed uniform superiority in environments with and without rust. Group B genotypes performed favorably only in the environment without rust. Group C genotypes yielded relatively well only in a rust environment and Group D genotypes performed poorly in environments with and without rust. Seasonal variations appear to influence the intensity of rust, which may influence STI. The inbred lines listed in Table 2 have been identified to be tolerant to soybean rust.

Table 1. Relationships between disease reactions and IWGSR ratings for soybean rust.

Disease reaction	IWGSR rating
Immune	111
Resistant (R)	122, 123, 132, 133, 222, 223
Moderately resistant (MR)	142, 143, 232, 233, 242, 243, 322, 323
Moderately susceptible (MS)	332, 333
Susceptible (S)	343

Table 2. Soybean rust-tolerant lines

No.	Entry	Pedigree
1	AGS 129	Shih Shih × SRF 400
2	GC 00138-29	(CH #1 × Anoka) × (Clark 63 × 64-4)
3	GC 60020-8-7-7-18	PI 194647 × TN #3
4	GC 84051-32-1	TN #4 × (Shih Shih × SRF 400)
5	GC 84058-18-4	PI 79712 613 × (PI 79712 613 × SJ #4)
6	GC 85037-2-3-5-1	Ankur × PI 230970
7	GC 86017-170-1N	(Shih Shih × SRF 400) × PI 153240
8	GC 86018-427-3	(Shih Shih × SRF 400) × PI 194647
9	GC 86049-35-2-1-1-8-1N	(PI 157424 × KS #8) × (Shih Shih × SRF 400)
10	GC 87012-10-B-5	(SRF 400 × Tzuzunoko) × KS #1
11	GC 87016-11-B-2	KS #1 × Mainland China
12	GC 87021-13-B-2	KS #1 × 269
13	GC 87021-26-B-1	KS #1 × 269
14	SRE-B-15A	Akiyoshi × PI 68728
15	SRE-B-15C	Akiyoshi × PI 68728
16	SRE-C-56A	PI 68728 × Shih Shih
17	SRE-D-11A	(UPSL-85 × PI 230971) × PI 68728
18	SRE-D-14A	Sanga × Akiyoshi
19	SRE-D-14B	Sanga × Akiyoshi
20	SRE-D-14C	Sanga × Akiyoshi
21	SRE-D-14D	Sanga × Akiyoshi



Stress Intensity (SI) = 0.34, where SI = 1-Ys/Yp

Figure 1. Grouping of soybean materials using stress tolerance index (STI), yield potential (Yp), and yield under rust stress (Ys) under moderate conditions[1]; advanced yield trial, Autumn 1992

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VII WORLD SOYBEAN RESEARCH CONFERENCE

MARCH 4

Molecular breeding for resistance to defoliating insects and root-knot nematodes

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Abstract

In the past decade the molecular linkage map of soybean, *Glycine max* (L.) Merr., has continued to develop and now contains over 1800 linked markers on 20 linkage groups. The application of marker-assisted selection (MAS) to soybean improvement has directly contributed to the release of several improved cultivars by both public and private breeding organizations. The backcross development of lines with unique combinations of soybean insect resistance genes and an insect resistance transgene from *Bacillus thuringiensis* utilized MAS for the resistance alleles of the donor parent. DNA markers were used in the discovery of a rare resistance gene (rare in the ancestors of elite U.S. cultivars) to the southern root-knot nematode (*Meloidogyne incognita*). Markers tightly linked to this gene are currently being employed in the early generation selection of resistance to southern root-knot nematode in crosses between resistant and susceptible parents.

Introduction

Molecular marker technologies have facilitated the construction of genetic maps of many organisms including important crop species. An analysis of the association of phenotypic trait and genetic marker data from a population of lines segregating for genes conditioning a quantitative trait provides the unique ability to dissect the quantitative trait into a set of discrete quantitative trait loci (QTL) (Tanksley et al., 1989). Genetic marker analysis can measure the effect of an individual QTL at the allelic level (i.e., the contribution of an allele from each parent at a particular QTL can be determined separately from the other QTL affecting the trait). This important attribute of genetic markers allows pyramiding of the desirable alleles for a complex or otherwise intractable trait into an adapted genetic background.

The current consensus linkage map of soybean contains 1845 markers and a total genetic distance of 2527cM (unpublished data, 2003, P.B. Cregan, USDA-ARS, Beltsville MD). A new type of DNA marker, single nucleotide

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polymorphisms (SNPs) offers advantages, such as ease of genotyping, not currently matched by any other category of DNA marker (Rafalski, 2002). SNP markers are currently being developed for targeted regions of the soybean genome (Zhu et al., 2003).

Marker screening for southern root-knot nematode resistance

Root-knot nematodes (*Meloidogyne* spp.) are economically important plant pathogens that cause extensive crop losses all over the world (Sasser, 1977). They have extensive host ranges, their distribution is worldwide, and they interact with fungi in disease complexes. In the southeastern USA, the southern root-knot nematode [*M. incognita* (Kofoid and White) Chitwood] (Mi) is the most common root-knot nematode. In a Florida field infested with Mi, soybean yields of susceptible cultivars were suppressed by 53 to 90% (Kinloch, 1974). Yield losses in the southern USA due to root-knot nematode were estimated at $80 million in 1996 (Pratt and Wrather, 1998).

The development and use of nematode-resistant soybean cultivars result in reduced yield losses and increased grower profits (Boerma and Hussey, 1992). Thus, many soybean cultivars with root-knot nematode resistance and high productivity have been developed in the USA over the past few decades. 'Forrest' was shown to possess a single additive Mi-resistance gene, *Rmi1* (Luzzi et al., 1994). In a cross of PI96354, which possesses a high level of resistance to Mi (Luzzi et al., 1987), and 'Bossier' (Mi susceptible), a major QTL was identified which mapped to LG-O of the public soybean genetic linkage map (Tamulonis et al., 1997). Using SSR markers, Li et al. (2001) found the major Mi QTL on LG-O was flanked by SSR marker Satt492 and Satt358. Tamulonis et al. (1997) speculated, based on segregation for gall number in a population of Forrest ´ PI 96354 and the level of resistance in Forrest, that the Mi-resistance QTL on LG-O was the *Rmi1* gene.

The availability of pedigree data and molecular linkage maps provides an opportunity to track genomic regions through the breeding process (Shoemaker et al., 1992). Narvel et al. (2001a,b) used a molecular pedigree analysis to track the *rxp* gene, which conditions resistance bacterial pustule (*Xanthomonas campestris* pv. *Glycines*), in elite North American soybean cultivars and to monitor QTL for insect resistance in 15 insect-resistant soybean genotypes.

The initial applications of MAS in soybean improvement were for traits conditioned by QTL with relatively large phenotypic effects and in which the positive alleles were rare in the ancestoral germplasm (Orf et al., 2004).

In these situations, breeders are usually aware of the QTL or genes conditioning the selected trait in their segregating populations and the parental source of the positive alleles at these QTL.

In the case of Mi, two tightly linked SSR markers tagged a rare resistance allele on LG-O which was found to condition most of the Mi resistance in a pedigree analysis of selected elite cultivars (Ha et al., 2004). Codescent analysis of markers and phenotypes across six cycles of breeding showed that Mi-resistant cultivars inherited a 200-bp band at Satt358 and a 238-bp band at Sat_132 from 'Palmetto' (Fig. 1). The tight linkage of both Satt358 and Sat_132 to the Mi QTL on LG-O indicates that selection for the Mi-resistant parent's allele at either of these markers should be highly effective in identifying Mi-resistant plants or lines.

Pyramiding insect resistance transgenes and native genes

More than three decades of breeding to develop high-yielding insect-resistant soybean lines have met with limited success (Boethel, 1999). Breeding programs in the USA have tried to exploit the resistance identified in the Japanese plant introductions (PIs) PI 171451, PI 227687, and PI 229358. These were initially identified as showing resistance to the Mexican bean beetle [*Epilachna varivestis* (Mulsant)] (Van Duyn et al., 1971). Further testing showed that these PIs exhibit both antixenosis (non-preference) and antibiosis types of resistance to a number of coleopteran and lepidopteran soybean pests. Unfortunately, these three PIs possess many undesirable agronomic traits, and some of the genes controlling these traits are linked to genes associate with insect resistance (Boethel, 1999). This results in linkage drag when the insect resistance genes are backcrossed into elite germplasm (i.e., selection for insect resistance genes results in inadvertent co-introgression of undesirable agronomic trait genes at linked loci). The quantitative nature of insect resistance in these PIs complicates efforts to introgress resistance genes into elite cultivars and increases the likelihood that some inferior agronomic alleles will also be transferred. Although three insect resistant cultivars have been released, none of them yield as well as the most productive non-resistant cultivars.

Advances in biotechnology have made alternative approaches to developing an insect-resistant soybean possible. A transgenic soybean expressing a synthetic *cry1Ac* gene from *Bacillus thuringiensis* (Bt) was developed at the Univ. of Georgia (Stewart et al., 1996). The Cry1Ac protein is highly toxic to the most important lepidopteran pests of soybean grown in the southeastern USA. The transgenic line, Jack-Bt, proved resistant to corn earworm [CEW; *Helicoverpa zea* (Boddie)], velvetbean caterpillar (*Anticarsia*

gemmatalis (Hübner)), soybean looper (SBL; *Pseudoplusia includens* (Walker), and the lesser cornstalk borer [*Elasmopalpus lignosellus* (Zeller)] (Walker et al., 2000). Although the level of protection that a Cry1Ac protein provides plants against sensitive pests can be dramatic, Bt transgenic plants have two limitations. One is that most of the proteins which have been engineered into plants confer resistance to either lepidopterans or coleopterans, but not both. Another limitation is that many sensitive lepidopteran pests have demonstrated the ability to evolve resistance to one or more Bt proteins, so effective resistance management strategies must be implemented to delay the development of Bt-resistant insect populations in the field (Gould, 1998). Alternative sources of resistance are therefore needed to both broaden and prolong the resistance of Bt-expressing crops.

Rector et al. (1998; 1999; 2000) used RFLP markers to investigate the number and contribution of QTL associated with CEW resistance in the three Japanese soybean plant introductions. Subsequent studies have used SSR markers to more precisely map the location of the QTL and to identify tightly linked markers useful for MAS (Narvel et al., 2001a). These markers also make it easier to confirm the effects of resistance QTL in other populations. Narvel et al. (2001a) showed that a region of the PI 229358 genome on LG-M that contains a major CEW resistance QTL was retained in at least 13 out of 15 breeding lines and cultivars that were developed through phenotypic selection for resistance.

In the QTL mapping studies of Rector et al. (1998; 1999; 2000) resistance was measured as percent defoliation on whole plants (antixenosis) and as larval weight gain on detached leaves (antibiosis). The previously mentioned major QTL on LG-M (229-M) is involved in both types of resistance in PI 229358 as well as in PI 171451. PI 229358 also has an antibiosis QTL on LG-G and antixenosis QTL on LG-H, which contributes to resistance in the other PIs as well. The LG-G QTL may contribute to resistance in the other two PIs, but this could not be determined in the populations used.

The 229-M QTL is of particular interest because of its relatively large contribution to both modes of resistance, and because it was identified in two of the PIs. MAS with SSR markers has been used to pyramid 229-M with the Bt *cry1Ac* transgene to develop a resistance gene pyramid in the genetic background of the cultivar Jack (Walker et al., 2002). Averaged across $BC2F_3$ lines with various combinations of the two resistance genes, 229-M and Bt caused a reduction in larval weight for both CEW and SBL, and there was a significant interaction in the case of CEW.

From the $BC2F_3$ lines we developed a series of $BC2F_{3:5}$ and $BC_2F_{3:6}$ lines carrying various combinations of Bt, 229-M, and the PI 229358 antixenosis

QTL on LG-H (229-H). These lines were evaluated in the field for defoliation by CEW and SBL in 2000 and 2001. The presence of Bt or 229-M reduced defoliation by both insects, but no effect was detected for 229-H. These pyramid lines were also evaluated for antibiosis resistance to tobacco budworm [*Heliothis virescens* (Fabricius)] in detached leaf studies. Two strains were used in the bioassays, one which had been selected in the laboratory for resistance to Cry1Ac (YHD2), and the other a related strain which had not been exposed to Cry1Ac (YDK). The presence of the 229-M QTL decreased larval weights in both strains, whereas Bt only affected the YDK strain, as expected. Based on these results, we concluded that combining a Bt transgene with one or more native resistance genes like 229-M could be an effective strategy to delay the development of Bt-resistant pest populations.

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Genomic approaches to molecular breeding of resistance to soybean sudden death syndrome and cyst nematode in elite cultivars

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Abstract

Marker assisted selection for resistance is used for millions of selections annually. We have developed methods for high-through-put selection in soybean for 5 genes for resistance to SCN and seven for resistance to SDS. However, the efficiency of marker selection is limited by using linked markers instead of the genes themselves. We have developed markers from gene sequences following positional cloning of *Rhg1, Rhg4* (for resistance to soybean cyst nematode) and *Rfs2* (for resistance to *Fusarium solani* f.sp. *glycines* causal agent of sudden death syndrome). The gene alleles are much more useful than conventional markers. Isolating the remaining genes will be challenging without a whole genome sequence for soybean. Physical map based sequencing of gene rich islands in complex genomes provides an alternative strategy to whole genome sequencing. During the last 3 years we have developed a fingerprint based method developed physical maps of the soybean genome, and integrated it with gene maps. The soybean physical map is most highly developed for linkage group G where 10 contigs encompass the chromosome. These are being used to identify gene rich islands by cDNA hyridization and sequence the gene rich islands within the chromosome. Four genes for resistance, were mapped to a 1240 Kbp contig G200 and sequenced 300 kbp to isolate the *Rhg1* and *Rfs2*. Using genetic markers we mapped two genes to a second 300 kbp interval of the same contig, *Rfs6* and *Rfs1*. Integrating EST libraries (60% of the genes) and predicted paralogs (40% of the genes) with the soybean physical map identified this interval as one of several gene rich islands on linkage group G. The interval was sequenced using anchored BACs from two cultivars. A high predicted gene density has been observed (1 gene per 7 kbp). Predicted genes have been annotated by position in the genome and presence in EST libraries or in the transcriptome. Comparing cultivar sequences we have found about 1 SNP per 2.1 kbp in genomic DNA, but two fold more SNPs around resistance genes. Sequencing of 10

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Mbp to encompass one chromosome arm, Rfs, Mi, Rps5, Rps6 and white mold resistance QTL is proposed. Contigs were prepared for 1 Mbp of genomic DNA encompassing R*fs4* and R*fs5* on linkage groups C2 and I. Combining genome sequencing and substitution maps will annotate and isolate the 300-400 economically important soybean genes efficiently within the next decade. Marker assisted selection efficiency will be increased and the yield of new cultivars improved faster that ever before. This work was supported by NSF project #9872635, the ISPOB and the USB.

Introduction

Resistance to sudden death syndrome of soybean (SDS) is a partial disease resistance (Hnetkovsky et al., 1996). In agriculture the stability and yield compatibility of partial disease resistance provides advantages over complete resistance (Webb et al., 1995; Yuan et al., 2002). The molecular basis of partial resistance has not been determined for any plant. SDS, a rapidly appearing leaf scorch syndrome, is often measured solely by a percentile, leaf symptom derived, disease index (DX %; Hnetkovsky et al., 1996) or disease progression (Stevens et al., 1993). However, SDS is primarily a root disease, caused by *Fusarium solani* f. sp *glycines* (Fsg). The pathogen is strictly restricted to infection of the roots (Rupe *et al.*, 1993; Gibson *et al*, 1994; Njiti *et al*, 1996). Leaf symptom appearance is environmentally dependent (Hnetkovsky *et al.*, 1996; Njiti *et al*, 1996) but has a genetic component (Stevens et al., 1993; Hnetkovsky et al., 1996) related to toxin synthesis (Baker and Nemec, 1994; Jin et al., 1996). Root infection severity (IS %) is environmentally stable and may be more consistent and reliable for determining the location of genes conferring resistance to SDS (Njiti *et al*, 1997; 1998).

Soybean linkage groups G, C2, I and N contain QTL for resistance to SDS (Iqbal et al. 2001; Njiti et al. 2002). Linkage group G (chromosome 18; Zhou et al., 2003) contains a cluster of loci for resistance to SDS comprising *QRfs, QRfs*1, *QRfs*2 and *QRfs*3. Based on DNA marker maps each is located in 2-5 cM intervals that are mutually linked. The genes are 0.25 to 30± 2.5cM from *rhg*1 the major gene for resistance to *Heterodera glycines*, the cyst nematode (Meksem et al. 1999; 2001; 2001a). *QRfs* is 30 cM from *rhg1* and probably corresponds with *Rfs*, a gene identified without DNA marker association (Stevens et al., 1993; Lightfoot unpublished). *QRfs*2 is about 0.2-0.5 cM from *rhg1* and reduces the DX (Meksem *et al.* 1999). Low map resolution means *QRfs*2 may be a separate gene linked to *rhg*1 or a pleiotropic effect of the gene. One goal of this study was to dissect the gene clusters by recombination.

Analysis of pachytene chromosome spreads predicts that the architecture of the soybean genome consists of 31 euchromatic regions and 21 heterochromatic regions among 20 chromosomes (Singh and Hymowitz, 1988). The short arm of chromosome 18 (linkage group G) is entirely heterochromatic. Euchromatin represents 50% of the chromosome and the entire long arm. The soybean genome shows conserved microsynteny with the Medicago genome (Yan et al., 2003) where gene rich islands are common. Therefore, gene-rich islands are expected in this portion of the soybean genome (Lightfoot and Meksem 2001). Extensive soybean genome sequence has been released to Genbank for regions of this chromosome (Hague et al., 2001; Lightfoot and Meksem 2001; Shultz et al., 2003). One region, encompassing 463 Kbp (in two segments separated by ~2 cM) on linkage group G, was posted due to the presence of a major gene (*rhg1*) for cyst-nematode resistance and patenting requirements (Hauge et al., 2001; Genbank AX196301-42; Lightfoot and Meksem 2001; GenBank AF506516-AF506518). These sequences were submitted to Genbank without annotation. Subsequently, two groups (Foster-Hartnett et al., 2002; Triwitayakorn, 2002 and this paper) estimated a gene density for this region of 1 ORF/ ~5-7 kb a density roughly equivalent to that found in Arabidopsis (The Arabidopsis Genome Initiative, 2002) and tomato (Alpert and Tanksley 1996; Ku et al., 2000). A second goal of this study was to determine the extent of the gene rich island.

The soybean genome (Xiaozhu *et al.* 1994) and the region of linkage group G studied herein, previously has ranged from 100 kbp to 1,000 kbp per cM (Danesh *et al.*, 1998; Foster-Hartnett et al., 2002). Therefore, the degree of marker saturation and extent of fine mapping required for each QTL in this region are expected to vary. The heritability of traits underlying QTL will also affect the extent of fine mapping necessary (Kearsey & Farquhar, 1998). The SDS resistance trait is highly heritable (90-100%) compared to many field traits (Hnetkovsky et al., 1996; Njiti et al., 1996). A third goal was to determine whether genetic resistance to SDS can provide a model for detailed analyses of field trait QTL. In this paper the location of the genes *QRfs2* and *QRfs1*, genome structure, gene density and the use of candidate resistance genes are shown.

Results and discussion

A genetic map that included 240 polymorphic microsatellite markers selected from 1,012 DNA markers was used to identify loci that segregated in the NILs (Iqbal *et al.*, 2001; Cregan *et al.*, 2002 Soybase (2002) http://soybase.agron.iastate.edu.). About 8% of the genome separated into 2-4 regions segregates in each NIL pair. The 10 cM region of linkage group G

between BARC-Satt309 and SIUC-Sat122 was the only region that was associated with quantitative trait loci for SDS in ExF34 derived NILs. Markers SATT371(C2) and SATT354(I) that segregated in the NILs are at least 10 cM from QTL for resistance to SDS (Iqbal et al., 2001) because the resistance to SDS of the NILs was only associated with the markers on linkage group G as judged by ANOVA ($P>0.05$).

Construction of a high resolution map on linkage group G between BARC-Satt309 and SIUC-Sat122

A fine genetic map within the interval between markers BARC-Satt309 and SIUC-Sat122 on linkage group G was constructed. The map was derived from 12 recombination events among 96 RILs and 24 recombination events among 80 NILs. Five microsatellite markers and four SCARs were mapped. The most probable map of this interval spanned 7.5 cM (Fig. 1).

Construction of 0.2 cm resolution map of sds resistance genes using near-isogeneic lines with recombination events

Seven recombination events were identified from within 2,000 plants screened. Less than predicted from map distances measured in RIL population. Stepwise selection for Forrest alleles at SATT309 and the absence of selection favoring the preservation of heterozygotic loci in intercross derived NILs may have resulted in the identification of fewer recombination events than expected from genetic distance (Meksem et al., 1999). Consequently, the earlier estimates of map distance were used. Marker order was adjusted to minimize double recombinant within all NILs. Marker order was consistent with DNA sequences. The alleles at regions of the genes of the lines from both recombination events in the ExF34 derived NILs were lined up to construct substitution maps as shown in Figure 1. The recombination events were located on both sides of the loci of interest.

Substitution mapping of the *Rft*/*QRfs*2 gene using DX.

Disease phenotypes were compared with the recombination events in genomic regions to establish the position of the disease resistance loci, *QRfs*1 and *QRfs*2 (Figure 1). All genotype groups (1-4, 6 and 7) carrying a Forrest allele at SIUC-Sat1 showed resistance to leaf scorch by SDS. This marker identified the only region all resistant lines had in common. Therefore, the *QRfs*2/*Rft* gene is probably located in the region encompassing Satt309, TMD and SIUC-Sat1.

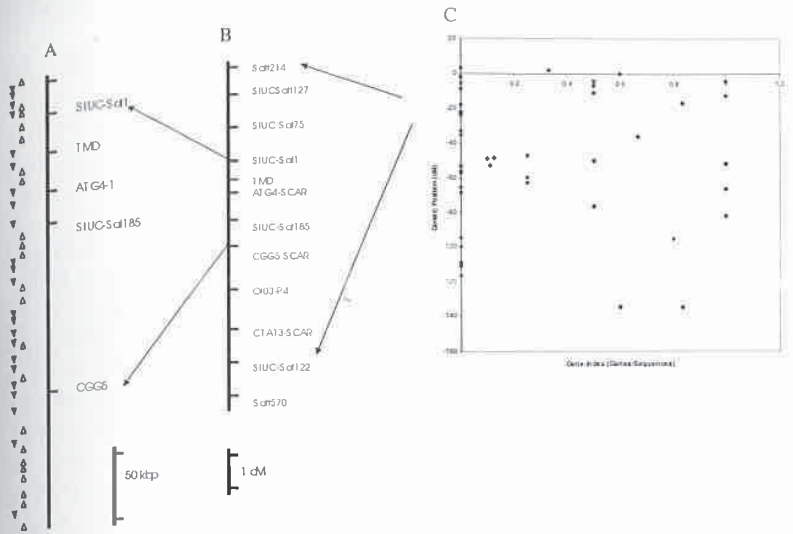


Figure 1. Genetic map of linkage group G (chromosome 18). A. Relative positions of the DNA markers and 56 genes encoded in a 312 kbp DNA sequence. Genes are shown as open triangles when encoded on the lower strand and filled triangles when encoded on the upper strand. B. A genetic map showing positions of marker used in this analysis within the 7.5 cM BARC-Satt309 to SIUC-Sat122 interval based on 96 RILs of ExF and 80 NILs (ExF 11-1 to 40 and ExF34-1 to 40). C. A preliminary gene index for the linkage group. The gene density was calculated from 56 loci, that anchored 352 BAC end sequences in which were found 67 predicted genes (http://bioinformatics@siu.edu).

Substitution mapping of the *QRfs*1 gene using IS

Among the genotype set (Fig. 1) the majority of the genotype groups (1-4 6 and 7) were susceptible to root infection. However, no single locus or marker model could explain the segregation observed. The consistent model was that susceptible genotypes did not carry the beneficial alleles from Forrest for the region encompassed by OI03-P4 to SIUCSATT122. Therefore, *QRfs*1 may be a locus comprised of several genes located between OI03-P4 and the CTA13-SCAR. In addition, when *QRfs*1 was present and in the absence of *QRfs*2/*Rft*, disease incidence was very high suggesting that *QRfs*1 may have pleiotropic effects or be linked to gene(s) that cause leaf scorch to be more evident (Njiti *et al.*, 1998).

Gene island inference and gene prediction within intervals

Intervals containing *QRfs2* and *QRfs1* were examined for structure and gene content using the physical map and BAC end sequence database published sequence (Figure 1; Hauge et al., 2001; Meksem et al., 2001) and published hybridization data (Foster-Hartnett et al., 2002). There were 20 contigs with BAC end sequence data within the 7.5 cM region examined and a high percentage (24%) showed significant homology to known genes. No contig sequences identified only repeat, non-geneic sequence. Therefore, the entire region contains gene rich islands and these may be contiguous.

Gene density was further explored using composition and homology searches. Within both published sequences there were seventy-four predicted genes at a density of one gene per 7 kbp (AX196295). Sixty two aligned with ESTs, the best hits were judged identical (31), paralogous (26), or orthologous (5) by sequence. Orthologs were from *A.thaliana, O. sativa and M. truncatulata* but there was no clear evidence of microsyntenic regions between soybean and other partially or completely sequenced genomes (Ku et al., 2001; Yan et al., 2003). Lack of conserved microsynteny may caused by the presence of a major disease resistance gene in the region in soybean. Data suggest 77% of soybean genes appeared to be paralogs of the existing soybean ESTs. About 83% have orthologs among plant ESTs. Identification of the remaining 17-23% of genes will be dependent on further legume genome sequencing.

Mean gene coding region was 2.56 Kbp (range 6 bp to 10 Kbp). The mean intron number was 3.4 (range 0- 24). Intergeneic distance was 5,309 bp but no large intergenic distance were observed (all <12 kbp). Genetic markers matched the sequenced regions at seven coordinates. Twenty BACs were expected to overlap the sequenced region based on a marker anchors to a BAC fingerprint derived physical map. BAC end sequences matched for all those tested. Clones with large inserts and a minimal overlap with the sequence regions were identified. Therefore, the region contained genes at a 10-20 fold higher frequency than expected for the entire soybean genome, that the annotation pipelines developed for *A.thaliana* (TIGR) and Plant ESTs (ERGO) are effective for soybean DNA and that orthologs are rarely the only evidence for annotation. Taken together the gene prediction data suggest the gene rich island extends for 7.5 cM or 3.5 Mbp. Therefore, the sequencing of soybean genome may be highly efficient if a rigorously edited physical map is used to select BAC clones (Shultz et al., 2003; Wu et al., 2003).

Candidates for the *QRfs2* gene clustered with the *Rhg1* gene for resistance to cyst nematode (genes 1-8). Laccase (gene 9) was also considered a

strong candidate for *QRfs2* because it is polymorphic between resistant and susceptible cultivars and may be involved in cell wall strengthening (Iqbal et al., 2003). Independently derived resistant cultivars Forrest, Pyramid and Hartwig (Prabhu et al. 1999; Njiti et al., 2002) all share the same haplotype for laccase (not shown). Independently derived susceptible cultivars Essex, Douglas, Flyer and Asgrow 3244 all share the second haplotype. These cultivars represent 5 of eight possible haplotypes at *Rhg1* (Hague et al., 2001; Lightfoot and Meksem 2001). Therefore, selection may have favored recombination, mutation or gene conversion in this gene pair.

A SNP within the laccase gene alters amino acid sequence within a sequence motif that is also found in *Rhg1* (Hague et al., 2001; Lightfoot and Meksem 2001). The sequence is not a recognized motif in current databases. However, it may represent an uncharacterized allosteric binding site that contributes to the interaction between *Rhg1* and *QRfs2* and therefore SDS and SCN resistance (Chang et al., 1997). Finally, laccase is a diphenol oxidase and many of the toxins produced by Fsg are phenolic in structure (eg. Monorden; Baker and Nemec, 1994; Jin et al., 1996). Plant transformation and protein activity assays will be pursued in future.

Conclusions

Based on the fine map the QRfs genes within separate loci appear to interact. Therefore, MAS for resistance to Fsg toxin (*QRfs2*) using BARC-Satt309 alone may not be effective unless TMD and SIUC-Sat1 are included. Using all three markers together will be able to predict for resistance to SDS or Fsg toxin more precisely. Moreover, selection of *QRfs1* for resistance to Fsg root infection by OI03-P4 or SAT122 alone will predict the phenotype of about 66-99% because this marker is not mapped exactly on the gene. Therefore, more markers derived from contigs between OI03-P4 and SIUC-Sat122 region may provide the better selection tools for this gene. Isolation and unequivocal identification of the *QRfs2* and *QRfs1* gene sequences will provide perfect markers for selection and provide basic information addressing the molecular nature of partial resistance in plants.

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The simplicity and complexity of the interaction of soybean mosaic virus and resistance genes in soybean

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Abstract

The interaction of soybean genotypes [*Glycine max* (L.) Merr.] with *Soybean mosaic virus* (SMV; Genus *Potyvirus*; Family *Potyviridae*) is summarized in this report. Genetic studies over the last 30 years have demonstrated that resistance in soybean to SMV is conditioned by single dominant genes at three independent loci. SMV isolates are differentiated as seven pathotypes on cultivars carrying alleles at the *Rsv1* locus. A necrotic, or hypersensitive response-like, host response occurs with many strain-allele combinations. The *Rsv3* locus confers resistance to SMV strains which overcome resistance of several *Rsv1* alleles, but not to strains for which *Rsv1* is effective. Resistance to virus accumulation and movement is conferred by *Rsv4*, providing protection from all strains. All three genes are molecularly mapped to the soybean genome, and can be detected with specific markers. Deployment of cultivars with durable resistance is the most effective means of managing the virus and reducing losses. This can be facilitated by understanding the soybean-SMV pathosystem, knowing the strain identity and diversity in a geographic area, and designing adapted cultivars with appropriate multiple resistance genes, to provide genetic protection to a wider range of SMV strains than can any gene alone.

SMV: the disease and the virus

Worldwide, SMV is considered the most prevalent and damaging virus of soybean, as its seed-borne and aphid-transmitted properties provide means of distribution and dissemination. Early infection results in decreases in yield and seed quality, including viability, germinability, and seedling vigor. Seed-coat mottling is often associated with virus-infected plants, as is foliage reduction, delayed maturity, poor seed set, reduced nodulation, and increased susceptibility to other pathogens. SMV strain diversity was first discussed in the USA by Ross (1987). Cho and Goodman (1979; 1982) collected many isolates of SMV in the USA from germplasm collections and experimental plots and inoculated them to a set of cultivars known to have genetic resistance to SMV. The isolates were classified into seven strain groups, based on the set of responses of the soybean differential

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cultivars 'Ogden', 'Marshall', 'York', 'Kwanggyo', and 'PI96983'. The isolate designated by them as the type isolate for each strain group has become known as SMV strains G1 through G7. Cultures of these strains have been maintained and deposited in the American Type Culture Collection (www.atcc.org), and used by geneticists in inheritance of resistance studies. New isolates of SMV in the USA and in several other countries are often tested on this differential cultivar series to determine the strain (Lee et al., 1991; Almeida et al, 1995). Variants with resistance-breaking properties are being identified with increasing frequency (Cho et al., 1977; Almieda et al., 1995; Fayad and Tolin, 2003a; Kim et al., 2003).

SMV isolates cannot be distinguished serologically, as the coat protein is quite similar (Domier et al., 2003). The total RNA sequence is now available for several SMV strains including G2 and G7 (Jayaram et al., 1992; Hajimorad et al., 2003), N (Genbank Accession No. NC 002634), G5 and G7H from Korea (Lim et al., 2003), an attenuated isolate Aa15-M2 from Japan (Genbank Accession No. AB100443; AB100442), and a severe strain from China (Genbank Accession No. AJ312439). Partial sequences are available for many more SMV isolates, and are beginning to be used to differentiate strains as well as to locate domains of the virus that may be associated with the resistance-breaking phenotype (Omunyin et al., 1996; Hajimorad and Hill, 2003).

Three genetically distinct loci for SMV resistance

The gene at the *Rsv1* locus, conferring resistance to SMV G1-G6, and its allelic forms, conferring different strain-specific responses, have been widely reported in inheritance studies (Kiihl and Hartwig, 1979; Buzzell and Tu, 1984; Buss et al., 1989). Allelism tests of the differential cultivars showed that each possessed a single dominant gene resistant to SMV-G1 strain at locus *Rsv1*. The alleles were designated as *Rsv1*, *Rsv1*-t, *Rsv1-y*, *Rsv1-m*, and *Rsv1-k*, respectively (Table 1) (Chen et al., 1991). Additional alleles are present in 'PI 486355' (*Rsv1-s*), 'Suweon 97' (*Rsv-sk*), 'Raiden' (*Rsv1-r*), and 'PI 507389' (*Rsv1-n*) (Ma et al., 1995; Chen et al., 2002; Chen et al., 2001; Ma et al., 2003). Each allele gives resistance to some of the strains of SMV, while being necrotic or susceptible to other strains. Only *Rsv1-sk* is resistant to SMV G1 through G7 (Chen et al., 2002). *Rsv1-n* is the only allele conferring lethal necrosis to SMV-G1 and no resistance to any other SMV strain (Ma et al., 2003).

Soybean plants with *Rsv3* are resistant to higher strains of SMV (G5-G7), but susceptible to lower strains (G1-G4), making it a good candidate for combining with *Rsv1* to confer resistance to a broader range of SMV strains.

Rsv3 was proposed by Buzzell and Tu (1989) as the symbol for a gene from cv. Columbia, on which G1 and G4 induced stem-tip necrosis. Later studies showed Columbia has two independent dominant genes, probably at *Rsv3* and *Rsv4* loci (Ma et al., 2002). A gene allelic to the *Rsv3* of Columbia is carried by 'L29' (Bernard et al, 1991), an isoline of cv. Williams containing a gene from cv. Hardee, but no necrosis is induced by G1 or G4 (Buss et al., 1999). The L29 gene is also allelic to *Rsv3* genes in 'OX670' and 'Harosoy' (Gunduz et al., 2001).

The *Rsv4* gene for SMV resistance was revealed as one (the other was *Rsv1-s*) of two R genes in PI 486355 by Ma et al. (1995), and was separated into a line released as 'V94-5152' (Buss et al., 1997). Gunduz et al. (2002) later found the single dominant gene in 'PI 88788' was allelic to the non-Rsv1 gene in PI 486355, and both were non-allelic to *Rsv1* and *Rsv3*. The SMV resistance gene in cv. Peking is also at this locus.

Molecular genetics of Rsv genes

Rsv1 was mapped using RFLP and SSR markers to molecular linkage group (MLG) F in a cluster of other resistance genes (Yu et al., 1994). Considerable fine structure analysis of this genomic region, and response of recombinant inbred lines to SMV strains, has shown its considerable complexity (Jeong et al., 2001; Gore et al., 2002; Hayes et al., 2004). *Rsv3* mapped, in a cross between L29 and cv. Williams, to MLG B2, and was flanked by AFLP markers with the nearest, pA591, 0.9 cM away (Jeong et al., 2002). The *Rsv4* locus of PI 486355 maps to MLG D1b and is flanked by the microsatellite markers, Satt542 at 4.7 cM and Satt558 at 7.8 cM (Hayes et al., 2000).

Interaction of SMV strains with Rsv1 and alleles

On *Rsv1*-carrying soybeans, SMV causes either mosaic (S = susceptible), necrosis (N), or no symptoms (R = resistant), depending upon the allele (Table 1). No one allele of *Rsv1* gives resistance to all the strains of SMV. In the necrotic reactions induced on Marshall and Ogden by SMV G5 and G6, virus accumulation is limited and only necrotic veins occur on inoculated and uninoculated leaves. Necrosis is much more extensive, and often lethal, on Ogden inoculated with G3, York or 'Hutcheson' with G4, Kwanggyo with G5, and PI96983 with G7. In genetic studies, plants with necrotic reactions are classified as resistant even though the necrosis may be limited locally to a lesion, systemically to a few veins, or may be unlimited and cause tip necrosis and plant death. Heterozygous plants often exhibit necrosis, suggesting partial dominance. Virus accumulation and rate of movement are reduced in necrotic plants, compared to susceptible plants. Resistant plants having *Rsv1* alleles inoculated with SMV G1-G6 show no symptoms and have no detectable virus,

though retain the potential for a hypersensitive response-like mechanism of resistance (Hajimorad and Hill, 2001).

Table 1. Reaction of soybean genotypes to seven strain groups of *Soybean mosaic virus* (SMV)

Source	Gene	Symptom Induced by SMV Strain Group							Reference
		G1	G2	G3	G4	G5	G6	G7	
PI 96983	*Rsv1*	R[1]	R	R	R	R	R	N_L	Kiihl and Hartwig, 1979
Ogden	*Rsv1-t*	R	R	N_L	R	R	R	N_L	Chen et al., 1991
Marshall	*Rsv1-m*	R	N_V	N_V	R	R	N_V	R	Chen et al., 1991
York	*Rsv1-y*	R	R	R	N_L	S	S	S	Chen et al., 1991
Kwanggyo	*Rsv1-k*	R	R	R	R	N_L	N	N	Chen et al., 1991
PI 507389	*Rsv1-n*	N_L	N	S	S	N	N	S	Ma et al., 1994
LR1[2]	*Rsv1-s*	R	R	R	R	N	N	R	Ma et al., 1995
Raiden	*Rsv1-r*	R	R	R	R	N	N	R	Chen et al., 2001
Suweon 97	*Rsv1-sk*	R	R	R	R	R	R	R	Chen et al., 2002
OX686[3]	*Rsv3*	N_L	-	-	N_L	-	-	R	Buzzell and Tu, 1989
Harosoy	*Rsv3-hs*	S	S	S	S	R	R	R	Gunduz et al., 2001
L29[4]	*Rsv3-hd*	S	S	S	S	R	R	R	Buss et al., 1999
LR2[2]	*Rsv4*[5]	R	R	R	R	R	R	R	Ma et al., 1995
Peking	*Rsv4*	R	R	R	R	R	R	R	Gunduz et al., 2004
PI 88788	*Rsv4*	R	R	R	R	R	R	R	Gunduz et al., 2004

[1] R = resistant symptomless; S = susceptible (systemic mosaic symptoms); N = necrotidpcal and systemic; N = limited veinal necrosis; N = extensive tip necrosis, near lethal by 914 days after inoculation.

[2] LR1 and LR2 are derived from cv. Essex x PI 486355

[3] OX 686 is derived from cv. Columbia (*Rsv3* + *Rsv4*) x cv. Harosoy

[4] L29 is a cv. Williams BC$_6$ isoline with SMV resistance derived from cv. Hardee

[5] For *Rsv4* lines, no symptoms develop, but some local infection foci develop which can be detected serologica (Fayad and Tolin, 2003b)

Interactions of SMV strains with Rsv3 and Rsv4

The response of cultivars carrying *Rsv3* alone to SMV strains is either S or R, with some local necrosis, depending on the allele and strain. Inoculation of L29 with SMV-G7 induced a few infection foci detectable in leaf immunoprints, whereas SMV-G1 induced a severe chlorotic mosaic. In contrast, *Rsv4* appears to provide resistance (R) to all seven strains by restricting virus accumulation and limiting virus movement to cells immediately surrounding the infection site (Fayad and Tolin, 2003b; Gunduz et al., 2004). In heterozygous plants with *Rsv4*, a unique late susceptible (LS) phenotype develops no sooner than 4 weeks after inoculation. Virus movement into vascular tissue is delayed. Chlorotic symptoms and virus accumulation are limited to cells near vascular tissue in uninoculated trifoliolate leaves. Cultivars such Columbia and PI 486355 which naturally carry multiple R genes were resistant to all SMV strains.

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Economical and environmental benefits of inoculation and biological nitrogen fixation with the soybean: situation in South America

M. Hungria and R. J. Campo[1]

Abstract

Biological nitrogen fixation is a key process allowing the supply of this nutrient to many plants belonging to the family Leguminosae. One main example of an efficient symbiosis is that between the soybean and rhizobial strains belonging to the species of *Bradyrhizobium japonicum* and *B. elkanii*, that can supply up to 95% of the crops needs for nitrogen. Rhizobial inoculants have been produced for more than 110 years, and quality of the products commercialized in South America has considerably improved over the last decade. The success of the biological process with the soybean crop in South America results from breeding programs searching for both plant and *Bradyrhizobium* genotypes with higher capacity of N_2 fixation, such that today higher yields are obtained in the total absence of N fertilizers. The importance of this approach is shown when we consider that in both China, considered as the genetic center of soybean, and in USA, where the low cost of N fertilizers does not stimulate the search for efficient symbiotic partners, the rates of N_2 fixation are far below from those reported in South America. In South America increases in yield due to inoculation are verified even in soils with a high population of soybean bradyrhizobia, thus the commercialization of inoculants reaches about 25 million doses per year in the main producing countries. The economy related to the biological process with soybean grown in Brazil is estimated at U$ 2.87 billion per year, and similar results are reported for Argentina, therefore growers need both to maintain their focus on N_2 fixation as an economic N source for this crop, and to ensure that land quality is maintained or improved by continued study of soil quality and the factors affecting it.

Biological N_2 fixation: a key process for agricultural sustainability

Meeting food needs worldwide has often led to the adoption of agricultural practices that can degrade the soil, and to the use of land that is marginal for crop production. Thus progressive soil chemical, physical and biological degradation is a major factor affecting crop sustainability. The situation is most serious in the tropics, due to the fragile soil structure, erosion, low

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soil organic matter and inappropriate farming technologies and in those areas nutrient depletion may be accentuated by the high cost of fertilizers, especially nitrogen (N) sources, the majority of which are imported (Cassman, 1999; Hungria & Vargas, 2000; Giller, 2001).

The Leguminosae (Fabaceae in the USA) is one of the largest families of plants, with over 18,000 species classified into around 650 genera, representing approximately a twelfth of all known flowering plants, occupying nearly all terrestrial biomes and including important grain, pasture and agroforestry species (Polhill & Raven, 1981). Many of those species can establish symbioses with a group of bacteria collectively known as rhizobia, in which the bacteria derive energy from the host for growth and N_2 fixation, and are protected from external stresses inside typical structures known as nodules, while the host accesses a form of N it could not otherwise utilize. Worldwide some 40 to 60 million metric tons (Mt) of N_2 are fixed by agriculturally important legumes annually, with another 3 to 5 million Mt fixed by legumes in natural ecosystems, providing nearly half of all the N used in agriculture. However, increases in the overall need for N in agriculture are projected for the following three decades, and could lead to significantly enhanced environmental damage, by nitrate contamination of groundwater, or by greenhouse effect. Reduced dependence on N fertilizer, and farming practices that favor the more economically viable and environmentally prudent N_2 fixation will benefit both agriculture and the environment (Smil, 1999; Graham & Vance, 2003; Hungria et al., 2004b).

The use of legumes in agriculture dates back from a long time, *e.g.*, there are reports of use of domestication of lentils (*Lens esculenta*) in Iran some 9,500 to 8,000 BC, and reports of domestication of soybean in the Shang dynasty (1,700-1,100 BC) in China (Hymowitz & Singh, 1987; Graham & Vance, 2003). Indeed, the benefits of the N_2-fixation process were recognized hundreds of years before the biological process was elucidated. The Romans developed the idea of crop rotation with legumes and non-legume plants to improved soil "health" and quality. However, only in 1813 the interest in chemistry of Sir Humphrey Davy led him to report that the legumes "seemed to prepare the ground for wheat" and speculated that the N came from the atmosphere. In 1838, Boussingault, a French agricultural chemist showed in a series of experiments that legumes had higher N levels than cereals and concluded that the atmosphere was the source of this N. Unfortunately, in 1848, Liebig, an eminent chemist with a high reputation, argued that the source of N was ammonia and not the atmosphere. Finally, in 1886 two German scientists, Hellriegel and Wilfarth demonstrated that the ability of legumes to convert N_2 from the atmosphere into compounds which could be used by the plant was due to the presence

of swellings or nodules on the legume root, and to the presence of particular bacteria within these nodules. The first rhizobia were isolated from nodules soon after, in 1888, by the dutch microbiologist Martinus Beijerinck, and shown to have the ability to reinfect their legume hosts, and to fix N_2 in symbiosis (Fred et al., 1932; Cooper, 2003; Hungria et al., 2004b).

Inoculants and inoculation

The practice of transferring soil from a field where legumes have been grown to new areas being planted to the same crop was known long before the description of the N_2 fixation process. Inoculation was thus a common practice and one example was the recommendation in England of transferring soil from established pastures of alfafa (*Medicago sativa*) to new areas. Soon after the isolation of the first rhizobia from nodules by Beijerinck, two other German scientists, Nobbe and Hiltner, demonstrated, in 1890, the advantages of adding pure bacteria with the seed at planting (Voelcker, 1896; Fred et al., 1932). In 1896 those two scientists submitted the first patent for pure cultures of rhizobia and for artificial inoculation, describing the use of pure cultures of the desired *Rhizobium* grown in flat glass bottles containing only a small amount of gelatin medium. This led to the beginning of the commercial inoculant industry and the establishment of the first inoculant company, Nitragin, in 1898, in USA (Smith, 1992). Progresses were achieved in the inoculant industry in the following decades and bacteria carriers for a large scale production had to be found.

In general, desirable properties of a good inoculant carrier can be summarized as: 1) readily available, uniform in composition and cheap in price; 2) non toxic to the rhizobia; 3) high water retaining capacity; 4) easily sterilized; 5) readily corrected to a final pH of 6.5 to 7.3; 6) permitting good initial growth of the target organism, and the maintenance of high cell numbers during storage (Hungria et al., 2004b). Peat has been the most suitable carrier for inoculant production, since it usually meets these requirements, but different sources vary in their capacity to support rhizobial multiplication and survival (Roughley, 1970; Balatti and Freire, 1996; Hungria et al., 2004b). Among the best sources are peats from Argentina (Tierra del Fuego) and Canada, each with an organic matter content of 40-50%, but for other sources the quality of the peat can be improved by addition of humus. Before processing, peat must be drained, dried, ground, the pH should be corrected to 6.5-7.0 and sterilized. Sterilization is a difficult andt key step, because a sterile carrier allows a high concentration of rhizobial cells; furthermore, it reduces the frequency and level of contamination, and thus the risk of introducing and disseminating plant, animal and human pathogens (Lupwayi et al., 2000; Catroux et al., 2001; Hungria et al., 2004b).

Unfortunately there are still products manufactured with non-sterile peat, which may contain up to 1,000-fold fewer rhizobia than those made with sterilized peat, reducing shelf life. In countries with high-quality products, as Australia, Canada, Czechoslovakia, France, The Netherlands, New Zealand and South Africa, sterile products are the general rule or are mandated by legislation. Argentina and Uruguay have been in that list of high quality inoculants and in the last three years the legislation in Brazil has established that inoculants must be free of contaminants at the 10^{-5} dilution. As those are the three countries producing inoculants in South America, we may consider that the quality of the commercialized products has improved.

However, it is not always possible to use peat as a carrier. Many soybean farmers, for instance, complain that peat-based products are time-consuming to use, especially when planting large areas, and some countries may not have natural deposits of peat, or the peat available may not be suitable. A number of materials fitting the characteristics of a good carrier have been alternatively used with different degrees of success, including vegetable oils, mineral oils, plant materials such as bagasse, silk cocoon waste, sawdust, rice husk, corncob, various clays including vermiculite, perlite mixed with humus, diatomaceous earth, lignite and derivatives, coal, filter mud and charcoal-bentonite, among others (Hungria et al., 2004b). However, in South America, the only alternative carriers used in large scale are liquid or gel-based preparations. Those inoculants show varied composition (magnesium silicate, potassium acrylate-acrylamide, grafted starch, hydroxyethyl cellulose products) and usually include additional proprietary substances to protect the rhizobia. Several studies report good performance of these preparations when compared to peat-treated seeds, however, in spite of good cell numbers in plate and most probable number (MPN) counts, many of them have failed to reproduce this performance under field conditions, especially under environmental stressing conditions, normally found at planting times in South America (Campo & Hungria, 2000b; Hungria et al., 2001, 2004b). The situation is more critical in new areas without established rhizobial populations, as shown in Figure 1. The performance of liquid inoculants is even more critical when the seeds are treated with fungicides and micronutrients (Campo & Hungria, 2000a,b; Hungria et al., 2001, 2002). However, as soybean are cropped in large areas in Brazil, Argentina and Paraguay, the increasing preference of the farmers for liquid inoculants is evident, as shown in Figure 2. One must consider that some of the farms are more than 10,000 ha and that the sowing period is very short, thus the facilities of using liquid products are a main characteristic considered by the farmer.

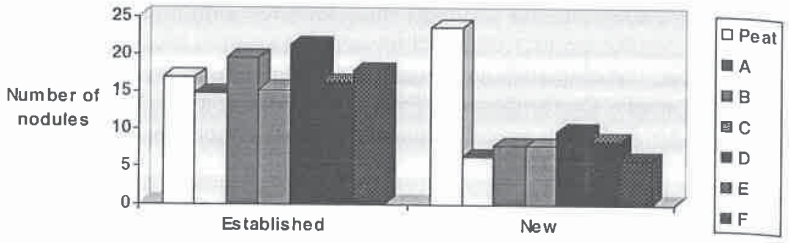


Figure 1. Effects of the inoculant carrier on nodule number (n°/plant) of soybean plants at 30 days after emergence. Inoculation was adjusted for the same concentration of cells per seed, with peat inoculant or with six commercial liquid inoculants. Experiments performed in Brazil, in a soil with established population of soybean bradyrhizobia (10^5 cells/g of soil), where treatments did not show statistical differences, or in a new area void of bradyrhizobia, where the nodulation was significantly lower (Duncan, $p \leq 0.05$) with the liquid inoculants.

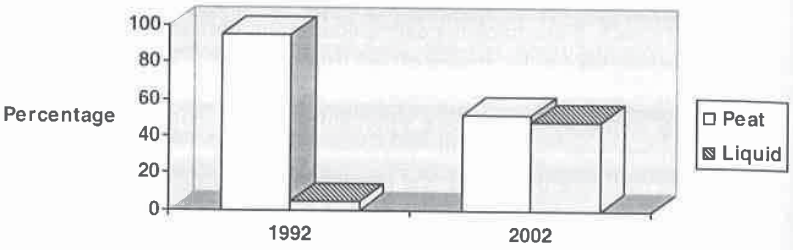


Figure 2. Percentage of peat and liquid inoculants for the soybean crop commercialized in Brazil. In 2002, of a total of 14 million doses.

Soybean inoculation and N_2 fixation in South America

Soybean was introduced in South America at the end of the 19th century, but crop expansion started in the 1960s in Brazil, and in the 1970s in Argentina and Paraguay. Crop expansion reached such magnitude that Brazil, Argentina and Paraguay are today responsible for about 45% of world soybean production with a significant increase over the last few years, whereas that from the USA has declined from 47% of world production in 1999/00, to 39% in 2001/02 (Hungria et al., 2004a).

The historical reports in South America are that inoculation was needed to guarantee nodulation and N_2 fixation. Indeed, in Brazil, careful studies have shown that the soils are void of rhizobial strains able to establish an effective

symbiosis with the soybean (Ferreira & Hungria, 2002). Strains were thus brought from foreigner countries in the 1960s and 1970s, but the performance with local cultivars was evaluated, searching for those more effective for the environmental and genotypic conditions. Furthermore, in Brazil and Argentina selection of parental genotypes active in N_2 fixation was always emphasized. As a result, efficient and competitive strains are used in commercial inoculants, *e.g.*, In Brazil there are four recommended strains [*Bradyrhizobium japonicum* strains SEMIA 5079 (=CPAC 15) and SEMIA 5080 (=CPAC 7) and *B. elkanii* strains SEMIA 587 and SEMIA 5019], and each can fulfill the crops need for N at yields greater than 4,000 kg (Vargas & Hungria, 1997; Hungria & Vargas, 2000; Hungria et al., 2001, 2004a). However as the national mean yield for soybean has increased from 1,166 kg/ha in 1968/69 to 2,765 kg/ha in 2002/2003, crop demand for N has also increased. (Hungria et al., 2004a). Furthermore, more than 90% of the areas cropped to soybean today have been previously inoculated, and have established bradyrhizobial populations of at least 10^3 cells g^{-1} of soil. Nowadays, the reinoculation with the four strains used in commercial inoculants results in increases in nodulation, N_2 fixation rates and yield, as shown in Table 1. Additionally, reinoculation is also important for replacing persistent strains with more efficient ones, as has been recently demonstrated from studies with strains belonging to serogroup of *B.japonicum* SEMIA 566 in Brazil (Mendes et al., 2000). In Argentina there are also strains very effective for local cultivars, with an emphasis on E109 (=SEMIA 5085, =USDA 138), while in Paraguay and Bolivia the inoculants usually carry the strains used in Brazil or in Argentina.

Table 1. Mean and maximum percentage increases in yield (kg/ha) and total N in grains (kg N/ha) due to the inoculation with the combination of strains *Bradyrhizobium elkanii* SEMIA 587 and *B. japonicum* CPAC 7 (=SEMIA 5080), when compared to the non-inoculated control. The increases were obtained in thirteen experiments performed in two Brazilian Regions, in soils with established population of soybean bradyrhizobia[1,2].

Region	Grain yield (% increase)		Total N in grains (% increase)	
	Mean	Maximum	Mean	Maximum
Central West	7.8	23	8.1	25
South	3.8	20	4.3	24

[1]After Hungria et al. (2000).
[2]Each experiment was performed with four to six replicates and after the multivariate analysis the data presented in this table were statistically significant (Duncan, $p \leq 0.05$).

However, more productive cultivars, soils with establish populations of soybean bradyrhizobia, and new technologies establish imply in the need for a continuous search of more efficient and competitive strains (Vargas & Hungria, 1997; Hungria & Vargas, 2000; Hungria et al., 2001). Unkovich & Pate (2000) and Giller (2001) suggest a potential for N_2 fixation of soybean of 360-450 kg of N/ha, levels that would allow yields of up to 5,500 kg/ha, thus the perspectives to reach higher yields without applying N fertilizer are very positive. There are few studies about quantification of N_2 fixation under field conditions in South America, and in a recent survey of data obtained in Brazil using ^{15}N techniques the contribution of N_2 fixation rates ranged from 72 to 94% of the total N accumulated by soybean plants (Hungria et al., 2004a). Those rates are appreciably higher than the results achieved in 33 commercial soybean fields in Australia, in which the percentage of N derived from fixation was estimated at 53% (Unkovich & Pate, 2000).

As soybean represents a high profit crop, there is increasing pressure for farmers to purchase N fertilizer. However, starter N doses as low as 20-40 kg of N/ha may decrease nodulation and N_2 fixation under Brazilian conditions, with no benefits to yield (Vargas & Hungria, 1997; Crispino et al., 2001; Hungria et al., 2001; Mendes et al., 2003). Indeed, in more than 50 experiments where inoculation and fertilization with 200 kg of N/ha have been compared (split application of N at sowing and flowering), no increase in yield due to N-fertilizer use has been reported. Similarly there were no benefits when N fertilizer was applied at a rate of 400 kg N/ha, split across ten application times. Finally, no benefits were observed also in several experiments when 50 kg of N/ha were applied at R2 or R4 (Hungria et al., 2001; Crispino et al., 2001; Loureiro et al., 2001). It is amazing when we consider that in northern and central China, center of genetic origin of soybean, N_2 fixation alone cannot meet the N requirement for maximizing yield (Gan et al., 2003). In those areas the best results were obtained with top-dressing of N fertilizer (50 kg/ha) at stages V4, V6 or R1. (Gan et al., 2002, 2003). As pointed by these authors, often the inefficient use of N fertilizer results in high costs of soybean production, and the reported yields are lower than those found in South America. Moreover, van Kessel & Hartley (2000) reported that for trials undertaken mainly in the USA and Australia the percentage of N derived from fixation has declined from 65% to only 54% since 1985, and the authors attribute that to a higher use of N fertilizers, decreasing the capacity of N_2 fixation. Such data emphasize the importance of the management that has been taken place in South America, maximizing the contribution of the biological process.

Economical return of soybean inoculation

Economic returns of inoculation and biological N_2 fixation with soybean in South America are outstanding. Brazil is used here as an example. For each

1,000 kg of soybean produced, soybean will require approximately 80 kg of N (65 kg allocated to seeds having ~40% protein; 15 kg N left in roots, stems and leaves). Considering the national mean yield in Brazil in 2002/2003 of 2,794 kg/ha, N requirements would be slightly more than 220 kg/ha of N. Where this is supplied through N_2 fixation, N losses are minimal. In contrast, the N-use efficiency of N-fertilized plants in the tropics is rarely as much as 60%, meaning that such plants would require ~360 kg N or 800 kg of urea (45% of N) to achieve equivalent yields. At US$ 0.20/kg of urea, the cost of applying N fertilizer would be at least US$ 160/ha, or in excess of US$ 2.87 billion nationally (17.95 million ha). As Brazilian transportation costs are very high, but total production costs are about 40% lower than in the USA, it is evident that biological N_2 fixation plays a key role in lowering Brazilian production costs. It is also important to emphasize that the application of only 30 kg of N/ha would imply a cost of US$ 13.3/ha to the farmer, and of US$ 239 million to the country per growing season, certainly decreasing competitiveness of the national product (Hungria et al., 2004a).

The success of N_2 fixation and the positive results obtained with inoculation and reinoculation result in an increasing use of inoculants by the farmers, as shown in Figure 3. In Brazil and Argentina it is estimated that reinoculation is practiced by about 60% and 50% of the farmers, respectively. In contrast, in USA the use of inoculants is a common practice in just 15% of the soybean production area (Hungria et al., 2004a). Therefore soybean growers in South America need both to maintain their focus on N_2 fixation as an economic N source for this crop, and to ensure that land quality is maintained or improved by continued study of soil quality and the factors affecting it.

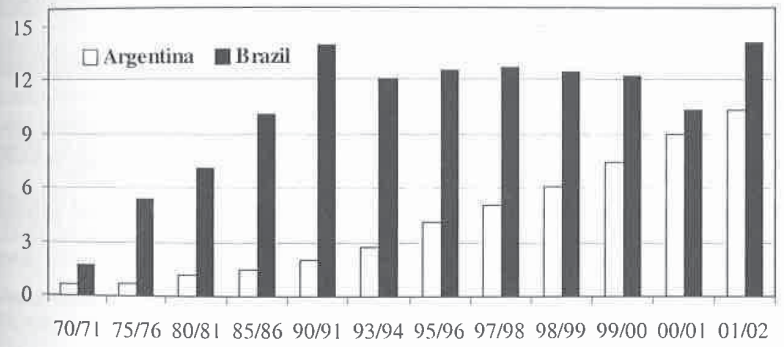


Figure 3. Units of soybean inoculant (x 10^6) sold in Argentina and Brazil (Dr. Enrique R. Moretti, Laboratórios BIAGRO, Dr. Solon Araújo, ANPI and Dr. Laura Machado Ramos, Ministério da Agricultura, Pecuária e Abastecimento, personal communication).

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Competition an old unsolved problem of rhizobia nodulating soybean

P. A. Balatti[1]

Introduction

This revision does not intend to be a thorough review about competition. Instead the idea was to raise some questions about the phenomenon of competition and to think how research can help to solve the problems farmers are confronted to every time they cultivate soybean.

Soybean is a native plant from Asia whose place of origin and domestication was North and Central China (Hymowitz, 1970). Soybean, like other legumes, establishes a symbiotic association with gram-negative soil bacteria. As a result of the interaction the plant develops nodules, which are the laboratories where rhizobia transform molecular nitrogen into nitrogenous compounds that are taken up by the host plant.

At the beginning of the twentieth century soybean was known in Europe as an exotic food plant from the orient (Hymowitz 1970) and its production was mostly confined to China, Indonesia, Japan and Korea. In the late's 1940 and early 1950's the US became the most important soybean producer worldwide. By 1968 approximately 28 million Ha of soybean were sown in nearly 25 countries (Hymowitz, 1970). It has been assumed that soybean materials and symbiotically compatible mycrosymbionts most probably coevolved within different areas of Asia, which is the center of origin of the soybean-rhizobia symbiosis. Furthermore, the introduction and proliferation of rhizobia that may naturalize, in new environments, might have been due to the release of viable rhizobia from decaying nodules and to the survival ability of these organisms (Garman H. and M. Didlake. 1914, Kuykendall, 1989).

The cultivation of soybean in new soils around the world requires the addition to the seed of a bacterial suspension of highly effective rhizobia, what is known as inoculation. In order to successfully inoculate soybean, the introduced strain must be competitive, that is it should compete against soil microorganisms and also against less effective resident rhizobia, a process that is under the influence of the environment.

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The word competition, according to the Oxford American Dictionary, means **"a friendly contest in which organisms try to do better than their rivals"** and rival is **"an organism that can equal another in quality"**. In ecological terms, two especies are competing when they are using the same resource so that any advantage that a species has over another one, over time, will lead to the elimination of the other one, being competence a destabilizing relationship (Margalef, 1977) Although competition between rhizobia soybean has been the concern of scientists for many many years, the molecular basis of it still remain obscure.

Fast and slow growing organisms induce nitrogen-fixing nodules on soybean

Until 1980, *Bradyrhizobium japonicum* was considered the sole symbiont of soybean (Fred et al., 1932). However, the isolation of rhizobia from new areas around the world and the availability of molecular techniques allowed researchers to identify and or to classify already known rhizobia as new rhizobial symbionts. Among the genus *Bradyrhizobium*, three different species have been described two slow growing and one extra slow growing rhizobia, *Bradyrhizobium japonicum* (Fred et al., 1932) *Bradyrhizobium elkanii* (Kuykendal et al., 1992) and *Bradyrhizobium liaoningense* (Xu et al., 1995), respectively. Beginning in 1980, several researchers also reported the isolation of fast growing organisms that developed nitrogen fixing nodules in soybean. *Rhizobium* sp. NGR234 (Trinick et al., 1980; Saldaña et al., 2003) *Sinorhizobium fredii* (Keyser et al., 1982), *Sinorhizobium xinjiangensis* (Chen et al., 1988; Peng et al., 2002) *and Mesorhizobium thianshanense* (Chen et al., 1995). All these fast and slow growing rhizobia interact with soybean in a different manner (Saldaña et al., 2003). It appears that the scenario for rhizobia-soybean interaction is more complex than already thought, since several species that differ among other things in their growth rate, presence of symbiotic plasmids, etc., might be competing for the colonization, infection and nodulation of the legume root. In addition, it has already been described that inefficient rhizobia might also compete with rhizobia for nodulation (Diatloff and Brockwell, 1976; Cregan et al., 1989; Lohrke et al., 1995). An extreme situation is nodulation blocking (Dowling et al., 1988). Balatti and Pueppke (1993) described nodulation blocking on soybean, that is a non-nodulating wild type strain of *S. fredii* inhibits nodulation by a mutant that develops nitrogen fixing nodules on soybean. In addition to this, the soils also host non-nodulating rhizobia though their effect on rhizobia survival and competitive ability remain to be determined.

Description of the legume-rhizobia interaction

The legume-rhizobia symbiosis is most probably the symbiotic system that has been studied in more detail. Many researchers around the world

contributed in one way or another to the actual knowledge of the symbiosis. Plants signals, the flavonoids, interact with a regulatory protein NodD. This interaction turn on the expression of common nodulation genes and host specificity genes, conducing this to the synthesis of nod factors, compounds with hormone like activity that induce root hair deformation and curling and nodulation. Infection threads grow by invagination of the root hair cell wall and membrane and carry rhizobia into the root cortex, where they are released close to the meristem of the developing nodule and infect nodule cells. Then, nitrogen fixation genes are expressed and as a result of this nitrogen fixation begins (Balatti, 1994). The alteration of any of the bacterial or host components that contribute to the development of nodules on legumes might change the organism ability to infect and nodulate efficiently. Although most of the molecular basis of the processes already mentioned are known, the most important technical problem of legume inoculation that is to succeed in inducing nodulation of soybean by the highly effective strain introduced through the inoculant, remains to be resolved. The reason is that competition is the result of a complex interaction between the bacterial and host plant genome and the effect of the environment.

The genome of the host contribute to the Bradyrhizobium-soybean interaction

Plant responses to rhizobium infection are diverse. While some soybean cultivars are fully incompatible with rhizobia others might exclude or restrict nodulation by bacteria belonging to serogroups of *Bradyrhizobium japonicum* or they also might be highly promiscuous like soybean cultivar Mambloxi.

Several genes controlling soybean response to inoculation with bradyrhizobia have been described. The rj_1 allele (Caldwwell, 1966) conditions a restriction of nodulation with a broad range of rhizobia. Other genes like Rj_2 (Caldwell, 1966), Rj_3 (Vest, 1970), Rj_4 (Vest and Caldwell, 1972), Rj_5 and Rj_6 (Pracht et al., 1993) restrict nodulation with a defined member or serogroups of bradyrhizobia. Therefore, the background of the host genome has a strong influence in the future structure of the rhizobia soil population, since the cultivar might not only be restricting highly effective nitrogen-fixing rhizobia but it might also be releasing rhizobia from senescent nodules. It has already been demonstrated that rhizobia are able to reproduce from crushed nodules of soybean (Zhou et al., 1985). Mutants that fail to infect legumes presumably might arise frequently but it is unlikely that they displace symbiotic rhizobia in soils where legumes are grown. Therefore rhizobia that infect legumes often leave more progeny than mutants that only colonize the rhizosphere.

The impact of the compatibility of the host genome with symbiotic bacteria was evidenced by several studies related with isolation of new rhizobia,

Cregan et al (1989) found a positive correlation between restricted nodulation and reduced competitiveness. In 1982 Keyser et al., reported the isolation of fast growing rhizobia that develop nitrogen-fixing nodules in *Glycine soja* and an old Chinese cultivar Peking. Later, Dowdle and Bohlool (1985) while isolating new fast growing rhizobia from China found that in soils that has been under soybean cultivation fast growing rhizobia predominated over slow growing ones. Since several evidences suggest that growth rate is unrelated with the competitive ability of the isolates (Smith and Wollum, 1989; de O churie and Hungría, 1997; Bhagwat et al 1992), rhizobia predominance in soils might have been the result of highly stringent cultivar-strain interactions.Van Resburg et al (1983) and Balatti and Pueppke (1992) confirmed that soybean genotypes with Chinese cultivars in their background are mostly compatible with fast growing rhizobia, resulting in the development of nitrogen fixing nodules. Therefore, utilization of soybean cultivars with Chinese or African materials in their backgrounds might have enhanced their number compared to slow growing rhizobia.

In the US and other countries, soybeans were most probably introduced along with slow growing rhizobia and this might have resulted in the enhancement of the number of slow growing rhizobia (Garman and Didlake, 1914), through the release of organisms from senescent nodules, Indeed, Kuykendall (1989) confirmed that host plant nodulation plays a positive role in the establishment of populations of *B. japonicum* in soil. Damirgi et al (1967), Coutinho et al., 1999 and Chen et al 2002 evaluated the diversity of rhizobia inhabiting soils in the US, Brazil and Paraguay, respectively and found that US soils host competitive representatives of *B. japonicum.* In the soils of Brazil both slow and fast growing rhizobia were found and management practices were found to alter rhizobia population structure. In the soils of Paraguay among 78 isolates collected from 16 different sites, 58 were slow growing organisms and 20 were fast growing organisms though evidences suggest that they were not *S. fredii* strains. Martinez Alcántara et al (2002) isolated rhizobia from alkaline and acid soils from different areas in Argentina and by means of PCR reactions (Videira et al, 2002; Pastorino et al., 2003) found that among 20 isolates 18 were slow growing *Bradyrhizobium elkanii or B. japonicum* and only two were fast growing organisms. Therefore, it appears that in the eastern hemisphere including the place of origin of soybean, fast growing rhizobia outnumbered slow growing ones whereas in the western soils like USA and Latin America, slow growing rhizobia are naturalized. The fact that growth rate was found to be unrelated with the ability of rhizobia to colonize the rhizosphere (Jjemba and Alexander, 1999) and with the strain competitiveness, suggest that the number and serogroup of naturalized rhizobia in the soils are the result of the ability of the host to restrict nodulation. Furthermore, whether they

are fast or slow growing organisms they will be the organisms that will be challenged by the exotic strain of rhizobia introduced by inoculation.

Rhizobia are soil bacteria and they have to compete either with other rhizobia or soil microorganisms. Rhizobia compete not only for water and nutrients but they are also challenged by inhibitors synthesized by antagonistic microorganisms that may release antibiotic like compounds. However, Hicks and Loynachan, 1989 found that although the population of *B. japonicum* antagonistic organisms was greater than the number of stimulatory microorganisms, they were unrelated with the population of *B. japonicum*.

Rhizobial genes involved in competition

Genetic alterations of rhizobia may alter competitiveness. The presence of plasmids and/or multiple copies of a gene as well as the inactivation of genes involved in EPS (Zdor and Pueppke, 1991) or myo-inositol dehydrogenase synthesis (Jiang et al., 2001) resulted in organisms that differ in their competitive ability on soybean (Beattie and Handelsman, 1993). The inactivation of the myo-inositol dehydrogenase gene drastically affected both the ability of *S. fredii* to fix atmospheric nitrogen and the ability of rhizobia to compete for nodulation compared to the wild type strain.

Other genes have been directly related to competition like the nodule formation efficiency (*nfe)* genes identified in *Sinorhizobium meliloti* GR4. San Juan and Olivares (1989) identified a DNA fragment involved in nodulation formation and efficiency with three ORF *nfe*A, *nfe*B and *nfe*D genes (Soto et al., 1993) which are involved in the nodulation efficiency and competitiveness of this strain on alfalfa roots (San Juan and Olivares, 1989; García-Rodriguez and Toro, 2000). In 1988 Deshmane isolated a gene in *B. japonicum* USDA110 that appeared to be important for the ability of *B. japonicum* to efficiently nodulate soybean that has many features in common with *nfe* genes from *R. meliloti* and because of this was designated *nfe*C (Chun and Stacey, 1994).

Bradyrhizobium elkanii is a slow growing bacteria that nodulates soybean and is distinguished from *B. japonicum* because, among other things, it shows a high intrinsic antibiotic resistance, it synthesizes Indol Acetic Acid, does not have hydrogenase and produce rhizobitoxine (Kuykendall, 1992). Rhizobitoxine (2 amino-4- (2-amino-3-hydropropoxy)-trans-but-3-enoic acid) is a compound that provokes chlorosis in soybean due to it's translocation from root nodules (La Favre et al., 1988). A correlation was noted between rhizobitoxine production and the ability of *B. elkanii* to infect soybean c.v. Clark rj1 rj1. Later, it was found that rhizobitoxine act as an inhibitor of

ethylene synthesis and because of thist it enhances nodulation. Yuhashi et al. (2000) and Okasaki et al. (2003) found that *B. elkanii* exhibited a greater competitiveness than *B. japonicum* on *Macroptilum atropurpur*eum roots. Furthermore, they also found that *B. elkanii* lacking the ability to synthesize rhizobitoxine were impaired in their ability to compete with *B . japonicum*. Based on these findings they suggested that rhizobitoxine production was correlated with nodulation and the competitive ability of the strain.

Other genes related with motility, bacterial adhesion, bacterial cell surface, etc have been found to contribute to nodulation competitiveness (Tripplet, 1990). However, the ecological implicancies of releasing recombinant bacteria in soils, suggest that it is very unlikely that mutations or recombinant bacteria could be used as a tool to generate more competitive organisms against naturalized or native organisms. Furthermore, Beattie and Handelsman (1993) found that genetic changes that increase competitiveness may occur spontaneously in soils, suggesting that strain selection is probably the best alternative to obtain highly competitive nitrogen fixing symbionts of soybean.

Conclusions

The most dramatic effect competition has is that efficient nitrogen fixing rhizobia which are added through the application of an inoculant most often fail to induce most the nodules developed by the host the plant. Even if rhizobia are inoculated at concentrations three times higher than the native populations, only 50% of the nodules are expected to be induced by the inoculated rhizobia (Weaver and Frederick, 1974). How can we help inoculant producers and the farmers to achieve a more successful inoculation?

It is possible that by knowing the identity and number of native rhizobia in the soil, farmers may succeed in introducing an efficient nitrogen fixing strain either by altering the number of viable rhizobial cells applied and/or by using soybean cultivars that may restrict nodulation of native strains but not of the inoculated nitrogen fixing strain. Therefore, governments should support not only basic studies to learn about the molecular basis of competition but also projects aiming at building maps of the soils of the country with a description of the number and species of rhizobia present, whether they were cultivated with soybean before or not. This means that if there is going to be a competence between native or naturalized bacteria and an exotic strain, the number of the former ones and their characteristics should be known in advance in order to establish the best strategy to achieve nodulation by the exotic strain of rhizobia. In addition, today practices in the field recommend not only to inoculate soybean seed but

also to treat it with fungicides and agrochemicals, compounds that by their toxic efect upon rhizobia may decrease the numbers of viable cells that are left on the seed surface. Hynes et al 2001 found that rhizobia hardly survive on the surface of the seed for a long time due to the effect of water stress and fungicides may contribute even further to bacterial cell death. Therefore, more studies concerning the use of technology and its compatibility with nitrogen fixing rhizobia should be carried out. Furthermore, the advent of molecular biology provided researchers with more accurate and informative tools to study the ecology of rhizobia in the soil and this should give answers to many old unsolved problems like rhizobia survival and competitiveness.

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Bradyrhizobium japonicum genome sequence: will this information help solve field problems?

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Introduction

Bacteria of the genera *Bradyrhizobium, Sinorhizobium, Mesorhizobium,* and *Rhizobium* (collectively rhizobia) are all members of the family Rhizobiaceae. These bacteria have the ability to infect legume roots, induce the development of a novel organ (nodule) and establish a nitrogen-fixing symbiosis. The steps involved in rhizobial infection of legume roots are complex and have been extensively discussed in other reviews (e.g., Cohn et al., 1998). Suffice it to say here that establishment of the symbiosis requires close coordination between the host and symbiont that is mediated by the exchange of diffusible chemical cues that result in gene expression essential for nodulation. A systems biology approach to understanding these processes requires a full knowledge of the bacterial and plant genome. Ongoing projects are now sequencing the gene-rich regions of two model legume species, *Medicago truncatula* and *Lotus japonicus* (reviewed in Young et al., 2003*)*. However, three complete genome sequences have been obtained for *Mesorhizobium loti* (symbiont of *L. japonicus;* Kaneko et al., 2001), *Sinorhizobium meliloti* (symbiont of *M. truncatula;* Galibert et al., 2001) and, most recently, *Bradyrhizobium japonicum* (symbiont of soybean; Kaneko et al., 2002). The latter is the focus of this review and is clearly the genome of most agronomic importance since its host, soybean, is now the most important leguminous crop and the world's foremost provider of protein and oil (Statistics, American Soybean Association). Bacteria within the Rhizobiaceae family (i.e., rhizobia and *Agrobacterium* spp.) are members of the á-proteobacteria phylogenetic group. Over the past few years, the complete genome sequences of several bacteria in this group have been elucidated. These include *A. tumefaciens, Rickettsia prowazekii, Brucella suis, Brucella melitensis, Caulobacter crescentus,* the above-mentioned rhizobial bacteria, and others (Andersson et al., 1998; DelVecchio et al., 2002; Goodner et al., 2001; Nierman et al., 2001;; Ogata et al., 2001; Paulsen et al., 2002; Wood et al., 2001). Thus, a significant amount of genomic information is available for this group of bacteria, allowing interesting comparisons.

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Summary of the *B. japonicum* genome.

The most recent member of the á-proteobacteria to be completely sequenced is *B. japonicum* strain USDA110 (Kaneko et al., 2002). Unlike many á-proteobacteria where multiple replicons are common, this bacterium has a single, circular chromosome of 9,105,828 bp predicted to encode 8,317 proteins.

All rhizobia contain nodulation (*nod, nol, noe*) genes, whose major function is to synthesize the lipo-chitooligosaccharide (LCO) Nod signal (Cohn et al., 1998). The Nod signal when recognized by the legume root induces many of the early plant responses necessary for nodulation. In *S. meliloti*, these *nod* genes are found on a megaplasmid (pSymA, 1.35 Mb; Barnett et al., 2001). However, in both *M. loti* and *B. japonicum*, the *nod* genes are encoded on the chromosome. Sullivan and Ronson (1998) found that the *nod* genes of *M. loti* were located on a 500 Kb segment of the genome, a symbiotic island that likely resulted from horizontal transfer and subsequent insertion into a phe-tRNA gene. The complete *B. japonicum* genome sequence reveals a symbiotic island of 681 Kb encoding 655 genes (Kaneko et al., 2002). Similar to the case in *M. loti,* the end of this symbiotic region terminates in a val-tRNA gene. Insertions into tRNA genes in the *B. japonicum* genome are common with no less than 14 occurrences with insertions of 4-97 Kb, resulting in each case in a duplication of tRNA sequences. This indicates a significant plasticity of the *B. japonicum* genome. This is also supported by the occurrence of 167 transpose genes, 104 of which are associated with insertion sequences.

Genome evolution in the á-proteobacteria.

Although *B. japonicum* has only one, albeit quite large, chromosome, a notable feature of the á-proteobacteria is the presence of more than one chromosome. For example, in *B. melitensis* such house-keeping functions as DNA replication, transcription, translation, core metabolism and cell wall biosynthesis are distributed on both chromosomes (DelVecchio et al., 2002). It is also interesting to note that genome size increases as one moves from obligate intracellular pathogen (i.e., *Rickettsia*), to facultative animal pathogen (i.e., *Brucella*), to free-living bacterium (e.g., *Caulobacter*), to facultative plant symbiont (e.g., *B. japonicum*). The genomes of *R. prowazekii* and *R. conorii* show clear signs of evolutionary degeneration with genome sizes of only 1.1 Mbp and 1.3 Mbp, encoding a predicted 834 and 1300 proteins, respectively (Andersson et al., 1998; Ogata et al., 2001). In contrast to these obligate intracellular pathogens, the genomes of other á-proteobacteria have clearly expanded in size. Thus, there appears to be two, opposing evolutionary pathways for the á-proteobacteria. One, exemplied by the *Rickettsia*, with perhaps *Brucella* as intermediate, is a

lineage of genome degeneration exemplified by biased loss of regulatory and biosynthetic genes. The other lineage, exemplified by *B. japonicum*, is exemplified by sequence acquisition by gene duplication and horizontal transfer, likely selected for by the need for environmental adaptation. For example, the size of protein families generally increases as genome size increases. For example, there are 67 predicted LysR regulators and 86 two-component response regulators in the *B. japonicum* genome.

A case study: *B. japonicum* polysaccharide synthesis.

The surface polysaccharides (SPSs) of rhizobia consist of extracellular, capsular, and lipopolysaccharides (EPSs, CPSs, and LPSs). Depending on the rhizobial-legume system, some or all of these SPSs have important functions in establishing an effective symbiosis (Carlson et al., 1999; Kannenberg et al., 1998). From the *B. japonicum* genome sequence, it is possible to locate the genes and/or gene regions responsible for the synthesis of the SPSs such as the EPS and both the lipid A and polysaccharide portions of the LPS.

The biosynthetic pathway of the lipid A portion of the LPS for enteric gram-negative bacteria as well as for *R. leguminosarum* has been worked out and recently reviewed (Raetz and Whitfield, 2002). Using the known sequences of the genes in *E. coli* or *R. leguminosarum* which encode these biosynthetic steps, the *B. japonicum* genes predicted to convert UDP-GlcNAc into the lipid A precursor, Kdo_2Lipid-IV$_A$ include those that encode for LpxA (UDP-GlcNAc acyl transferase, ORF bll4849), LpxC (UDP-acylGlcNAc de-N-acetylase, bll6595), LpxD (UDP-acylGlcN *N*-acyltransferase, bll4852), LpxB (the disaccharide synthase, bll4847), LpxK (the 4'-kinase, bll7514), and WaaA (the kdo transferase, bll7515). *lpxA* (bll4849), *lpxB* (bll4847), and *lpxD* (bll4852) are quite close to one another. The lipid-A of *B. japonicum* LPS has a disaccharide of 2,3-diaminoglucose rather than *N*-acetylglucosamine as is the case for enteric lipid-A (Mayer et al., 1989). The genes encoding the 4'-kinase and Kdo transferase, *lpxK* and *waaA* (bll7514 and bll7515), respectively, are adjacent to one another, while that encoding the de-*N*-acetylase, *lpxC* (bll6595), is not located near the other lipid-A biosynthsis genes. A unique feature of the lipid-A of rhizobia is the presence of a very long chain fatty acid, 27-hydroxyoctacosanoic acid (27OHC28:0), as the single lipid-A acyloxyacyl residue (e.g., Bhat et al., 1991; Que et al., 2000). This very long chain fatty acid requires a unique acyl carrier protein, AcpXL, and transferase, LpxXL. Genes encoding for both of these proteins were readily found in the *B. japonicum* genome sequence; *acpXL* (bsl3811) and *lpxXL* (bll3807). Three other genes were found in between these two genes; bll3808 (1.28 Kb) which encodes a 3-

oxoacyl-(ACP)-synthase II, bll3809, which encodes a second 3-oxoacyl-(ACP)-synthase (possibly synthase I), and bll3810, which encodes a 3-OH-acyl-(ACP)-dehydratase. Thus, it is likely that these five genes encode enzymes that are uniquely required for both the synthesis and transfer of 27OHC28:0. The arrangement of these five genes is very similar in other rhizobial genomes (e.g., *S. meliloti* (www.sequence.toulouse.inra.fr), *M. loti* (www.kazusa.or.jp/rhizobase/Mesorhizobium), and *R. leguminosarum* (Vedam et al., 2003)

There are no reported structures for the polysaccharide portion of *B. japonicum* LPSs. However, several compositions have been reported, including that for *B. japonicum* LPS (Carrion et al., 1990; Puvanesarajah et al., 1987). In the case of *B. japonicum*, a 18 Kb region (bll5919 through bll5932) may encode for O-antigen polysaccharide biosynthesis since at least one of the genes in this region encodes for a protein with sequence similarity to LpsQ, a UDP-4-keto-6-deoxyGlcNAc reductase (Forsberg et al., 2003) that is involved in the synthesis of quinovosamine, a glycosyl residue reported to be present in the *B. japonicum* O-antigen polysaccharide (Puvanesarajah et al., 1987). This 18 Kb region also contains 13 additional genes that putatively encode for five glycosyl transferases, five proteins involved in the synthesis of various NDP-sugars, three proteins with unknown functions, and one ABC transporter. One of these unknown proteins contains a domain for *wzy*, the gene which encodes O-antigen polysaccharide polymerase.

Other gene regions that appear to encode for the biosynthesis of various polysaccharides include a 15 Kb region (bll6303 – blr6312), a 10 Kb region (bll7571 – blr7578), and a 6 Kb region (blr6003 – bll6008). The 15 Kb region contains two genes that encode proteins with sequence similarity to polysaccharide export proteins (bll6303 has a domain for WzxC, an O-antigen transport protein, and bll6309 encodes a protein with similarity to ExoP that functions in the export of the *S. meliloti* succinoglycan) (Reuber and Walker, 1993); one gene that encodes a glycosyl tranferase (bll6306); two genes encode NDP-sugar synthesis (bll6307 contains both 2-epimerase and dehydrogenase domains, and bl46312 encodes a protein with similarity to GalE a UDP-Glc-4-epimerase); three genes encode proteins with unknown functions (bll6304, bll6305, and bll6308); and two adjacent genes (blr6310 and blr6311) encode proteins with similarity to ExoY and ExoF, respectively, which are thought to initiate *S. meliloti* succinoglucan synthesis by transferring the initial glycosyl residue to the lipid carrier (Glucksman et al., 1993, Reuber and Walker, 1993). The 10 Kb region contains three genes (bll7571, blr7576, and blr7577) whose products have sequence similarity to ExoQ, ExoP, and ExoT, respectively, which are involved in the polymerization and export of succinoglycan in *S. meliloti* (Glucksman et

al., 1993, Reuber and Walker, 1993); three genes (bll7572, blr7573, and bll7574) that encode putative glycosyl tranferases of which blr7573, and bll7574 show similarity to ExoU or ExoO, and ExoM, respectively, transferases involved in the synthesis of the succinoglycan of *S. meliloti* (Glucksman et al., 1993, Reuber and Walker, 1993); one gene (blr7578) which encodes a putative ExoB protein, a UDP-Gal epimerase; and one gene (blr7575) that encodes a methyl transferase. The 6 Kb region contains two genes (blr6003 and bll6006) that encode for putative glycosyl transferases; two genes (blr6004, and bll6007) that encode for a putative dTDP-Glc-4,6-dehydratase and a UDP-Glc-4-epimerase, respectively; one gene (bll6008) that encodes a putative acetyl transferase; and one gene (blr6005) that encodes for a protein of unknown function.

From this one case study, it is very clear that knowledge of the complete *B. japonicum* genome sequence will allow the rapid identification of genes and gene regions involved in a variety of cellular functions, many of which have relevance (e.g., SPS synthesis) to the symbiosis.

Knowledge of *B. japonicum* genomics can lead to strategies for inoculant improvement

We have long argued that basic knowledge of the *B. japonicum*-soybean symbiosis will ultimately lead to rationales for inoculant improvement and enhancement of the agronomic potential of this symbiosis. For example, research in our laboratory has recently revealed a means by which competition between *B. japonicum* strains can be manipulated by chemical cues. Competition between inoculant strains and indigenous soil rhizobia is a significant impediment to the application of commercial inocula.

B. japonicum uses members of three different global regulatory protein families to control *nod* gene expression. A LysR-type regulator, $NodD_1$, and a two-component regulatory system, NodVW, positively regulate *nodYABC* gene expression in response to plant produced isoflavone signals. In addition, NolA, a member of the MerR-type regulatory family (see below), appears to act indirectly to repress *nod* gene expression. Recent data shows that NolA is a central regulatory component that allows the cell to respond to a variety of signal molecules and finely regulate *nod* gene expression. For example, NolA represses *nod* gene expression in vitro and in planta in response to the presence of an iron-responsive molecule, bradyoxetin (Loh and Stacey, 2003). In the presence of low iron (e.g., after prolonged batch culture or within the nodule), the bacteria produce bradyoxetin. This compound is sensed by the bacterium leading to induction of NolA production, which in turn leads to induction of $NodD_2$ production and

subsequent repression of *nod* gene transcription. Mutations in either *nolA* or *nodD2* result in an inability to repress *nod* gene expression at high cell density or in planta. Addition of bradyoxetin can significantly shift competition between two strains if one of the strains is more resistance to the effects of bradyoxetin.

The *nod* genes encode for the production of a lipo-chitin nodulation signal (Cohn et al., 1998). Recent research by Nitragin Corp. (see abstracts of this meeting) have shown that inoculant preparations that incorporate the Nod signal have significant effects of the agronomic performance of soybean. Again, this research would not have been possible without the basic research which led to the elucidation of the *B. japonicum* Nod signal structure (Sanjuan et al., 1992).

Conclusions

The recent completion of the *B. japonicum* genome adds to a growing list of á-proteobacteria for which the complete genome sequence is available. The á-proteobacteria are of particular interest since members span the spectrum from obligate intracellular animal pathogens (of significant animal and health concern), to facultative intracellular animal and plant symbionts, to free-living bacteria. Thus, comparative genomic analyses of this group of bacteria should allow insights into the important features involved in animal and plant infection and growth/ maintenance in an intracellular environment.

With regard to the *B. japonicum*-soybean symbiosis, knowledge of the complete sequence now allows rapid prediction and identification of those genes involved in pathways postulated to be critical for symbiotic establishment (e.g., the case study above on polysaccharide synthesis). This knowledge will not only increase our basic understanding of *B. japonicum* and its fascinating relationship with soybean but will also lead to new approaches to apply this knowledge to inoculant improvement. Promising results along these lines have already been achieved from basic research that examined the regulation of *nod* gene expression in *B. japonicum*. Further insights into the basic biology of the *B. japonicum*-soybean symbiosis will likely lead to real improvements for field applications.

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On the origin and evolution of cultivated soybean (*Glycine max* (L.) Merr.): a review and discussion

J. Y. Gai and T. J. Zhao[1]

There appeared in the literature several hypotheses on the center of origin of cultivated soybean, i.e. Northeast China origin, Middle and Lower Huang River valley origin, Changjiang River valleys and southern China origin, and multiple-center origin in China as well as southern Japan origin, respectively. In the present presentation the evidences of different hypotheses of soybean origin in phylogeny of the genus *Glycine*, archaeological remains, ancient literature and comparative experimental biology were reviewed. The problems such as the relationship between the center of diversity and center of origin and the methodology usually used in studies of origination and evolution of cultivated species were discussed. The authors' data tended to support the hypothesis that the cultivated soybean originated from the ancient population of annual wild soybean in somewhere of Changjiang River valleys and southern China. The annual wild soybean supposed to be domesticated in the southern part of China at least 5000 years ago. Then the primitive cultivated soybeans were moved gradually northward to the Yellow River valleys, further to North and Northeast China, and upward to highland and mountain areas. In the southern part of China, the old time cultivated soybean was planted as full season crop, then changed into short season crop when the multiple cropping systems were invented. Along with the multiple cropping systems were extended to Yellow River valleys, the soybeans evolved also into short season cultivars. At present, there exist spring planting type, summer planting type and fall planting type of soybeans in southern China, the former two types exist in Yellow River valleys or North China, but only the first type exists in Northeast China. The evolution tendency was from short-day sensitive to less sensitive even insensitive, no matter for geographical evolution or seasonal evolution. Another direction of evolution after soybean being well domesticated was evolving from grain soybean into vegetable soybean (maodou). The earliest literature of maodou was found to be in 10th century in lower Changjiang valley. In addition, the methodologies of study on soybean evolution were also discussed. Among them, comparative biological approach with the assistance of molecular technique might be of important significance.

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Diversity within the perennial *Glycine* spp.

T. Hymowitz[1]

Abstract

The genus *Glycine* Willd. is divided into two subgenera *Glycine* (perennials) and *Soja* (Moench) F. J. Herm. (annuals). The domesticated soybean is a member of the subgenus *Soja*. Thus far, soybean breeders have been restricted to the subgenus *Soja* for improving soybean cultivars and have not exploited the wealth of genetic diversity found in the wild perennial *Glycine* species. The perennial species have been screened for many physiological and biochemical traits as well as for sources of resistance to economic pathogens. Of particular interest are the sources of resistance located for soybean rust and soybean cyst nematode. In addition, accessions of wild perennial species have been located that lack the Bowman-Birk protease inhibition.

The genus *Glycine* Willd. is divided into two subgenera *Glycine* (perennials) and *Soja* (Moench) F. J. Herm. (annuals). The list of species in the genus *Glycine* is presented in Table 1. Thus far, soybean breeders have been restricted to the subgenus *Soja* for improving soybean cultivars and have not yet exploited the wealth of genetic diversity found in the wild perennial *Glycine* species. These species are extremely diverse morphologically, cytologically, and genomically, grow in very diverse climatic and soil conditions, and have a wide geographic distribution (Table 1) (Singh and Hymowitz, 1999). This paper will review the literature concerning the identification of useful as well as interesting traits found within the wild perennial *Glycine* species.

Plant pathogens

Table 2 contains the scientific name, common name, and literature source(s) for resistance located for various pathogens within the wild perennial *Glycine* species. Major current interest lies in the sources of resistance found for soybean rust and soybean cyst nematode.

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Table 1. List of species in the genus *Glycine* Willd., three letter code, 2*n*, genome symbol and distribution as of July, 2003. (Hymowitz, 2004)

	Code	2*n*	Genome[1]	Distribution
Subgenus *Glycine*				
1. G. *albicans* Tind. & Craven	ALB	40	II	Australia
2. G. *aphyonota* B. Pfeil	APH	40	?	Australia
3. G. *arenaria* Tind.	ARE	40	HH	Australia
4. G. *argyrea* Tind.	ARG	40	A_2A_2	Australia
5. G. *canescens* F. J. Herm.	CAN	40	AA	Australia
6. G. *clandestina* Wendl.	CLA	40	A_1A_1	Australia
7. G. *curvata* Tind.	CUR	40	C_1C_1	Australia
8. G. *cyrtoloba* Tind.	CYR	40	CC	Australia
9. G. *dolichocarpa* Tateishi and Ohashi	DOL	80	?	(Taiwan)
10. G. *falcata* Benth.	FAL	40	FF	Australia
11. G. *hirticaulis* Tind. & Craven	HIR	40 80	H_1H_1 ?	Australia Australia
12. G. *lactovirens* Tind. & Craven	LAC	40	hh	Australia
13. G. *latifolia* (Benth.) Newell & Hymowitz	LAT	40	B_1B_1	Australia
14. G. *latrobeana* (Meissn.) Benth.	LTR	40	A_3A_3	Australia
15. G. *microphylla* (Benth.) Tind.	MIC	40	BB	Australia
16. G. *peratosa* B. Pfeil & Tind.	PER	40	?	Australia
17. G. *pindanica* Tind. & Craven	PIN	40	H_2H_2	Australia
18. G. *pullenii* B. Pfeil, Tind. & Craven	PUL	40	?	Australia
19. G. *rubiginosa* Tind. & B. Pfeil	RUB	40	?	Australia
20. G. *stenophita* B. Pfeil & Tind.	STE	40	B_3B_3	Australia
21. G. *tabacina* (Labill.) Benth.	TAB	40 80	B_2B_2 Complex[2]	Australia Australia, West Central and South Pacific Islands
22. G. *tomentella* Hayata	TOM	38	EE	Australia
	TOM	40	DD	Australia, Papua New Guinea
		78	Complex[3]	Australia, Papua New Guinea
		80	Complex[4]	Australia, Papua New Guinea, Indonesia, Philippines, Taiwan
Subgenus Soja (Moench) F. J. Herm.				
23. G. *soja* Sieb. & Zucc.	SOJ	40	GG	China, Russia, Taiwan Japan, Korea (Wild Soybean)
24. G. *max* (L.) Merr.	MAX	40	GG	Cultigen (Soybean)

[1]Genomically similar species carry the same letter symbols
[2]Allopolyploids (A and B genomes) and segmental allopolyploids (B genomes)
[3]Allopolyploids (D and E, A and E, or any other unknown combination)
[4]Allopolyploids (A and D genomes, or any other unknown combination)

Table 2. Resistance to pathogens located within the wild perennial *Glycine* species.

Scientific Name	Common Name	Literature Source(s)
Phakopsora pachyzhizi	Soybean Rust	Hartman et al., 1992; Schoen et al., 1992; Burdon, 1988
Sclerotinia sclerotiorum	Sclerotina stem rot	Hartman et al., 2000
Fusarium solani	SDS	Hartman et al., 2000
Phytophthora sojae	Phytophthora root rot	Kenworthy, 1989
Heterodera glycines	SCN	Riggs et al., 1998
Septoria glycines	Brown spot	Lim and Hymowitz, 1987
Bean pod mottle virus	BPMV	Chen et al., 2003
Alfalfa mosaic virus	AMV	Horlock et al., 1997
Yellow mosaic virus	YMV	Singh et al., 1974
Microspaera diffusa	Powdery mildew	Mignucci et al., 1978

Physiological and biochemical traits

Table 3 summerizes information about research conducted to locate physiological and biochemical traits within the wild perennial *Glycine* species. Of particular interest is that the only source of Bowman-Birk protease inhibitor nulls within the genus *Glycine* reside in the subgenus *Soja*. These are excellent sources of genetic material to improve upon the nutritional status of soybean meal when fed to monogastric animals.

Investigations on the wild perennial *Glycine* species were restricted in the past by the lack of experimental material. However, within the past 25 years, plant exploration teams from several countries have made extensive collections that resulted in the naming of new species.

Secondly, several incompatibility barriers in intersubgeneric *Glycine* hybrids (extremely low crossability rate, early pod abortion, low recovery rates of putative F. hybrids, poor parental genomic affinity) have hindered the introgression of useful genes. Thus far, only one intersubgeneric hybrid (*G max* x *G. tomentella*) (2n=40 x 2n=78) produced fertile derived diploid soybean lines (Singh et al 1998) and SCN resistance successfully was transferred into the soybean.

Table 3. Physiological & biochemical traits of interest located within the wild perennial *Glycine* species.

Trait	Literature source (s)
Herbicide tolerance	White et al., 1987; Hart et al., 1991
Salt tolerance	Pantalone et al., 1997; Shannon et al., 1989
Bowman-Birk Inhibitor nulls	Domagalski et al., 1992
Leaf flavonoids + isoflavonoids	Vaughn and Hymowitz, 1984
Phytoalexin	Keen et al., 1989
Lectin nulls	Mettu et al., 1995
Glycinin composition	Staswick et al., 1983; Alexsenko et al., 1985
Ease of regeneration	Newell and Luu, 1985; Hammatt et al., 1986; Hymowitz et al., 1986
Rhizobium specificity	Pueppke, 1988
Flowering response to photoperiod	Kenworthy et al. 1989

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Soybean production in the midwestern USA: technologies for sustainable and stable yields

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Abstract

The two-year maize-soybean rotation is the predominant cropping system of the US Corn Belt (the upper Midwest), and there is evidence that soybean yield in this cropping system has not increased over the past decade. Possible reasons for this include changes in weather patterns, buildup in soybean diseases and insects, and temporarily decreased breeding progress resulting from rapid incorporation of glyphosate tolerance into cultivars. Yields of glyphosate-tolerant cultivars currently equal or exceed those of "conventional" cultivars, however. Breeding for tolerance to diseases and insects has generally provided only partial tolerance, and pests continue to reduce yields annually. Studies suggest that planting date targets are generally being met in most cases, and that seeding rates are within optimal ranges in most fields. There has been an increase in soybean produced using reduced or no tillage, in order to decrease both soil loss and production costs. Research has shown that tillage has little effect on soybean yield, however. Seed inoculants and different methods of providing plant nutrients have been found to have little effect on yield. Glyphosate is used on some 75 percent of the soybean area, and provides excellent weed control and little crop injury, though glyphosate-tolerant weed biotypes have begun to appear. Reducing the intensity of soybean in the cropping system might increases yields and yield stability, especially in combination with improved IPM practices, and may offer one way to increase yield.

Introduction

Soybean yield in the US rose from about 1.5 tons ha^{-1} in 1950 to 2.5 tons per ha^{-1} by the start of the 21st century (Fig. 1). The US Corn Belt, identified as the region where soils and climate are favorable for producing maize, is comprised of eight or nine states, but for purposes of discussion we will identify it as six states – Iowa, Minnesota, Missouri, Illinois, Indiana, and Ohio – that currently produce about two-thirds of the US crop, on about 60 percent of US soybean area. These states have average yields about 10 percent higher than the average of the US, and this yield advantage has remained consistent over recent decades (Fig. 1).

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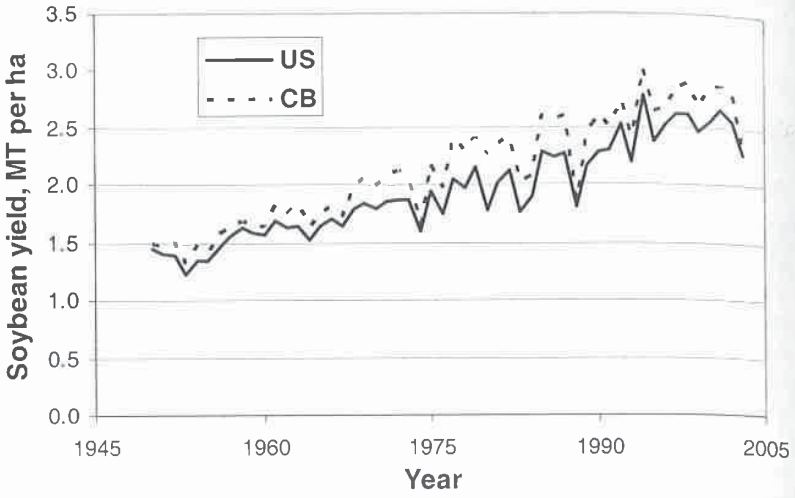


Figure 1. Soybean yield in the US and in six Corn Belt states, 1950-2003. Source: http://www.usda.gov/nass/

While yields have increased some 40 percent over the past 50 years, the upward trend in soybean yield in the Corn Belt seems to have slowed during the past decade (Fig. 1). Most of the soybean in the US Midwest is grown in a two-year rotation with maize, and the fact that maize yields have continued to increase in recent years has increased the concern about current yield level and sustainability of soybean production. During the ten years from 1994 to 2003, the yield ratio of maize to soybean was 3.2 in the Corn Belt, but in 2003 this ratio was 4.2, due to high maize yield and low soybean yield, mostly related to weather patterns. Such disparity, even if for only one year, leads many producers to consider increasing maize and decreasing soybean in their cropping systems.

The purpose of this paper is to examine factors related to soybean yield stagnation in the US Midwest, and to see what current research can tell us about raising and stabilizing soybean yields.

Cultivars

Wilcox (2001) reported rates of improvement for yield of publicly-developed soybean cultivars to be about 30 kg ha^{-1} y^{-1} for maturity groups (I, II, and III) common in the Corn Belt. While most of the breeding for commercial cultivars takes place in the private sector in the US, source germplasm and

breeding techniques are generally similar for all breeding programs. Recent patent protection and restricted use of commercial cultivars for breeding purposes may eventually provide commercial breeders with the ability to make faster progress, however.

On the other hand, the rapid incorporation of glyphosate tolerance into cultivars through backcrossing over the past decade has perhaps decreased the overall rate of breeding progress. Elmore et al. (2001) also reported lower yields of sister lines with the glyphosate tolerance gene. Recent cultivar tests, however, show that glyphosate-tolerant cultivars yield about the same as non-GM soybean cultivars (Fig. 2). While this does not address the question of breeding progress during the shift to glyphosate tolerance, it does suggest that, as some breeding programs begin to include only cultivars with glyphosate tolerance, the restraint on breeding progress from use of this gene should disappear. Producers can still improve yields substantially by carefully choosing superior cultivars using data generated through cultivar testing, both public and private. Most trials show top-to-bottom yield ranges of 1 MT ha^{-1} or more, indicating the substantial advantage that can be gained from careful cultivar selection.

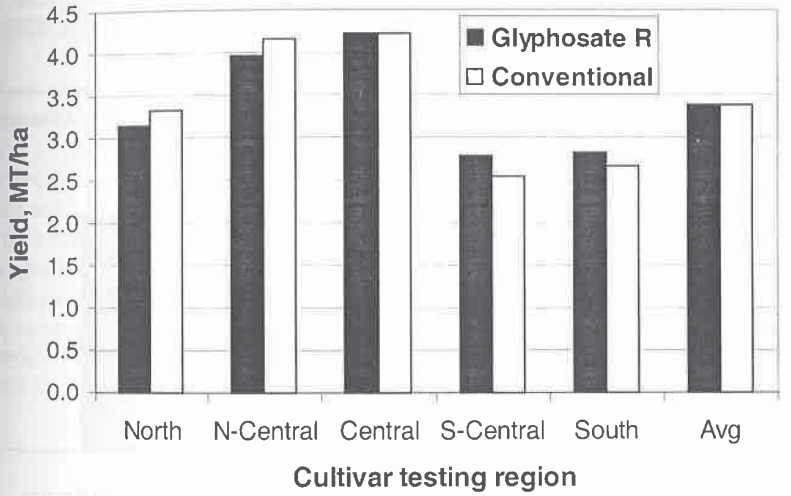


Figure 2. Yields of conventional (non-GM) and glyphosate-tolerant cultivars in the 2002 cultivar trials in Illinois. Data are averages of the most commonly-used maturity group within each region. Source: http://vt.cropsci.uiuc.edu/soybean.html

Tillage

In 2002, about 33 percent of the soybean crop in the Midwest US was planted without tillage and 24 percent was planted using a form of conservation tillage, defined as having more than 30 percent residue cover after planting. The remaining area was about evenly divided between reduced tillage, with 15 to 30% residue cover, and conventional tillage, with less than 15% residue cover after planting (http://www.ctic.purdue.edu/CTIC/CRM.html). The advantage of no-till or reduced tillage on preventing soil erosion varies considerably depending on topography and soil. For the most erosive fields, there are government-mandated conservation plans. Soybean usually follows maize in the rotation, and the high amount of crop residue from maize helps meet soil conservation goals even if some tillage is done.

Recent data from studies in Illinois show that tillage has little if any effect on yield (Fig. 3). In contrast, Pederson and Lauer (2003a) found that no-till increased yield by about 5 percent. No-till has worked well, and for many producers it represents the lowest-cost tillage option, but it appears to offer only limited ability to stabilize soybean yields against weather-related problems, such as too much or too little water.

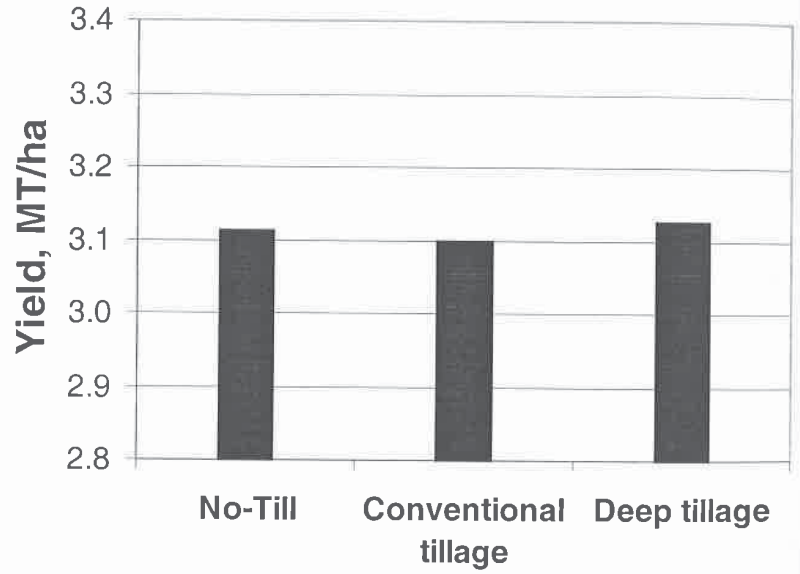


Figure 3. Effects of primary tillage on soybean yield, averaged over six Illinois locations and three years. Data are from the author.

Planting time, seeding rate, and row spacing

Most soybean producers in the US Midwest plant both maize and soybean, and they often use the same planting equipment for both crops. The size of the planter is usually based on the need to plant maize rapidly, up to 100 or more ha per day per planter, with the goal to complete maize planting by late April. Soybean planting usually starts after maize planting is complete, and most producers hope to finish soybean planting by mid-May. Wet weather can delay planting of both maize and soybean. Recent planting date studies have shown that the response to planting date is relatively flat from late April to mid-May, but that planting either earlier or later than this can decrease yield (Fig. 4). Similarly, Pedersen and Lauer (2003b) found small and inconsistent effects of planting date, from early May to late May, on soybean in Wisconsin.

Our results show that soybean yield responds little to plant density within the range of 300 000 to 600 000 plants ha^{-1} (Fig. 4). Ennin and Klegg (2001) showed similar results in Nebraska. While 250 to 300 thousand plants ha^{-1} may be adequate to maximize yield, there is also little or no yield penalty for having plant densities up to twice this minimum level. Hence, many producers plant 500 000 to 600 000 seeds ha^{-1}, in order to insure adequate stands in the event that stand loss from unfavorable conditions exceeds the normal loss of 10 to 20 percent of planted seeds.

Row spacing used for soybean in the Midwest has shifted over the past decades. The most commonly-used row spacing was 76 cm (planted with the same planter used for maize) in the 1970s. As herbicides improved, there was a shift in the 1980s and 1990s to drilled rows, spaced 15 to 25 cm apart. More recently, there has been a shift to 38-cm spacing, in which the same planter used for maize is used in a "split-row" configuration for soybean. Rows 38 cm apart are now the most common for soybean, followed by drilled rows, with less than 20 percent of soybean now planted in rows more than 50 cm apart. While Pedersen and Lauer (2003a) found little effect of row spacing on soybean yield, data from Illinois studies show a small increase in yield as row spacing is decreased (Fig. 5).

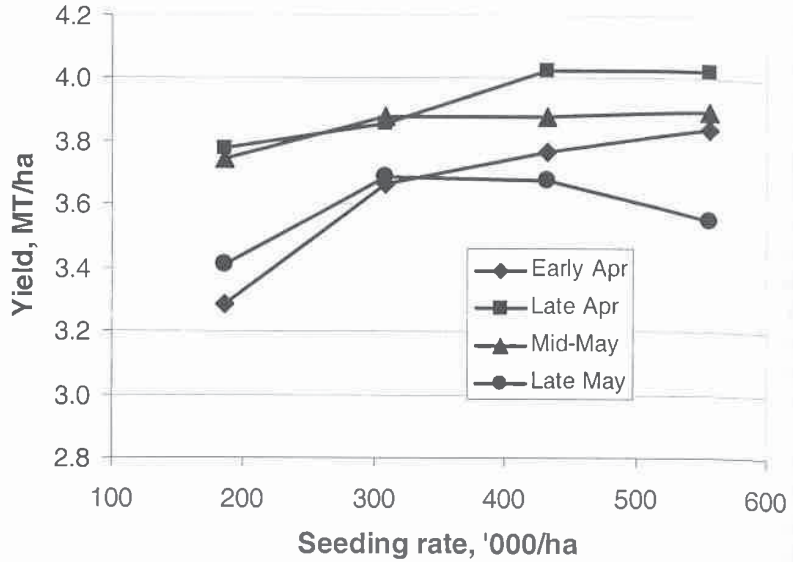


Figure 4. Planting date and seeding rate effects on soybean yield. Data are averages over three years at Urbana, Illinois. Data from the author.

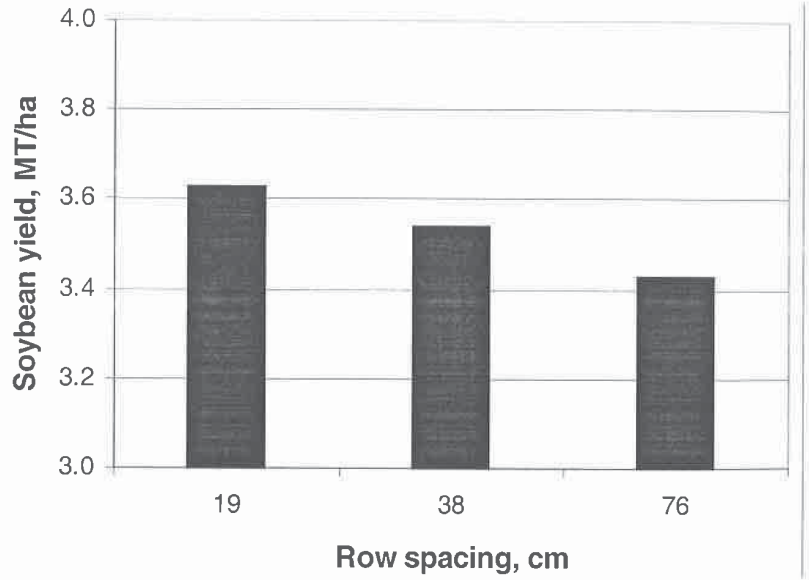


Figure 5. Row spacing effects on soybean yield, averaged over 14 environments in Northern Illinois. Data compiled by E. Adee, University of Illinois.

Seed treatments and mineral nutrition

There is little evidence that seed-applied fungicides or Bradyrhizobium inoculants provide consistent yield increases in soybean in the US Midwest. Recent research has similarly shown little or no effect of nitrogen fertilizer (Scharf and Wiebold, 2003) or of foliar micronutrient supplements on soybean yield.

Crop rotation

Recent fluctuations in soybean yields in the US Midwest, and intensifying pressure from international production, have led many producers to consider decreasing soybean area and increasing the area of maize and other crops. While we do not expect this to be a rapid shift, such a decrease in cropping intensity of soybean provides an opportunity to lengthen the rotation, to include soybean less frequently than in alternate years. There is evidence that this could increase yields and yield stability. Pedersen and Lauer (2003a) and Porter et al. (1997) showed that the first year of soybean following five years of continuous maize yielded more than soybean in a 2-year maize-soybean rotation. In an Illinois study, we saw a modest increase in soybean yield when the rotation was lengthened to three years, including wheat as the third crop (Figure 6). Hence more years between soybean crops in the same field may increase soybean yield and yield stability, probably by decreasing the levels of diseases (especially soybean cyst nematode) and other pests that decrease soybean yields in intensive rotations. At the same time, increasing the frequency of maize in the same field will tend to decrease maize yields, partly offsetting the positive effect on soybean. Thus any decision to change cropping patterns will be complex.

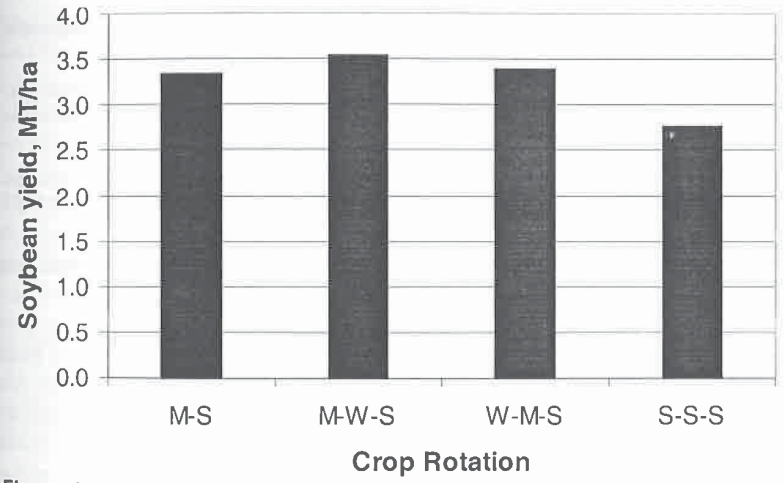


Figure 6. Crop rotation effects on soybean yield, averaged over two Illinois locations and four years. In the rotation designations, M = maize, W = wheat, and S = soybean. Data from the author, compiled by E. Adee.

Summary

From our studies and those reported in the literature, we must conclude that there is no clear-cut, easy way for most soybean producers in the Midwest USA to increase and sustain yields. Weather during the growing season clearly influences yield to a greater extent than do most management practices, though it is important to have a properly-managed crop in place in order to take advantage of favorable weather when it occurs. Many producers manage soybean at close to optimal levels with regard to cultivar selection, tillage, planting date, seeding rate, row spacing, and other management factors. Changing the crop rotation to less-intensive soybean production (i.e., fewer soybean hectares annually) may be one way to help stabilize soybean yields, but this may have negative consequences with regard to yield stability of crops that replace soybean, and so will be beneficial only if supported by economic conditions.

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Soil management and soil carbon contributions in Brazilian soybean production systems

J.C. Franchini; O. F. Saraiva; G. G.Brown and E.Torres[1]

Soil management is a generic name that includes a pool of cultural practices such as plowing, use of disc harrow, sowing, crop rotation, fertilization, and correction of soil acidity, among others. Soil management interferes directly with the capacity for carbon storage in soil, through the interactions between the quantity and the quality of plant biomass produced and the soil physical, chemical and biological processes. These interactions affect both the decomposition of residues and the ability of the soil to store carbon, that is further influenced by the soil texture, type of clay mineralogy, the presence of aluminum, and the quantity and activity of the soil biological fraction (microbial biomass, macrofauna).

To increase the organic carbon content in soil is a difficult task due to factors such as warm and moist climatic conditions and soil tillage, that favor losses instead of gains in soil carbon. In general the recovery of soil carbon is associated with practices such as the addition of fertilizers (mineral or organic), especially nitrogen, the adoption of no-tillage, and crop rotation with cover crops and green manures to increase the plant biomass produced.

The adoption of the no-tillage system in Brazil dates from the early 1970's and has increased since then, so that today the area under this management system occupies close to 18 million ha (FEBRAPDP, 2001). Eighty percent of the area under no-till is cropped with soybean, with 30% under continuous no-tillage (winter and summer).

The use of cover plants and green manures in crop rotation systems increases the quantity of carbon produced; commercial crops like soybean and wheat produce only about 2-3 Mg ha^{-1} of dry weight, while oat, lupine and radish can produce up to 10 Mg ha^{-1}. Furthermore, no-tillage can decrease the rate of decomposition of residues, decreasing the losses of carbon in the form of CO_2. Maintaining crop residues on the soil surface in general reduces the decomposition rate by as much as 20%, doubling the life-time of remaining of the residues under field conditions (Franchini *et al.*, 2002). Maize crop residues may stay on the soil surface on average 250 days, while those of oat will remain only 70 days. The relationship between the

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time that the residues remain on the soil surface, the qualitative properties of the residues and the soil carbon storage is still not well known and should be better explored.

The rate of carbon sequestration by the conversion of conventional (disk) tillage to no-tillage varies with the type of soil, cropping systems, ecosystems and climate conditions, ranging from 100 to 1,100 kg ha^{-1} yr^{1}. The rates of carbon fixation of the main soil types and ecosystems have been established through the monitoring of carbon dynamics in long term experiments (Table 1).

It is important to point out that these results represent mean values – variation of carbon content between different management systems as a function of time of adoption – showing that the rates for the initial period of adoption of non-tillage can be higher, reaching a new equilibrium in a relatively shorter period. In a field experiment performed at Embrapa Soja, Londrina (PR), the rate of carbon accumulation after 4, 8 and 12 years of adoption of no-tillage was of 775, 325 and 182 kg $ha^{-1}yr^{-1}$, respectively (Table 1). Those results show the importance of the production of straw in the first years of adoption of no-tillage to reduce the time required to reach the new equilibrium.

Table 1. Rates of carbon accumulation under different soil management and crop rotation systems in different Brazilian sites (State in parenthesis).

Site	Crop sequence or tillage practice	Time (yr)	Treatment	Depth (cm)	Carbon accumulation (Mg ha^{-1} yr^{-1})	Ref
Eldorado do Sul (RS)	O/M	9	CT1 vs. NT	30	460	a
	O+CV/M+C	9	CT1 vs. NT	30	640	a
	CT1	9	O/M vs. O+CV/M+C	30	560	a
	NT	9	O/M vs. O+CV/M+C	30	740	a
Santa Maria (RS)	NT	8	F/M vs. V+M	20	678	b
Brasília (DF)	n.d.	15	CT1 vs. NT	40	1060	c
			CT2 vs. NT	40	970	c
Ponta Grossa (PR)	n.d.	22	CT1 vs. NT	30	863	d
Londrina (PR)	O/S/L/M/O/S/W/S/L/M	5	CT1 vs. NT	40	580	e
	O/S/W/S/L/M/O/M/W/M	5	CT1 vs. NT	40	-200	e
	W/S	4	NT	40	777	e
	W/S	8	NT	40	325	e
	W/S	12	NT	40	182	e

References a: Bayer et al., 2000; b: Amado et al., 2001; c: Corazza et al., 1999; d: Sa et al., 2001; e: Franchini et al., unpublished; CT1: conventional tillage, disk plow; CT2: conventional tillage, hard disk; NT: no tillage; S: soybean; W: wheat; M: maize; O: oat; V: velvet bean; CV: common vetch; F: fallow; L: lupine; n.d.: not available or too complex to detail.

Corrections of the values of increases in carbon contents as a function of soil mass have also shown decreases in the estimated values, as no-tillage tends to increase soil bulk density and thus the soil mass in the surface soil layers, resulting in higher calculated carbon contents. After proper corrections for higher bulk density and soil mass, the values for two crop rotations in Londrina after five years changed from 580 to 310 kg $ha^{-1}yr^{-1}$ for the first and from 200 to 340 kg $ha^{-1}yr^{-1}$ for the second crop rotation.

The rates of carbon conservation in the systems (organic carbon in crop residues and carbon accumulation in the soil organic carbon) are also low when we consider the quantity of crop residues generated in some crop rotation systems (Table 2). Estimates indicate that the relationship between the carbon input as shoot and root residues and the carbon accumulation in soil organic matter ranges from 10 to 20%. Conversely, the reversion of the systems to more intensive management and soil disturbance can result in a fast loss of the sequestered carbon. This occurs mainly due to the loss of the light fraction of the organic matter derived from crop residues that contributes greatly to the increase in soil carbon under no-tillage. In a study performed by Bayer et al. (2001) the light fraction represented 10 to 30% of the increase in soil carbon content.

Studies comparing crop rotation systems under no-tillage and conventional tillage systems in Brazil have always shown a higher accumulation in soil carbon under no-tillage. In a five-year study performed in Londrina using no-tillage and conventional tillage (Table 2), the predominance of legumes in one of the rotations favored carbon accumulation, while the excess of grasses in the other rotation led to net carbon loss. These results confirm that the presence of legumes favors carbon accumulation (Bayer et al., 2000; Amado et al., 2001). The carbon lost under the no-till and crop rotation systems with the predominance of grasses can result from a longer period of low quality plant residues on the soil surface. The rate of decomposition of the residues would be then much lower than the rate of decomposition of the soil organic matter, reducing the soil carbon content. On the other hand, the same low quality of the residues would also decrease the rate of their decomposition after incorporation, contributing to the increases in soil carbon under conventional tillage management.

Table 2. Quantities of dry matter and carbon added in crop rotation systems, differences in soil carbon between no-tillage and conventional tillage, and the rate of conversion of carbon from crop residues into soil organic matter after five years in a clayey Oxisol at Embrapa Soja, Londrina (PR).

Crops	Crop systems					
	Cropping frequency	Dry mass	Carbon	Cropping frequency	Dry mass	Carbon
		kg ha^{-1}			kg ha^{-1}	
Oat	2	16.03	7,21	2	16.03	7.21
Lupine	2	14.75	6,64	1	7.38	3.32
Maize	1	5.30	2,39	2	10.61	4.77
Soybean	3	8.98	4,04	2	5.99	2.69
Wheat	1	2.35	1,06	2	4.70	2.12
Total		47.42	21,34		44.70	20.12
Soil carbon difference NT vs. CT kg ha^{-1}			3.09			-3.43
Plant carbon/Soil carbon %			14			-17

CT: conventional tillage, disk plow; NT: no tillage

The carbon accumulation in deeper layers than those affected by tillage implements can receive contributions from root residues, from movement of soluble organic compounds in soil or from the vertical transport by saprophytic soil macro-invertebrates (e.g., earthworms, scarab beetle larvae). The contribution of these mechanisms to carbon storage, however, still remain largely unknown.

Long term studies performed at Embrapa Soja have shown that use of no-tillage can result in lower yields in the first 4 to 6 years after its adoption, and thereafter the yields increase and become higher than those under the conventional tillage (Saraiva et al., 2002). The lower yields in the initial period under no-till may be related to the time that is necessary to modify the chemical, physical and biological properties of the soil. Crop rotations including cover crops and green manures will contribute greatly to increase the probability of success of no-tillage, especially by supplying more N to the soil biological processes, resulting in residues with a higher quality than those of commercial crops.

We may conclude that the adoption of no-tillage has positive effects on soil carbon content and yields many benefits to tropical agroecosystems. However, there is still a lack of information about carbon dynamics and the rates of carbon accumulation at different stages of adoption of no-tillage, as well as about the effects of plant species on carbon dynamics. The interrelationships

between changes in the soil biology under no-tillage and the increase/decrease in soil carbon storage or sequestration potential are also still poorly understood and deserve more attention, especially under moist-wet tropical climate conditions. Such information is essential to provide the farmer the tools needed to successfully adopt conservation agriculture, as well as to alert for possible factors that may become limiting at the initial and critical stages of the adoption process.

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Soybean production management for yield improvement in India

P. S. Bhatnagar[1]

Abstract

Adoption of soybeans in Indian agriculture in general and Madhya Pradesh in particular has revolutionized rural economy and has resulted in improved economic status of soybean farmers. Soybean has occupied important place in agricultural, edible oil and national economy of the country. Notwithstanding, Area expansion and growth in national production of soybean in India has been phenomenal (6.5 m. ha, and 6 m t. respectively), but yield has remained low. (1 t/ha). Nevertheless, productivity potential of 2 t/ha has been established under real farm conditions in farmer's fields. The major factors limiting yield of soybean in India could be encompassed as: i) environmental stresses; ii) technology gap; iii) information gap and 1v) seed gap. Narrowing the gaps and overcoming the constraints can improve productivity of soybean. For the result oriented action points to improve soybean yield, scrupulous preparation and implementation of road map with vision is necessary. All of this will require dogmatic efforts and increased investments from both public and private sector institutions/ companies.

Introduction

Acceptance of soybeans in Indian agriculture in general and Madhya Pradesh in particular has revolutionized rural economy and has resulted in improved economic status of soybean farmers. Soybeans have occupied important place in agricultural, edible oil and national economy of the country. Besides contributing about 8 per cent to the national edible oil supply , nearly 10 per cent of the export earnings of the entire agriculture sector and 70 per cent of the oilseeds sector are from soybean alone. Presently, they rank, second only to groundnut among the nine conventionally grown oilseeds crops in India. Apart from its role in easing edible oil situation in India, soybeans offer good prospects for the alleviation of pulse protein malnutrition which is wide spread in the country, specially among the weaker sections of the society.

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While the growth of soybeans in India was unprecedented, even faster growth rate of soybean processing industry resulting processing capacity of more than 3 times the production (over 15.6 Mt.) resulted critical situation for the Indian soy sector. The situation could be eased if gap between supply and demand is narrowed by harnessing the yield potential in conventional areas and extending its cultivation to non conventional but potential regions.

Area, production and productivity

Location specific production research coupled with development efforts and ensured market due to continuous establishment of soybean processing factories have resulted unparallel growth (15%) of soybean area and production in India. Starting from 3,000 hectares in 1969, soybeans have demonstrated steady increase in area over the years to estimated 6.5 million hectares in 2003. While, soybean production has more than doubled from 2.49 million tons in 1991-92 to 6.5 million tons in 2003, the productivity has remained low, hovering around 1 t per hectare (Fig. 1). To put this into context, average yields of soybean in India (0.9 t/ha) is about half of those in China (1.77 t/ha) and one third of those in the Western Hemisphere particularly Brazil (2.70 t/ha). India, however has only two decades of history of commercial cultivation of soybean. On the other hand, some of the Western Hemisphere countries have a century of experience while China has 20 centuries. Extensive research and development efforts, by both public and private sectors in these countries have been largely responsible for high yields of soybean. While growing conditions for soybean in India as rain-fed crop during monsoon are harsher than those in the Midwestern United States, the pampas of Argentina or the Savannah of Brazil, there is climatic similarity among these areas for growing soybean. However, the small farm size, the large number of farmers, limited mechanization, and the poor infrastructure in India make the task of overcoming the yield constraints more difficult.

Notwithstanding, the average yield of soybean for the country is hardly 1 t/ha, the easily harness-able potential, as demonstrated on farmers fields under real farm conditions over last 14 years is about 2 t/ha. (Table 1).The average highest yield under improved production technology ranged from 1875 to 3479 kg/ha with an overall average of 2406 kg per hectare. There are farmers in India, who are harvesting as high yield as 3 t/ha.(Kauffman et al, 2001). Thus, with appropriate production management and overcoming the yield constraints, national productivity of soybeans can be improved.

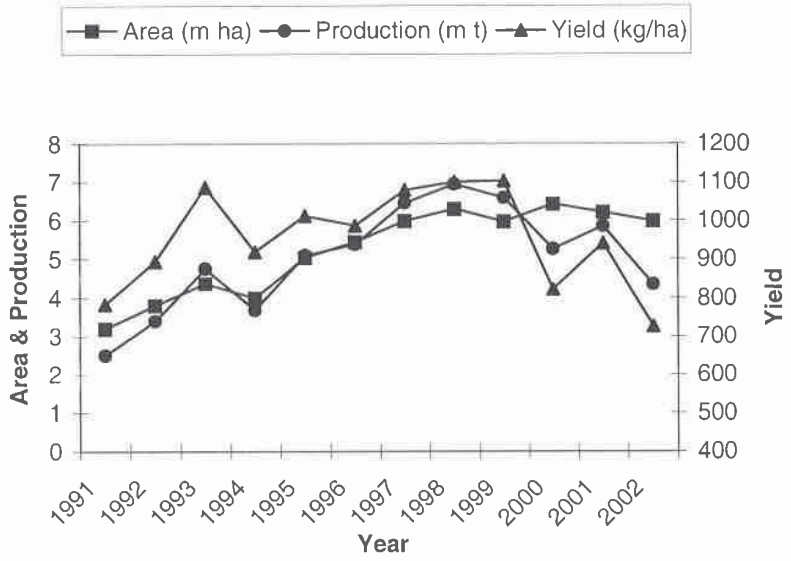


Figure 1. Area, production and yield of soybean in India

Yield constraints

Following are some of the major factors limiting productivity in India

- Abiotic stresses, mainly lack of adequate soil moisture or (occasionally) excess water due to the erratic nature of the monsoon and inefficient water management.
- Lack of genetic diversification, such that farmers do not have adequate choice of cultivars for their specific growing conditions.
- Limited availability of high quality seed of new or existing cultivars.
- Limited use of improved agronomic practices for tillage, planting, seed treatment, herbicidal weed management, spraying, IPM and harvesting.
- Lack of proper soil fertility management and resulting nutritional insufficiencies or imbalances.
- Biotic stresses due to inadequate control of diseases and insect pests.
- Lack of access to modern equipment resulting on problems with timely tillage, planting, weeding, and harvesting.
- Lack of information relating to weather and marketing.
- Limited investment in inputs due to uncertainty of profitability of soybean farming.

Strategies to increase soybean yield

To ensure sustainability of soybean sector in India, it is important to improve and stream line all aspects of the production system as well as develop strong links among all segments of the soybean value chain. Special efforts must be made to plug or narrow the technology and information gaps through domestic R & D as well as carefully evaluating technology and material from other major soybean producing countries that might be adapted under Indian conditions. Appropriate use of information technology that can provide timely information about the weather, inputs, and marketing can help farmers lower the risks from environmental factors. All of this will require dogmatic efforts and increased investments from both public and private sector institutions / companies to accelerate the modernization process

For the result oriented action points to narrow the gap between supply and demand of soybean in India, it is necessary to prepare a road map for maximization of productivity and production in the country. Besides harnessing the yield potential of soybean, concerted efforts for horizontal increase must be made, in conjuncture with development of remunerative soybean markets in non-conventional but highly potential areas of the country. Strategy and action points should be carefully planned and implemented with determination to subdue the yield constraints. Determined efforts with active partnership of public and private sectors, involving leaders of all segments of Indian soybean enterprise are essential to narrow the following three major gaps which encompass the yield limiting factors enumerated above, and that are directly and indirectly responsible for the Yield Gap.

Narrowing of technology gap

Various surveys conducted in major soybean growing regions have revealed that notably low percent of soybean growers follow the recommended package of appropriate production technology, standardized after years of systematic, applied research through the National Agriculture Research System (Kauffman et al, 2001). It is unequivocally agreed that the technology gap is largely responsible for low yield of soybeans in India. As indicated by front Line demonstrations conducted with appropriate production technology on farmer's fields under real farm conditions for 14 years (1989 to 2002), average yields of about 2 tons per hectare, more than double the current yields, are feasible if the technology gap could be narrowed (Table 1).

Table 1. Mean soybean yield (kg/ha) and yield gap II in Front Line Demonstrations

S. No.	Year	Number of Trials	Farmers' practice	Improved technology	Yield gap II
1	1989-90	150	901	1951	1050
2	1990-91	179	1280	1959	679
3	1991-92	179	1446	1991	545
4	1992-93	227	1427	1933	506
5	1993-94	179	1407	1899	492
6	1994-95	179	1360	1810	450
7	1995-96	220	1385	1839	454
8	1996-97	173	1501	1824	323
9	1997-98	186	1409	1852	443
10	1998-99	186	1245	1736	491
11	1999-2000	134	1292	1736	444
12	2000-2001	191	1191	1540	349
13	2001-2002	210	1420	1769	349
14	2002-03	218	1299	1646	347
Total / Mean	14 years	2611	1326	1820	494

Source: Annual Report 2003. All India Coordinated Research Project on soybean (ICAR)

Transfer of appropriate technology of soybean production is possible by organizing effective trainers' training programme, farmers' training programme, farmers' fairs, distribution of effective, self-explanatory pictorial literature, and conducting Front Line Demonstration on farmers' fields under real farm conditions in different soybean growing regions of the country. Creating awareness about the subsidies provided by Central and State governments, and helping the farmers to avail them would facilitate adoption of the recommended package of practices. Capturing the rich potential and synergies of Information Technology, including as an example creation of an Interactive Diagnostic Guide would go a long way in narrowing the technology gap. These activities are to be undertaken with missionary zeal by State Department of Agriculture, State agricultural universities, private companies, and NGOs like the SOPA Foundation, either independently or in active partnerships.

The adoption of recommended production technology, despite farmer awareness of the benefits of the cultural practices, is often limited due to some of the following requirements. Results-oriented, determined action to address these would yield the desired outcome.

i) Timely availability of need-based subsidies for soybean production provided by Central and State governments: There is a need to create awareness about the subsidies provided by governments and of the procedures to avail them. Provision should be made for need-based subsidies for certain essential but high cost items involving operations

for soybean production viz., fungicides for control of soybean rust, herbicides for control of weeds, sprinkler irrigation systems for avoiding wastage of water and ensuring availability of moisture at critical stage of plant growth of soybean

ii) Availability of inputs: It should be ensured that high-quality inputs namely fertilizers and agro-chemicals (herbicides, fungicides, insecticides) are available to the farmer at the right time and at reasonable prices. It has often been observed that some essential inputs are not available or that they may be of low quality. Such instances that result in conspicuous yield losses are long-remembered, and so can have an ongoing, negative consequence among farmers.

iii) Assurance for reasonable price of soybean: During the year 2002-03, the record-high price of soybean has rebuilt confidence among soybean growers. This has indeed been favorable for narrowing the technology gap. With the right approach and sincere efforts, farmers will adopt the recommended technology and will invest the resources for harnessing the yield potential of soybean. Nevertheless, sustained confidence of farmers in respect to getting remunerative returns from soybean cultivation is necessary. Unless this is attained, it would be unrealistic to expect farmers to invest the money necessary for adopting appropriate production technology. Price stability can be achieved by announcing minimum procurement prices of soybean in advance by the processing plants in their area of operation. This price could be estimated on the basis of the expected returns by the processors, and should be sufficiently remunerative and competitive. The declaration may indicate that the procurement of soybean would be made at the declared minimum price or the prevailing local market price, whichever is higher

Narrowing seed gap

Production of quality seed of improved cultivars of soybean and assured availability of such seed to farmers well ahead of optimum planting time are prerequisite for augmenting productivity and profitability of soybean. While some 79 cultivars of soybean have been released for cultivation in India (AICRPS, 2003) , limited quantities of seed of only two or three varieties are typically available to the farmers in a given area. This has resulted almost mono-variety culture on one hand, and use of leftover commercial produce (grains) from the previous season for planting of the new crop on the other. This situation is contrary to the basic requirement of sustainable farming. In light of the problems resulting from use of a single cultivar over wide areas, the varietal "cafeteria" approach has been recommended as a sound farming practice.

Effective narrowing of the seed gap is possible by advanced planning to meet the national requirements for soybean seed. Realistic stock of the situation regarding the availability of seed of different cultivars with public and private sector should be taken, and seed distribution should be regulated such that the farmers are not exploited. Ensuring optimum germination of seed by conducting reliable germination and seed vigour test, doing away with excessive seed rate of 100 kg or more per hectare instead of 70–75 kg. per hectare would enable planting of larger area with the available seed. The following strategy could go a long way to ensure availability of required quantity of seed of improved soybean cultivars:

Self reliance for Seed

The annual estimated requirement of soybean seed in India is about 60,000 tons, considering the seed replacement rate at 10 per cent. While it is difficult for seed producing organizations to meet such requirements, soybean farmers could be trained to produce and properly store good quality seed for themselves. This would require initial provision of foundation seed of improved cultivars in adequate quantities, but it could address the problem of non availability of high quality seed.

Conventional Seed Production Program:

Production of seed of the classes- nucleus, breeder, foundation and certified of the released soybean cultivars is to be systematically done. Timely indents for breeder seed in appropriate quantity of the following 13 soybean cultivars recommended by the GOI committee chaired by the Agriculture Commissioner should be submitted to NRCS / ICAR: JS-335, NRC-37, NRC-12, NRC-7, NRC-2, MACS-450, PUSA-16, MAUS-47, PK-416, PK-1024, PK-1029, JS-71-05 and JS-80 (Anon, 2001) Production of foundation and certified seed of the indicated varieties must be undertaken annually to ensure genetic purity. Tracking of breeder, foundation, and certified seed produced is needed to ensure continuous flow of soybean seed of various categories as well as certified seed of these cultivars to the farmers for cultivation.

Long term

Quite a few high yielding varieties with tolerance/resistance against biotic and abiotic stresses are in cultivation or are in the pipeline in countries with advanced breeding programs in soybean. The possibility of making available the seed of some of these varieties for testing and multiplication in India should be explored. This could be done by renewing the MoU or by signing MTA (material Transfer Agreement) with EMBRAPA and such other organizations. Just as the introduction of cultivars of Mexican wheat due to the vision of late Mr. C. Subramaniam and Prof. M.S. Swaminathan the

then Minister of Agriculture and D. G. ICAR, respectively in the 1960s triggered the era of high yielding wheat varieties and Green Revolution in India, so might introduction of soybean cultivars help to increase yield and yield stability in India

Narrowing information gap

Lack of timely information about the weather, inputs, and marketing increases the farmer's risks from environmental as well as biological factors. Appropriate use of information technology can provide timely information on the above aspects as well as on market trends for disposal of farmer's produce at the best price. Also, availability of the latest innovations related to soybean production technology as well as warnings about the outbreak of diseases and pests, including preventive and curative measures will definitely effect higher productivity and production of soybean.

The Information gap can be effectively narrowed by the use of conventional methods like farmer meetings, farm magazines, newsletters, radio, teleconferences and modern techniques of Information Technology (IT). The IT is the largest and richest "agricultural information system" in the world. Its massive holdings, covering all aspects of soybean production systems, will enable farmers to locate needed information to improve yields, plan for weather contingencies, access research information, visualize precision data, manage finances, buy inputs and sell outputs, and monitor prices in local as well as world markets It is surely the most promising way for Extension services to reach more farmers with better services. Interactive diagnostic guides and databases of probable questions at different stages of plant growth on the net would be most useful, in timely diagnosis of the problems and finding their appropriate solutions to prevent and cure the malady at right stage (Schmitz 2001 and Bhatnagar 2002).

Establishment of soybean clinics

The concept of Soybean Clinic is to set up a fully equipped unit as one stop resource centers that may offer to the farmers a wide range of services and solutions of any production-related problem, from pre-sowing to post-harvest management and marketing of their soybean at the best price. The Soybean Clinic will be very effective in narrowing the three gaps comprising the yield gap and will address the identified action points for attaining increased productivity and production of soybean in all regions. Soybean Clinics, staffed with subject matter specialists of all the disciplines of soybean production, will have the essential resources to be able to meet expectations. Farmers would receive guidance on a range of activities such as high quality inputs at a reasonable price and various subsidies provided

by the GOI and State Governments for soybean production. Beginning in the direction has been made by ITC ltd. in the form of the Soya Chaupal, groups established at the village level through which inputs and information can be provided to local farmers.

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The diversity of soybean producing systems in China and technologies applied for yield improvement

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Soybean has been planting in China for thousands years, which have different cultivars adapted to various environments. Unlike its progenitor of annual wild soybean, which germinates in spring and matures in autumn, soybean (Glycine max) have formed different planting types in different ecotype regions because of cultivation and selection for thousands years.

Diversity of soybean producing systems

Diversity of cultivation region and adapted cultivars

There are three basic sowing types of soybeans, including spring sowing soybeans, summer sowing soybeans and autumn sowing soybeans. In addition, winter sowing soybeans can be observed in Yunnan province located in the southern China. Soybean production in China are divided into three main ecotype regions, which are Northern Spring Soybean Region(NSSR), Huanghuaihai Summer Soybean Region(HSSR), Southern Soybean Region(SSR).

The soybeans planted in the complicated multiple tillage system, have different characteristics especially in the photoperiod (Table 1).

Table 1. Soybean cultivation and photoperiodicity in three ecotype regions in China

Ecotype Region	Sowing Type (Season)	Planting Time (Month)	Harvest Time (Month)	Photoperiod Sensitivity
Northern spring soybean Region (NSSR)	Spring (NSp)	April ~May	Late Aug. ~Early Sep.	Weak
Huanghuai Summer Soybean Region (HSSR)	Spring(HSp)	Late April ~ Early May	Sep. ~Early Oct.	Weak
	Summer(HSu)	June	Sep~Early Oct.	Middle
Southern Soybean Region (SSR)	Changjiang Spring(CSp)	Late March ~ early April	July	Weak
	Southern Spring(SSp)	Feb~ealy March	Middle June	Sensitive
	Summer(SSu)	May ~Early June	Late Sep ~ Oct.	Sensitive
	Autumn(SAu)	Late June~Early Aug	Early Nov.	Very sensitive

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All the spring soybeans except SSp soybean are weak for short photoperiodicity. Some of the soybeans from the high latitude region and part of CSp soybeans can even flower with the continuous lightening; and HSu soybean is moderate sensitivity to the short photoperiod. Relatively, SSu and SAu soybeans are sensitive and very sensitive to the photoperiod, respectively.

Genetic diversity of soybean cultivars among different cultivation systems

There are more than 20,000 soybean accessions in China. Most of the accession are landraces, taking about 92.3% of the total accessions with the range of the highest in the HSp soybean (100%) and the lowest in NSp soybean (87.2%); but bred cultivars has less accessions, ranging from 0~11.3% of the total accessions. These accessions were precious source of parents for soybean breeding. The genetic diversity of 180 landrace and 184 bred cultivars from Shanxi were compared at both phenotypic and molecular levels. The results showed that the landraces had higher genetic diversity relative to that in cultivars, and both types were obviously divided into two groups based on the 45 SSR loci. It indicated that soybean breeding could bring reduction of genetic diversity in some extent (Lin et al., 2003).

The genetic diversity center was found based on the agronomic traits of 22637 accessions. It was a stripe region from southwest to northeast, including Sichun, Shaanxi, Henan, Shanxi, Shandong and Hebei provinces (Zhou et al., 1998). In order to better understand the genetic diversity of these accessions, the soybean core collection was established (Qiu et al., 2003). By analyzing over 2600 primary core collection with 45 SSR loci, different soybeans were compared for both genetic diversity and richness, which were calculated by simpson index and average alleles per locus (Fig 1).

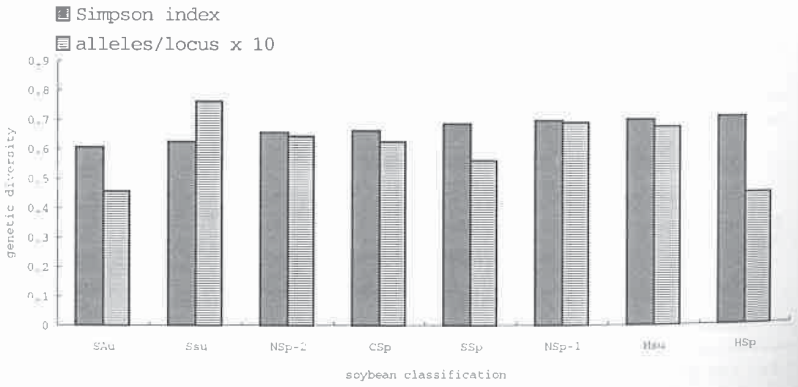


Figure 1. Genetic diversity of soybeans anong vari ous types

The genetic diversities of HSp and HSu in the HSSR were the highest among three ecotypes, then following with spring sowing soybean including NSp-1 (Northeast), SSp, CSp and NSp-2 (Northwest). Both SSu and SAu soybean were the lowest. However, the genetic richness was the highest for SSu soybean, and both SAu and HSp soybeans were the lowest. In each of sowing soybean among three ecotypes, the genetic diversity centers were located, which all but one for NSp-1 were the same, compared to the center of phenotypic diversities. Because NSp only had the higher genetic diversity at DNA level, but it's phenotypic diversity was lower.

The differentiation among various planting soybean types was detected by using SSR markers (Qiu et al., 2002). About 80 cultivars were selected from each of NSp, HSu, SSp, SSu and SAu in primary core collection, and analyzed with 67 SSR loci. The differentiate coefficient of genetic diversity ranged from 0.039-0.353 with average of 0.154, which means 15.4% variation existed among the five types. The X^2 tests for the distribution of alleles at each locus among five types were significant. And the relationship among five planting types was analyzed (Xie, 2002).

Li et al. (2003) used 45 SSR loci analyzing 107 Hsu soybean and 112 SSu soybeans, both planting types mainly grouped separately. For the HSu soybean, 90.5% of them grouped together and the rest 10 soybean cultivars dispersed among the SSu soybean cluster. However, 70.5% of SSu soybean was grouped together, which was lower than that of SSu, but there were 33 SSu soybean distributed among Hsu soybean group. It provides the evidence that there was genetic diversity among different planting types from various cultivation region at both phenotype and molecular level.

Technologies application for yield improvement

Breeding Technologies

Cultivar improvement

From 1950' to 1980', the improvement of yield potential depended much on the steady increase of 100 seed weight and weight per pod, harvest index, and biomass; besides, the shortening of plant height and decreasing of the numbers of nodes on main stem and branches also play a very important role (Feng et al., 1991)

Overcoming the twinning habit is the key to utilize favorite traits of wild soybean in the soybean improvement (Zheng, 1982; Wang, 1984; Li, 1987). The utilization of two small seeded cultivars of 'Jilin small seed no.1' and 'Dongnong small seed no.1' in the production provided advantageous evidence.

Because of short photoperiod sensibility, each soybean cultivar usually adapts narrow latitude ranging 1.0~1.5. In order to utilize diversity of adaptable among various soybean germplasm located in the different region, Meng (1982) indicated that Hsu soybean may broaden Nsp soybean genetic base. The crosses made between Nsp and Hsu had higher combine ability than that between Nsp soybeans, and Hsu from Shangdong had higher potential yield than ones from Shanxi (Tian, 1986). By using this strategy, the lines with high yield and high combine ability were developed such as Gongjiao 7335, Gongjiao7662 etc.

Introduced soybeans have been used to develop high yield cultivars. Soybean cultivar 'Hefeng 25' developed by crossing local cultivar with introduced Japanese cultivar 'Shi sheng chang ye' has been accumulatively planted over 850 million ha in Heilongjiang, Liaoning, Neimeng, Hebei and Xinjiang etc for more than 9 provinces within 12 years, the highest yield level can reach up to 319.5kg/667m²(Guo et al., 1997). Similarly, cultivar Liao dou no.3 was developed by the parents from geographical distant region and produced 300.05 kg in 11.9 ha(Wang et al., 1985).

Utilization of heterosis

The important strategy to increase the yield is utilization of the heterosis by using cytoplasmic male sterile soybean ZA, XXT (Zhao et al., 1998) and M type (Zhang et al., 1999) to produce the hybrid seeds. It had been indicated that recombination of distant parents might have higher heterosis parent through repeat tests and 39 high heterotic combinations were screened which average high parent heterosis and check heterosis were 39.6% and 33.2%, respectively (Wang et al., 2002).

By using M type cytoplasmic male sterile soybean as female parent to cross with 22 cultivars from Huanhuai and Southern region, the average heteroses were 22.9% for higher parent heterosis and 24.83% check heterosis (Li et al., 2002). The yield of 9 crosses can reach up to 260kg/667m². There are tendency to have higher heterosis between geographical distant (Li et al., 2002) or pedigree distant (Wang et al., 2002) soybeans.

Diverse Cultivation Technology

Diverse tilling system

In the northeast China, the tri-deepness and banding type tillage can be used in the poor physical and chemical features of the topsoil in waterlogged and flat lowland (Yang, 1997), the yield in three year averagely increased 15.1%, ranging from 13.7%~16%. This tillage pattern increased available

fertilizer of N, P, and K by 19.2%, 58.9% and 36.7% respectively. However, three Ridge Cultivation (TRC) has been widely adopted in the most fields. It is deep-tillage of ridge, stratified application of fertilizer in ridges, with urea and calcium, and combined with double row precise seeding on ridge, which can increase yield 27.6~153.8% compared to the traditional ridge cultivation. The other important tillage system is wide flat ridge; the mean increase of yield per year is about 12.7% during 1976-1983. The optimum population distribution for this method should be hill distance 15-20cm, with 3-4 plants per hill (Hu, 1985). The key of this system is narrow-row dense planting (NRD), which has been widely popularized. Combine TRC with NRD, the yield can increase up to 40%.

In the southern China, there is much hill upland field which plant maize, sweet potato or sugarcane, interplanting with the other crops result in harvesting three times a year. The SSp soybean is usually interplanted among the rows before wheat mature, and then interpolated with sweet potato before harvesting soybean. The yield for all three crops was 21624 kg/ha, with 1846.5 kg/ha for soybean. The SSp soybean also is intercropping with tea, seedling tree, or orchard, the average yield usually was obtained for more than 750kg/ha.

Integrated management

Except above major technologies to improve the yield, there are still some other technologies used in the soybean production, such as disease and pest control management.

The major diseases and pests problems around the country include soybean mosaic virus in national wide, soybean cyst nematode (SCN) in northern and huanghuai region, the Agromyzid fly (*Melanagromyza sojae Zehntner*) and soybean rust in the southern China, root-knot nematode in huanghuaihai and southern China, frogeye leaf spot (Cercospora sojina) and soybean pod borer (*Leguminirora glycinevorella (Mats.)*) in northeast China. Recently, the aphid become serious problem in Huanghuaihai and northern region, Limabean pod borer (*Eliellazinckenella*) in Huanghuaihai region. Few chemical is used to prevent the disease except using cultivars with disease resistances. The only effective way for preventing these diseases is breeding resistant cultivars. However, some pesticides have been applied in killing the insects, especially in southern region, and this will break down the biological balance.

The other important technique is to apply fertilizer. The ratio of soybean yield relying on soil was 74.9%~88.6%. After applying organic manure,

the dependence of soybean yield on soil declined significantly. By simply application of organic manure, the soybean yield increased by 22.3 ~ 33.6% and on the base of chemical fertilizer combining with organic manure, the soybean yield increase by 12.7 ~ 17.7 due to the effect of organic manure (Han et al., 2001).

For the new no tilliaging reddish yellow soil, the important methods to increase the yield is to utilize more fertilizer. But it is needed to add calcareousness about 1500 kg/ha firstly in order to neutralize the soil. The using manure mixed with phosphor as seed fertilizer, and then fertilize after manuring.

Combination of high yield cultivar with suitable cultivation method

The high yield record was created in the Huanghuaihai and northern region. Summer soybean germplasm MN413 created high yield of 4725kg/ha. Similarly, the yields of Shanning no.8 exceed 4500kg/ha for three continuous years.

In northern China, Nsp soybean cultivar Liaodou no.14 can produce 4875 kg seeds per hectar. The highest yield of 5955 kg/ha was created at Xinjiang autonomous region by using Nsp soybean cultivar 'Xindadou no.1'. This miracle was artificially created by integrated techniques, which included spraying growth regulator to decrease the plant height, irrigation during growth and seed development, and applied fertilizer. All of these high-yield records were created by the cultivars with high yield potential based on the evenly distribution of the seedling or plants.

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The expansion of biotechnologically modified soybean in Argentina: economical aspects and considerations

R. Muñoz[1]

Abstract

The expansion of the soybean crop in Argentina allowed the Country to become the main exporter of soybean ground products, meals and oils, which became the most important products, in agricultural values, and almost one third of the total Argentinean export figures. The transgenic "RR" soybean contributed to this so-called expansion by modifying and providing production systems with lower production costs and lower use of labor with agricultural machinery. This easiness of cropping and favorable economical equation imposed an economical shift in the rural environment, causing a transformation in the agricultural frontier and committing areas considered marginal or of environmental risk. The sustainability of the agronomical model leaded by the soybean crop concerns all the rural community, to which should be aggregated the economical and social consequences of a smaller employment capacity.

From the positive side, the rural sector was freed from the Argentinean economical recuperation right after the critical period of money convertibility. Moreover, the RR transgenic soybean has contributed to the enforcement of the no-till sowing, which is actually nowadays considered as one of the most important conservational agricultural changes. Some criteria of agricultural politics that contemplates some type of regulation in the expansion of the soybean crop, according to the vital interests of the rural sector and of the country, are expected.

Introduction

The soybean production in Argentina has reached a spectacular increasing from 1980 up to date. The favorable agronomical and economical characteristics of its cropping have been the main causes of its expansion, associated to a parallel development of the agro-industry of processing, until converging to the complex of the principal agro-industrial and exports branches of the country. According to Rebolini [1], for the year 2003, the soybean production of 35 million metric tons represented a figure of 7.900 million dollars or 49% of the total value of the agricultural production, an

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export value equivalent to 8.100 million dollars, or 73% of the total Argentinean exports of agricultural and derivate products, and approximately one third of the total Argentinean exports.

The reason for the soybean-increased production in the country has been the large increase in the soybean-cultivated area; witch expansion has been noticeable in the 80's and 90's decades. Undoubtedly, a new period of expansion occurred during the last six years as a consequence of high soybean international prices (since 1996/97) and to the uses of herbicide-resistant cultivars. The harvested area reached five million hectares in 1990, regulating its increasing to one million of additional increases since the 1995/96 cycle. From 1996/97 to the 2002/03, the growth went fast, increasing one million hectares per year and passing from 6 to 12,5 million of hectares. The production reached 11 million tons in 1990 to moderate the growth since 1995/96 cycle when it reached 12.5 millions. From 1996/97 to 2002/03, the increasing went faster, incrementing 3 million tons/year, until reaching 35.5 million tons.

The crop competitiveness as regards the national average yield has been a factor of the motivation of the cultivation in the country. During the last five years, a strong expansion of the area harvested was associated with stable production. The comparative analysis of the yields according to data of the USDA [2] shows that Argentina presented a very good competitiveness comparing with USA, main producer and exporter of soybean.

The increase of the production was accompanied by the equally important evolution of the mill capacity that was associated to strong agriculture-industrial investments that granted great competitiveness of the products, dedicated basically to the market of the export. Also, their growth was remarkable and in the last period, from 1996 to 2002, when its projection was duplicated from approximately 11 to 23,5 million tons.

The production of soybean in the country has been one of the most remarkable developments in the agriculture of world. In a little more than two decades 1980 and 1990, Argentina became the third world producer, after the USA and of Brazil, becoming the main exporter of the mill of the grain products, the meal and the oil. One of the reasons is the low level of domestic utilization of the soybean that makes the activity dedicated to the export. Considering the production of 2002/03 and according to the USDA [3] from a production of 35,5 million tons, 23.5 were dedicated to mill and 8,7 were exported, remaining a 1 million for seeds and other uses. From 18.6 million tons of meal production, 18.4 are exported and 0.2 millions were consumed. From a 4.4 million tons of oil production, 4.35

was exported and only 0.12 millions were consumed. Thus, in the group, the exportation index would be of 97% for the grain, 98,8% for the meal and 99,7% for the oil.

The previous indicators had no precedents in the world, regarding soybean trade, where the producing countries and exporters, except Argentina, have a relatively very important domestic use. This exportation is not comparable to most of the agricultural products in the world. From the strategically point of view, this constitutes the fundamental arguments of a country to be considered especially exporter structurally.

The soybean biotechnological modified

The acceleration of the expansion of the soybean in the Argentinean agriculture has largely been attributed to the development and diffusion of herbicides resistant soybean, known as RR, short name for Roundup resistant. This biotechnological innovation made possible to apply the glyphosate, a herbicide of wide spectrum, controlling the weeds in any moment, without affecting soybean cultivation. The combination with no-tillage and RR-soybean formed a technological package that sustained the advance of its cultivation, granting an annual increment of the planting area of almost one million hectares per year, covering 12,5 million hectares in the 2002/03 cropping season and the 13,7 in those projected for 2003/04.

It can be proven a change of tendency of the area cultivated with soybean in the country whose breaks took place exactly in 1996/97 and 1997/98, with the diffusion of that technological innovation. The projections remain upward for next years while the conditions of the international market is substantially stable.

The agronomic and economic advantages

The technological package of RR-soybean in combination with no-tillage, explained the advance of the cultivation, because the producers adopted the techniques massively. Among the explanatory reasons, the most important concentrated on the economic and agronomic aspects.

For the medium and large producers, the massive cultivation of the soybean, mainly of the RR-soybean, came from the combination between the high international prices and the drop in the production costs induced by this technology. The soybean prices for several year were above the other export cereals, at the same time that the production costs lowered.

The detailed analysis of the costs was shown in the Table 1, where is proven that the gross margin of the cultivation of soybean for the season

2003/04 and for the expected yield was superior to the main cultivations. In the Figure 1 and 2 an evolution of the previous indicators was shown, from 1990 to the projection of 2003. On the other hand in the Table 2, it is pointed out that, for the year 2003, using the same prices and yield, the differences of the economic indicators among the technologies of conventional soybean and of RR-soybean associated to no-tillage.

Table 1. Gross margin of the crop, in dolars. North Buenos. Ayres.

Date 09/01/2004		Wheat		Sunflower		Maize		Soybean	
Yield	qq/ha	35	45	18	25	75	95	28	38
Future prices	u$/QQ	12,8	12,8	20,3	20,3	8,8	8,8	20,3	20,3
Gross incomes	U$/Ha	446	574	365	508	660	836	568	771
G commercialization	%/IB	16	16	12	12	24	24	15	15
Net income	U$/Ha	375	482	322	447	502	635	483	656
Tillage	U$/Ha	35	35	32	32	27	27	38	38
Seeds	U$/Ha	22	22	20	20	90	90	21	21
Urea, FDA	U$/Ha	76	76	31	31	64	64	13	13
Agrochemicals	U$/Ha	17	17	29	29	27	27	29	29
Harvesting	U$/Ha	27	34	22	30	40	50	34	46
Direct costs	U$/Ha	-177	-184	-134	-142	-248	-258	-135	-147
Net margin	U$/Ha	198	298	188	304	254	377	348	508
Sowing Percentage									
Net margin-40%	U$/Ha	119	179	113	182	152	226	209	305
Rentals									
Average rental	qq/Ha	9	9	7	7	18	18	12	12
M B- rental	U$/Ha	83	183	46	162	96	219	104	265

Note: Prices without IVA; short distance 20 km, long distance 200 km freight
Source: INTA Pergamino. Informe de Granos, 6 (166), 2004 [8]

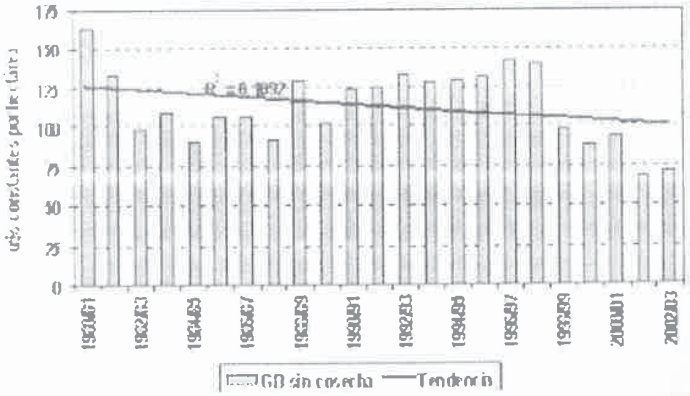


Figure 1. Evolution of soybean direct costs (excluding harvefting costs).

Table 2. Comparative analysis of net margins, conventional soybean versus no-tillage RR-soybean.

Yield		North of Bs As Conventional Group IV		North of Bs As No-tillage Group IV / RR		Difference In units and values	
Yield	Qq/ha	34	38	34	38	0	0
Soybean May, 04	U$/qq	18.9	18.9	18.9	18.9	0	0
Gross income	U$/qq	642.6	718.2	642.6	718.2	0	0
Commercialization.	U$/qq	80.6	90.0	80.6	90.0	0	0
Net income	U$/ha	562.0	628.2	562.0	628.2	0	0
Tillage	U$/ha	72.8	72.8	46.1	46.1	26.7	26.7
Seeds, inco, fungicides	U$/ha	25.8	25.8	29.3	29.3	-3.5	-3.5
Agrochemicals, Fertilizers	U$/ha	43.2	43.2	52.8	52.8	-9.6	-9.6
Harvesting	U$/ha	38.6	43.1	38.6	43.1	0	0
Total cost	U$/ha	180.4	184.9	155.6	160.2	24.8	24.8
Gross margin	U$/ha	381.7	443.3	406.4	468.0	-24.7	-24.7

Source: Márgenes Agropecuarios.[9].

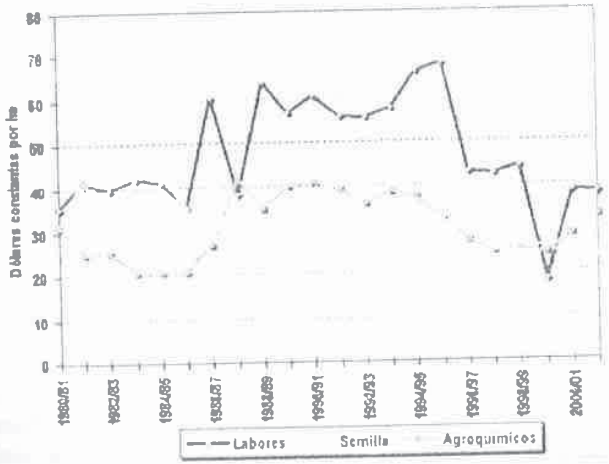


Figure 2. Evolution of soybean direct costs.

The Tables showed that the profitability of the soybean measured in gross margins has been superior to the alternative cultivations. During the used series, it was proven from 1996/97, a reduction of the costs of production of the soybean. The main drop was in the direct costs, leaded by the decrease of the works with machinery used in the conduction of the cultivation (Fig. 2).

The comparison among the technologies of production of conventional soybean versus the RR-soybean with no-tillage, showed that the substantial decrease of machinery-labor costs (almost 28 U$/ha), when one no-till, one fertilizer application (usually with the sowing) and the necessary, terrestrial or by air spraying. The changes measured in units of agricultural traction (UTA) fell of 4.66 up to 2.1 according to the productive models, mainly due to the decrease in the recruiting of this works. There was an important change in the form of conduction of the cultivation, where the hired labor prevailed because the park of machinery of the growers became obsolete when capable machinery for no-till was not available.

According to Teubal & Rodríguez [4], there was an important reduction of the necessary manpower for the mechanical tasks in the preparation of the soil and for the soybean sowing that caused an important social change with a deterioration of the work. Similar results were found by J. Tosi [5] studying the evolution of the economic results obtained in the 1994/95 at 2002/03 agricultural seasons, for the cultivation in conventional and no-till farm. The acceleration of the agriculture produced a change in the production modalities and in the cultural practices of all the cultivations, with an increase in the yields. Basically, there were changes in the tillage used and in the use of the inputs, the abandonment of soil management implements as plow, the chisel and harrow disk for no-till, increased the fertilization, the incorporation of more specific herbicides, new corn and sunflower, hybrid, RR-soybean, with addition of improvement in the technology processes.

Among results, the wide diffusion of the soybean cultivation is explained in the last years, for the big margin gross average, the small installation costs and the return for more invested money to its alternative ones.

Projections and considerations

The association between RR-soybean and no-till was a factor that strengthened the expansion of the soybean in the Argentina, causing an unprecedented change, ending up in 2002/03 occupying 50% of the total cultivated area. The agricultural expansion has advanced on marginal areas, colonizing new areas and explaining in great measure the advance of this cultivation in the region extra "pampeana". According to F. Begenesinic [6] in a study of the Secretary of Agriculture, Livestock, Fishes and Feeding (2002), based on the behavior of the soybean in both "pampeana" and extra "pampeana" regions, concludes that soybean displaced other cultivations (effect substitution) and, extended to areas previously considered marginal from the point of view ecological agriculture. The good results of the cultivation were attributed to the association of transgenic RR-soybean plus no-tillage.

The effects on the other cultivations have emphasized a competition for area starting from the appearance of the transgenic soybean development, all the cultivations diminished considerable their cultivated surface. Comparing the areas between 1996/97 and 2002/03, it was proven that the rice crop diminished its cultivated surface, 44,1%, the sunflower 34,2%, and the corn 26,2%. In the case of the wheat, its surface reduction was not very significant (3,5% less), but it have to be considered that it participates of systems in no-tillage of wheat and soybean.

The agricultural expansion of the soybean is generating in the Argentina a great debate on the monoculture of the soybean and their effects on the deterioration of the soils and the future productive capacity. Recently, positions and opinions regarding the sustentability [7] of the current productive pattern, in a process that requires an agricultural politics with some regulation power.

The obsolescence of the park of machineries for the conventional agriculture leads to the contractor's dependence, a type of producing with bigger financial capacity that incorporated the purchase of harvesting machinery and the necessary teams for no-tillage.

The sustentability includes in its secondary effects, the debate on the rural employment, the work loss and the rural emigration.

The productive model leads to a progressive transfer of fields to other farmers by means of legal contracts that assumed great dynamism. The reduction of growers, being a great part due to renting fields, in a context of a strong concentration in the rural areas.

One of the effects of no-till and RR-soybean technology was the augmentation of the capacity of the farmer's work that can sow without big inconvenient thousands of hectares. A strong demand for land was generate, originating the increase in price of the land as well as hired value; the price of the same one, reached values close to their historical maximum. Among the positive aspects, it can be inferred that the Argentina economy recovery was leaded by the agricultural sector and this for the soybean,; after the rejection of the convertibility, soybean became payment currency, financing and reservation of value. Also the soybean allowed the reactivation of rural sector. No-tillage was consolidated, with a decisive role in the technology of the biotechnologically modified cultivars, that parallely constitutes the more important movement conservationist of the last years.

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Adoption of genetically modified soybeans in the United States: lessons from the first decade

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Introduction

Genetically modified soybean varieties with resistance to the herbicide Roundup were first commercialized in the United States during the 1996 growing year. These Roundup Ready varieties contain a bacterial transgene that allows protein synthesis to proceed in the presence of Roundup (the trademarked name for glyphosate), an herbicide that normally shuts down this essential process and kills plants. The combination of glyphosate and Roundup Ready varieties provides highly effective weed control.

During their first year of availability, Roundup Ready varieties were planted on fewer than 400,000 hectares in the United States, less than 2% of the total (James, 1998). Acceptance by farmers was very rapid, and by 2000, more than half of the soybeans grown in the U. S. were glyphosate-tolerant (Carpenter and Gianessi, 2001). Today, more than 80% of the soybeans harvested in the U. S. are transgenic (Giles, 2003). This rapid rate of adoption of a new technology by soybean growers is unparalleled in American agriculture (Carpenter et al., 2002) and raises some intriguing questions about economics and the factors that influence a farmer's decision to use the technology.

One of the major drivers for technological innovation is profitability. Users will generally accept new technology if they can make money in doing so, and they will reject technologies that fail to provide an economic return. Roundup Ready soybeans have several obvious effects on the economics of production (Bullock and Nitsi, 2001). These include the relatively low cost of Roundup in comparison to alternative herbicides, as well as its ability to control a broad spectrum of weed species without the need to purchase other, more specific herbicides (Carpenter and Gianessi, 2001). Weighed against this are the cost premiums for Roundup Ready seed (the so-called technology fees), as well as progressively steep reductions in the prices of competing herbicides that could be used in conventional weed control programs (Nelson and Bullock, 2003).

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Farm level profitability is not the only factor that goes into the decision of whether or not to adopt Roundup Ready technology. Global marketing uncertainties persist. They are being driven by the unwillingness of consumers and governments in many parts of the world to accept foods that contain ingredients from genetically modified varieties (Yimin and Mervis, 2002; Mitchell, 2003), and a regulatory environment that remains complex and fluid, especially in Europe and Asia (Burrill, S. G., 2003).

Farmers balance all of these tangible issues with less tangible factors such as convenience and personal management preferences. The attractiveness of a simple and easy weed control program (Fernandez-Cornejo and McBride, 2002) is one such factor. Its importance was recognized soon after the technology had been introduced, when promotional material from the technology provider (Monsanto Company, 1997) quoted farmers as saying: "Roundup Ready gave us the cleanest beans we've ever had, and a great crop in general. To be honest, we didn't have to work that hard to get it." Or: "Roundup Ready is a terrific management tool for cleaning up ground." The relative value that farmers place on economic factors versus those of convenience is a personal decision that varies from individual to individual.

Experience in Illinois

Four groups of farmers in Illinois were recently surveyed to determine their selection criteria in choosing soybean varieties to plant (Bekric et al., 1999). These decisions are typically made in the winter, and for 1999, the most frequently cited sources of information were seed company dealers (87-100% of farmers) and test plots from seed companies (58-86% of farmers). No more than 53% of the farmers in any group relied on information from public sources—either variety performance data from the University of Illinois or recommendations of university extension educators. Thus in deciding which varieties to purchase and whether or not to adopt Roundup Ready technology, Illinois farmers apparently rely most heavily on recommendations from the companies that sell seed—and not on information from third party sources.

Norvell (2002) studied adoption of Roundup Ready technology in the soybean growing areas of the American Midwest by examining a comprehensive dataset from farmland owned by the University of Illinois. These lands are leased to commercial operators, who farm them to make a profit. Records from 45 farm tracts, ranging in size from 16 to 400 hectares, were analyzed between 1996 and 2000. The data included seed costs, the varieties that were planted, costs to control weeds, yields, and sales prices. In analyzing this information, Norvell (2002) sought both to measure economic impacts of the technology and to understand why farmers chose to adopt it.

Adoption rates on the University of Illinois farms were typical for the U. S. Midwest. The percentage of the acreage plated to Roundup Ready varieties increased from under 5% in 1996, to 24.3%, 49.1%, 52.0%, and 46.5% in 1997, 1998, 1999, and 2000, respectively. There was no evidence that the technology provided a consistent economic advantage over traditional soybean varieties. Average yields were virtually identical (3.28 tons per hectare for Roundy Ready varieties versus 3.26 for conventional varieties), and as expected, seed costs were higher and herbicide costs lower for the adaptors. Across all years and farmers, and in contrast to the conclusions of Mara et al. (2002), there was no statistically significant difference in net revenues per hectare.

Some individual farmers in Illinois can nevertheless achieve significant economic benefits by planting glyphosate-tolerant soybean varieties. Depending upon the weed pressure, savings can be as high as $33 per hectare (Bullock and Nitsi, 2001). It is noteworthy, though, that Illinois farmers apparently disregard experiences from the past year in deciding which varieties to purchase for the next year. This happens even though past performance of soybean varieties—as measured by yields and absolute returns—provides an accurate predictor for future performance, at least on University of Illinois farms (Norvell, 2002). For any given farmer and year, the decision to adopt or not to adopt is clearly complex, not yet fully understood, and mediated by intangible and difficult to measure factors that fall into the realm of personal preferences (Carpenter and Gianessi, 1999).

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Soil management and organic matter dynamics in the Cerrado of Brazil

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The cerrado ecosystem

The neotropical savannas of the north of South America cover approximately 267 million ha distributed as follows: Bolivia (14), Brazil (204), Colombia (17), Guyana (4), and Venezuela (28) (Borlaug and Dowswell, 1994).

In the Central Plateau of Brazil, the Savanna that is called Cerrado occupies 204 million ha, which is approximately 24% of the national territory and extends from 5°50'N to 21°26'S and from 40°47'W to 65°18'W. It includes the States of Goiás, Minas Gerais, Tocantins, Bahia, Maranhão, Piauí, Mato Grosso, Mato Grosso do Sul, Pará, Ceará, Rondônia, and Federal District, and the satellite areas in States of Roraima, Amapá, Pará, and São Paulo.

According to Azevedo and Caser (1980) the Cerrado has several climatic variations. About 80% of the rainfall occurs from October to March with a dry spell of 2 to 4 wk called "veranico", the common periods without rain that occur in January and February. The precipitation in 75% of the region ranges from 1,000 to 1,800 mm and the water deficit in 88% of the region lasts 4 to 7 mo. The annual average temperature varies between 18° and 23°C, depending on the altitude and latitude.

Most soils in the Cerrado (73%) are located between an altitude of 300 to 900 m while 22% are below 300 m, and only 5% are above 900 m (Adámoli et al., 1986). The toposequence, in general, is characterized by a peneplane localized on top of plateaus ending in steep scarps. From 100 to 200 m below this plateau there is another plane with almost flat to undulating relief, with slopes varying from 3% to 8%.

Latosols (Oxisols) occupy 46% of the region, Quartz Sand 15%, and Podzolics 15%. The former two occupy a plane and undulating topography on uplands, which are suitable for mechanization.

Latosols occuring on almost flat to undulating slopes facilitate intensive agricultural activities once their natural fertility limitations are corrected.

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These soils are considered of great potential for agricultural production either for annual or perennial crops and also for pastures. Latosols have two main constraints: 1) They are typically chemically poor and with low clay activity. Their mineral fraction is composed mainly of kaolinite, gibbsite, and iron oxides, associated with average organic matter contents varying from 25 to 30 g kg^{-1}. Also, they have a high aluminum content throughout the profile or at least to a 50-cm depth. 2) They are deep and very well structured with extremely high porosity (70%), equally distributed into macropores and micropores. However, the water availability, between -6 and -100 kPa water potential is low (about 10% by volume), which means plants have less than 1 mm of available water per each cm of soil horizon (Resck and Silva, 1990; Lopes and Guilherme, 1994).

Quartz Sand soils are not well developed, quite deep, acidic and with low nutrient availability, which occur on the flat to almost flat types of slope (0-3%). Their clay content is always less than 150 g kg^{-1} and they contain more than 800 g kg^{-1} sand; their structure is weakly developed, highly drained, and with low water availability, which makes them even more fragile than the Latosols when managed inappropriately. The organic matter content is generally low (~10 g kg^{-1}), but of extreme importance for conditioning most of the chemical and biological reactions of these soils.

Podzolics soils are moderately deep with accentuated differentiation between the horizons due to clay illuviation from the surface layer to the subsurface one. The fertility conditions are related to the nature of the parent material, mainly acid soils with low nutrient availability. They usually occur in undulating to rolling slopes (8-30%), which limit them for intensive farming.

The vegetation shows a physionomical diversity that according to its biomass, is called "Campo Limpo" where grasses dominate; "Campo Sujo" with some shrubs and trees and the dominance of grasses; "Cerrado" latu sensus where there is a balance between shrubs, trees, and grasses; and "Cerradão" where the trees dominate with some of them reaching 6 to 7 m high (Resck et al., 2000). The Cerrado biodiversity is still a little known natural resource. According to Almeida et al. (1998), there are more than 120 native species with ornamental, medicinal, forage, fruit, and lumber potential in the Cerrado ecosystem.

This region is crossed by five out of eight important watersheds of the country, ranked as follows by area: Tocantins-Araguaia (29%), Paraná (19%), São Francisco (19%), Paraguai (17%) and Amazônia (16%). Three of the most important hydroelectric plants depend on this region: Itaipu (Paraná River, 12,600 MW), Tocantins (Tocantins River, 7,745 MW) and

Paulo Afonso (São Francisco River, 3,986 MW). The potential capacity of these rivers could be at least four times greater than that mentioned but little by little bank beds are being formed due to soil erosion and sediments deposit, which diminish their capacity and affect the water quantity and quality (IBGE, 1989; Resck, 1991).

The Paraná-Paraguay Rivers system includes the area of the Southwest of the State of Minas Gerais, towards the south, until reaching Atlantic Ocean through Rio da Prata.

The São Francisco basin is the greatest fluvial system located inside the Brazilian territory. It runs 1,609 km towards the northeast to the Atlantic Ocean. Like the Paraná and Tocantins Rivers, the São Francisco River starts in the Cerrado, Central Plateau of the country. The upper river course is navigable by smaller crafts in some areas, but only the last 277 km are navigable by vessels and small ships.

Soybean cropping in the Cerrado

For the 21 year period from 1975 to 1996, soybean area increased from 235,736 ha (4% of the total national area) to 4,267,513 ha (40% of the total). There was an increase of 1,710% in the cultivated area of soybean. In addition to the area, soybean yield also increased by 50% from 1,463 kg/ha to 2,198 kg/ha. Contribution to the national production raised from 3% in 1975 to 43% in 1996 (~10 million t), it was a phenomenal increasing of 4,191 % (IBGE, 1997), considering only five states (Minas Gerais, Mato Grosso, Mato Grosso do Sul, Goiás, and Distrito Federal), which represent 66% of the Cerrado and 16% of Brazilian territory. The contribution to the national grain production raised to 50% in the cropping season of 2002/2003 (~25.3 million t), to 48% in area (~8.7 million ha), and to 104% with respect to yield, respectively (average 2,914 kg ha^{-1}, c.v.= 5%) (CONAB, 2003). Considering a soybean crop residue/grain ratio of 1.5, and a C/N ratio of 14 (carbon content= 580 g kg^{-1} and N= 41.4 g kg^{-1}), about 15 million Mg of carbon could be incorporated in the soil this year. Most of this carbon is decomposed easily by the soil microorganisms because of the low C/N ratio.

Soil management and soil organic matter dynamics

It is almost impossible for only one management system to counterbalance environmental restrictions related to climate and soil in any ecosystem and, therefore, it is necessary to consider instead a set of management systems.

To overcome the Cerrado environmental restrictions of chemical, physical, physicochemical, and climatic nature, the so-called "Tropical Paradox" is

established (Resck, 1998). First, soil must be mixed with lime as deeply as possible to improve its chemical status (higher pH and bases saturation) and to activate soil organic matter (SOM) that has low activity. The clay fraction of Latosols and Quartz Sands predominantly contains 1:1 type clay minerals (with little or even no isomorphic substitution) and iron and aluminum oxides. Consequently, these soils have a low net negative surface charge density and most of their CEC is attributed to SOM due to ionization of the carboxylic, enolic, and phenolic groups. Increase in soil pH increases charge on SOM and clays, but SOM has a much greater effect on CEC than clay (Resck, 1998; Helling et al., 1964). Effects of SOM and clay fraction on CEC are not additive because part of the clay charge is blocked by the SOM and it cannot be detected by the normal procedures of CEC determination. Silva et al. (1994) showed that SOM is responsible for about 75% and 81% of the total CEC in Latosols and Quartz Sands soils, respectively. Raij (1969) also showed that the average contribution of SOM to CEC in soils from the State of São Paulo was 74% for the surface layers compared with 35% for B horizons.

Latosols have medium to high SOM contents. About 17% of the Cerrado soils have less than 8.7 g kg^{-1} of organic carbon and these are mainly Quartz Sands (Lopes and Cox, 1977).

By using activated SOM more charge sites (CEC) are created, which improves soil aggregation and soil water storage capacity as a final result in the whole profile. In uncultivated soils, therefore, charge can be increased by liming and fertilization. Resck (1986) found that liming increased CEC in a virgin Dark-Red Latosol (Typic HaPlustox) by 93% compared to an increase of only 6% in a cultivated soil. The objective in cultivated soils is to improve aggregation and water storage capacity to increase nutrient supply to the plants. The more plant biomass that is produced and returned to the soil, the better are the soil physical properties due to enhanced microbial activity. The addition of biomass strengthens nutrient recycling mechanisms. These interactive effects can be summarized as follows: better soil chemical condition, more CEC, more plant biomass, more microbial biomass, more charge and more cementing by-products, more soil aggregation, more water storage, and more nutrients availability to plants.

Once the soil physical and chemical quality is restored, the soil management practices adopted must maintain or sustain the soil quality thus achieved. It is a paradox because in order to do that, soil chemical, physical, physicochemical, and microbiological properties must initially be built up. Undisturbed systems such as forest, pasture, fruit trees, and minimum tillage and no-till practices are better suited to maintain the desirable condition (Resck, 1998).

Carbon sequestration and CO_2 fluxes

Carbon transfer from the atmosphere to vegetal biomass and to soil is a natural process and its accumulation constitutes C sinks. It is believed that it is possible to attenuate or to overcome atmospheric CO_2 concentration increase by C sequestration and accumulation as C sinks in agricultural areas of tropical regions (Lal et al., 1995). Therefore, C accumulation in soil would compensate for CO_2 emissions, helping countries to reach emission levels established in the Kyoto Protocol, in 1997. Nowadays, there is a worldwide interest to understand the C fluxes behavior of tropical savannas, mainly as sink to atmospheric C. According to Parton et al. (1995), tropical savannas actually are a modest C sink, but those areas are precisely the ones subjected to management pressure from over-grazing, frequent fires, and conversion to agriculture for grain production. Soil C storage increased in Colombian pastures after the introduction of deep-rooted African forage grasses and legumes into native savannas, both with adequate soil fertilization (Fisher et al., 1994). In the Cerrado region in Brazil, about 49 million ha of native pastures have been substituted for cultivated African grasses (Embrapa Cerrados, 1999) and according to Scurlock and Hall (1998), they could be storing an additional 0.1 - 0.5 Pg C yr^{-1}. However, earlier, Macedo (1995) said that most of those cultivated African grasses in pastures were experiencing some degradation of soil chemical, physical, and biological properties due to poor management. Under these circumstances, tropical pastures could reverse their role as C sink, leading to C losses either by erosion or oxidation.

Changing conventional soil tillage systems (with soil disturbance) to minimum or no-till systems reduces C loss and contributes to its maintenance in the soil. Establishment of cultivated pastures can result in an increase in soil C, which also is possible in reforested areas. Research in a Cerrado soil by Corazza et al. (1999) showed that in areas under no-till system, pastures, and Eucalyptus forest, C content was kept higher that in native areas, while in areas managed with disk plow and heavy disk harrowing during 10 yr followed by 2 yr of pasture (*Brachiaria decumbens*), the C content was lower.

Carbon transfer (CO_2) from atmosphere to plant biomass (annual or perennial crops) is much faster (few years) than transfer from plant to soil. Changes in soil C are small and take many years to become detectable; net gains, if extrapolated to no-till, pasture, and forest areas in Brazil, will represent a small impact to mitigate the emission effect. The best and reasonable understanding of the above statement, is that, even having a beneficial effect and being highly recommended, these soil management systems can not be seen as the only solution to overcome the greenhouse gas effect.

However, in another study, in the same area, from April, 2000 until May, 2001, i.e., during a 398 day-period, Ferreira (2002) did not confirm the significant difference between no-till and disk management systems that produced in average 8,000 kg ha^{-1} of $C\text{-}CO_2$. This occurred because, throughout the year, during the dry season, there is more soil water under the no-till system and, therefore, more respiration that counterbalanced higher CO_2 emission by the conventional system after plowing at the beginning of the rainy season. Therefore, much more research is needed to evaluate the importance and the effect of improved pastures and soil management practices on the fluxes and C storage in soils of Brazilian savanna.

For Cerrado soils, the objective is to establish a management system that preserves soil organic C; removes atmospheric CO_2 by photosynthetic activity; and increases biomass, grain, fruit, fiber, wood, energy, and meat productivity. Adequate use and judicious management of soil-nutrient-water-plant system can enhance CO_2 sequestering potential, reversing the historical trend of C losses in cultivation of agricultural land. Integrated livestock and no-till farming systems must be stimulated due to their high C storage capacity and, hence, CO_2 sequestration.

Effect of tillage on soil physical, physicochemical, and microbiological properties soil aggregation

Tillage operations in the Cerrado region normally start in the dry season (July, August, or September). Generally farmers have large areas to prepare and few tractors. To speed up tillage operations, they use heavy disk harrows as the most common implement, which has many consequences. Most soils are well aggregated but are destroyed by this implement's action that cuts, exposes organic matter to microbial action, and forms a compacted layer at 12 to 15 cm below the surface.

In Latosols with 450 g kg^{-1} clay, non-disturbed areas under virgin Cerrado type vegetation, *Brachiaria* pasture and Eucalyptus (*Eucalyptus* spp.) forest had more than 80% of the aggregates > 2.0 mm in diameter in the arable layer. Disturbed areas, prepared with a heavy disk harrow, and cultivated for 10 yr either with a soybean-corn or rice-corn rotation, had only 20% of aggregates > 2.0 mm. Soil carbon loss in a soybean-corn rotation area corresponded to 13 Mg ha^{-1} (7 g kg^{-1}) [1 ha = 2,000 Mg] whereas in a rice-corn rotation that loss was 17 Mg ha^{-1} (9 g kg^{-1}). Areas cultivated with green manure for 10 yr and prepared with a rototiller gave no more than 42% of water-stable aggregates, demonstrating soil structure damage caused by these implements despite soil incorporation of large amounts of crop residues (about 10 Mg ha^{-1} in both cases). The organic carbon content

in > 2 mm diam. aggregates of these systems was: 16 g kg^{-1}, 13 g kg^{-1}, 13 g kg^{-1} and 15 g kg^{-1}, for Cerrado and pasture, forest and green manure, soybean-corn rotation, and rice-corn rotation, respectively (Resck and Silva, 1990). The failure to maintain a high percentage of aggregates > 2 mm in the disturbed area was caused by a decrease in the physically-protected organic matter pool (POM) (Duxbury et al., 1989) and, consequently, in its quantity that was several-fold less in the disturbed systems compared with those in undisturbed systems.

The effect of time of cultivation, type of soil tillage implement, and use of green manure on soil carbon is clearly shown in many circumstances in cultivated areas in the Cerrado region. A monitoring research on soil organic matter during 11 yr in a highly weathered clayey Oxisol (Red-Yellow Latosol, 700 g kg^{-1} of clay) showed that, just after clearing the vegetation, soil organic carbon content was 19 g kg^{-1} in the surface horizon. During the following 2 yr of upland rice cultivation, it increased to about 23 g kg^{-1}, presumably because of the decomposition of root residue of natural vegetation. Under continuous cultivation of soybean that soil prepared with heavy disk harrow, soil organic carbon fell to less than 17 g kg^{-1} because of a decrease in total residue inputs and an increase in microbial activity resulting from liming and fertilization. This loss was not greater because of the high clay content that makes the organic matter physically-protected pool even larger and more difficult to destroy than in loamy or sandy-textured soils. After 11 yr of cultivation, the soil surface horizon that originally contained 90% of > 2.0 mm soil aggregates under Cerrado vegetation had only 62% of > 2.0 mm aggregates and lower organic carbon content, which was less than 17 g kg^{-1}. As a consequence there was an even redistribution of soil aggregates into aggregate class diameters of > 1 mm, 0.5 mm, 0.25 mm, 0.1 mm and < 0.1 mm (Woomer et al., 1994). Water-dispersed clay content increased from 10% in soil under native Cerrado vegetation to 44% after 11 yr of cultivation. The heavy disk harrow pulverizes soil structure exposing the organic matter to microbial decomposition. Besides, that implement formed at the 12-15 cm depth a compacted layer clogged by incoming natural clays transported by the percolation of rain water. On the surface, it also leads to the formation of a thin crust that prevents water infiltration. Latosols are normally very resistant to erosion but after these processes there are considerable losses of soil and water, which carries nutrient enriched sediments: phosphorus (14 times greater), Ca and Mg (2 times greater), potassium (1 time), and organic matter (5 times greater) (Resck et al., 1991; Resck, 1981).

Deep lime incorporation is a necessary management practice because of the low water availability of Latosols and yet much less of Quartz Sands.

Without soil chemical correction down to 40 cm, plants are very susceptible to climatic events, especially to dry spells ("veranico"), which can last more than 25 d. Considering a 40-cm layer of a soil profile, the Quartz Sand stores only about 16 mm of water as compared with 36 mm by the Latosols. That implicates much less resistance to the veranicos by the plants grown in that sandy soil.

The soil conditioning process can take at least 3 to 4 yr to improve soil physical and chemical properties. Having accomplished that phase, farmers should proceed to the second phase by either choosing an implement that causes least soil disturbance such as a chisel plow for minimum tillage or shift to no-till depending on their technical skills and financial resources. Moreover, farmers can adopt pasture or forest systems to maintain and even improve soil properties over time (Resck, 1998).

Despite the fact that pasture systems have, in general, a high respiration rate, soil carbon physically protected pools (aggregates) are formed very rapidly (1 to 2 yr), which allows for carbon accumulation in the profile. This is due to voluminous and deep root systems in pasture. Pasture is capable to form and preserve those pools deep in the soil profile. As evidence, aggregates increase with depth under pasture and eucalyptus agroecosystems.

No-till systems also result in organic carbon accumulation in the profile mostly in the surface layer (0 to 5 cm); however, they do not form aggregates, but only maintain soil structure. For an area cultivated under center pivot irrigation using a heavy disk harrow during 10 yr and left as a *Brachiaria decumbens* fallow field for 2 more years (pasture after harrow), soil structure was recovered within that period (Guedes et al., 1996). There is not a direct relationship between carbon content and aggregates formation. High organic carbon content in no-till systems does not reflect in aggregates formation. In fact, it was comparable to the disk plow system. It was concluded that this high stock of organic matter in no-till systems is located in the labile pool that lies mostly in the soil surface, with less interaction with soil particles.

Mendes et al. (2003) studying biological properties from a Cerrado Oxisol under conventional (disk) and no-till (NT) management systems found that the the NT system favored higher levels of acid phosphatase and arylsulfatase in its aggregates. The highest activities of ²-glucosidase were also found in the NT macro and microaggregates.

Cation Exchange Capacity (CEC)

An example of inadequate soil management due to the destructive effect of the heavy disk harrow largely used by farmers for tillage of Quartz Sand and Latosols soils is the following. The decay of soil organic matter (SOM) with time of cultivation (TC), and its effect on cation exchange capacity (CEC) were analyzed in fields continuously cropped with soybean in the West Cerrado region of Bahia State. Soils were grouped accordingly to clay content: Group I (clay content lower than 150 g kg^{-1} and from 150 to 300 g kg^{-1}, joined because their similar behavior), and Group II (clay more than 300 g kg^{-1}). SOM decay with TC occurred in all soils and after 5 yr of cultivation the amount lost was lower in Group I (15.7 Mg ha^{-1}) than in Group II soils (18.9 Mg ha^{-1}). However, those losses represented 69 and 46% of the initial SOM stock in Groups I and II, respectively. SOM decay with TC was studied fitting the model SOM = SOME + (SOMI - SOME)*EXP(-K*TC) to the data, where SOM is organic matter content at a given time, SOME is SOM at equilibrium, SOMI is the initial stock of SOM (before cropping), and K is the SOM decomposition rate, according to Dalal and Mayer (1986). K values estimated for Groups I and II were 0.300 and 0.244 % yr-1 and t½ (half-life) values were 2.74 and 6.07 yr, respectively, showing that the SOM rate of loss was higher in soils with low clay content, mainly in Quartz Sand soils where K = 0.299 % yr-1 and t½ = 2.15 yr. SOM decay was followed by a decline in CEC (61%, and 53% for the loamy Latosol; the decline in the Group II soils was 29%) and both factors were linearly related with a correlation over 0.80 for both groups. Stepwise regression tests, introducing the clay content into the linear model, showed that clay was only significantly important in Group II soils. CEC decline per unit percent loss of SOM was higher in Group I than in Group II soils, probably due to the contribution of the clay fraction to CEC in clayey soils (Silva et al., 1994).

Microbial biomass carbon and nitrogen

Microbial biomas carbon was measured in a long-term field experiment (20 years), under a corn-soybean crop rotation (Ferreira, 2002). Six management systems were selected: disk plow before planting (ADPP); disk plow after harvesting (ADPC), no-till after disk plowing in the first year (PDAD); moldboard plow before planting (AVPP); moldboard plow after harvesting (AVPC), no-till after moldboard plowing in the first year (PDAV) and a virgin area of Savanna type vegetation, Cerrado (CE), as a reference. Sampling was made in five periods: 1- April, at soybean harvesting time; 2- May, after harvesting time; 3- August, in the dry season with no crop on the field; 4- November, at the begining of rainfall season and corn planting time; 5- February, at com blooming stage. These samples were taken at

five depths: 0-5, 5-10, 10-20, 20-30 and 30-40 cm of a clayey Dark-Red Latosol (Oxisol), with three replicates and microbial biomass was determined by the chloroform-fumigation-incubation method. Microbial biomass decreased with depth in all treatments and sampling periods, This decrease was much higher in the CE, followed by no-till treatment. Microbial biomass found in the CE treatment was greater than all management systems. Microbial biomass found in no-till treatments (PDAD and PDAV) was greater than the post harvest treatments (ADPC and AVPC), but not significantly different from ADPP and AVPP, averaged over all four sampling periods.

Another study to determine the effect of different management systems on microbial biomass carbon and nitrogen (C_{mic} and N_{mic}) was carried out in the same long-term field experiment (20 years) in a clayey Red Latosol cultivated with corn in the Cerrado (Figueiredo, 2003). Eight management systems comprised by different implements and crop residues incorporation time were studied. Soil samples were collected in five depths: 0 to 5, 5 to 10, 10 to 20,20 to 30, and 30 to 40 cm, with three replicates and in three sampling periods - before corn planting time, 30 days after germination., and at corn blooming stage.

Treatments under no-till presented differences on C_{mic} and N_{mic} content. Whereas in the no-till treatment, which soil was cultivated by moldboard plow in the first year (PDAV), had greater C_{mic} content, the other no-till treatment, which soil was cultivated by disk plow in the first year (PDAD), had greater N_{mic} content, indicating that even after 22 yr the effects in the soil profile caused by the disk and moldboard plowing remain. In general, the greatest differences between no-till (PDAD and PDAV) or minimum tillage (ESCAD and ESCAV; chisel after disk and moldboard plow in the first year, respectively), and conventional tillage (ADPP, ADPC, AVPP, and AVPC) occurred before planting time, therefore, before soil plowing and fertilization to establish the main crop.

No-till System

In the Cerrado, the evolution of cultivated areas under no-till system (NTS) was very rapid. From 300 ha in 1981/1982, in Rio Verde city, state of GO, it jumped to 1,500,000 ha in 1995/1996, an stupendous increase of 499,900%, whereas in Brazil that increase was of 2,290%. Today, in the Cerrado region of Brazil, of the 13 million ha being occupied by annual crops, approximately 5 million (38%) are under NTS, an impressive figure, considering that only 22 yr passed since the first experience in the region. In 2001, only the United States had a larger NTS area than Brazil. Earlier figures, in million ha, showed: United States - 21.12, Brazil - 14.33,

Argentina - 9.25, Australia - 8.64, and Canada - 4.08; Bolivia - 0.35, Venezuela - 0.15 and Colombia - 0.07 occupied the 8th, 9th and 12th places, respectively (Canalli, 2001).

This NTS adoption is an ongoing process and it has started in several states located in the Cerrado such as Piauí, Roraima, and Rondônia. Only pasture is better than the NTS to protect soil against the splash energy produced by raindrop impact, which causes soil erosion and losses. Despite its soil protection characteristic, there are considerable water losses with no-till systems. In a soybean field (crop whose canopy protects soil surface) under no-till, water losses per hectare due to runoff corresponded to 1,616 m^3 of water, whereas for conventional tillage with a disk plow, it was 2,610 m^3. In a rough estimate, the amount of water lost under no-till just from 1 ha will be enough to supply water, annually, to 81 people consuming 20 m^3 per capita. That represents a volume of water sufficient to supply 55 l $person^{-1}$ daily in a city with 29,382 inhabitants (Resck, 2002a, 2002b). Taking into account that in the Cerrado today there are about 5 million ha cultivated under this system, these water losses become highly significant. It means that physical barriers to water movement such as terraces and contour levels should also not be ignored where this system is being practiced, despite it's innumerable advantages over conventional systems, mainly those using a heavy disk harrow.

Despite the abundant availability of water from October to April, there is practically no precipitation during the months of May to September in this region. As a result, there is not enough soil water to establish cover crops ("safrinha") to produce either grain – as it occurs in the Southern Region of Brazil, in the State of Paraná, for example -, or biomass for mulching.

An experiment conducted in Embrapa Cerrados, in Planaltina, Brazil, where an average annual precipitation is 1,500 mm, several species and strategies to establish cover crops after soybean (the main crop) were tested as follows: soybean in a sequence with two varieties of sorghum (*Sorghum bicolor* (L). Moench): Sorghum BR303 and Sorghum BR304, millet (*Pennisetum glaucum*; Millet SE), a forage radish (*Raphanus sativus*), and a black oat (*Avena strigosa* Sckreb). Also tested was stylosanthes (*Stylosanthes guianensis)* cultivar "Mineirão" in consortium with soybean, millet before planting soybean as the main crop (Millet BP), and conventional tillage. For conventional tillage, all biomass produced by soybean and any other vegetation at the location was totaled for comparison with other treatments.

The mean dry matter production of soybean for all treatments was 1,820 ± 290 kg ha^{-1} (coefficient of variation, c.v.= 16%) whereas mean of dry

matter produced by cover crops in all management strategies tested was 3,090 ± 420 kg ha^{-1} (c.v. = 14%), including the conventional tillage (Silva and Resck, 2000). In the core area of the Cerrado region very little dry matter is produced due to poor rainfall distribution. Furthermore, the dry matter decomposes rapidly.

The complete success of the no-till system, including agropastoral systems, is dependent on developing early ripening and productive cultivars of corn and soybean, or of cover crops (millet, forages, and legumes) that give high crop yields and cover crop residues in seasons with a water shortage. In spite of those limitations the adoption of no-till has increased exponentially due to the innumerable advantages such as fuel saving, shortening of planting time, soil erosion control, organic matter accumulation, reduced emission of the so called greenhouse effect gases, and others.

In order to make these savanna areas able to produce grains, meat, fiber, and energy it will require large amounts of credit obtained from loans at low interest rates and/or from partially subsidized federal programs. High technology and new inputs already available should be used and applied to already deforested areas to preserve most of the still untouched area.

Achieving a sustainable agricultural business could be a very important economic and social developing factor mainly because the Savannas Ecosystem represents the last and the greatest agricultural frontier of the planet.

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Socio-economic impact of soybean in Indian cropping system

O.P.Goel[1]

Introduction

The green revolution in India in the late 60s and early 70s was not a stroke of luck but a result of an endeavour that began when the US President Lyndon Johnson in the 1960s thought of teaching India a lesson by providing food aid in driblets at a time that India's food stocks were hardly sufficient for two weeks.

Dr. Norman Borlaugs' dwarf wheat varieties and Dr. Swaminathans' National Demonstration programme helped usher in a productivity increase in wheat when acreage under high yielding varieties rose from four hectares in 1963-64 to over four million hectares in 1971-72. This small govt. " national demonstration programme" became a mass movement and came to be known as the green revolution: The success of wheat was later replicated in paddy and also recorded in other crops like sugarcane, cotton, millets and oilseeds.

However, India's green revolution was mainly confined to the irrigated lands of North and South India, and chiefly in wheat and paddy. It was left to the central Indian states to meet the nations requirements in Edible Oils, fruits, vegetables and coarse grains.

Soybean cultivation in India picked up in the late 70's in the barren and unirrigated lands of MP in Central India, around the same time the famous Paddock brothers, often referred to as "prophets of doom" concluded in their book "Famine 1975" that by the mid seventies alteast half of India would be led to a slaughter house.

Soybeans in Indian cropping system

India is a land of paradoxes. Though it may have one of the largest cultivable land areas in the world, only 30% is irrigated and the balance 70% is at the mercy and vagaries of the monsoon, often exacerbated by the super dramatics of El Nino and La Nina.

The drylands, constituting 70% of the cultivable lands have undulating topography, irregular rainfall pattern, produce about 42% of the country's food, 83% soybean, 81% pulses and 90% oilseeds.

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Introduction of soybean cultivation in the drylands of M.P. came like a godsend. It being a hardy crop, grew well in the rainfed areas and with minimal expenses. Though soybean in India is a rainy season crop (mid June to end September), it is highly dependent on the monsoon and a very risky gamble than most other agri commodities. In spite of all the risks, soybean cultivation picked up very well, first starting with the black variety and gradually switching over to the yellow one. In 1981-82, area under soybean was 0.455 million hectares which now stands over 6 million hectares (CMIE)

On an average, Indian food grain yields are at best half of the best world yields and only 35% in oilseeds and pulses. There remains a tremendous scope to boost yields and production with the right technology and appropriate agronomic practices.

Notwithstanding all the negative factors the cultivation of soybeans in India has helped the farmers achieve a level of prosperity which has greatly improved their economic and social status. It helped the dry land farmers come out of their morass of cultivating crops which had very low value and yields and has permanently altered the Indian cropping pattern in Central India states. Moreover, it has the potential to grow well in the southern states of A.P.Tamil Nadu and Karnataka as well.

The rainy season crops of soybean area are:

Main Rainy Season (Kharif) crop of Central India

		Irrigation	Non Irrigation /Rainfed
1	Cotton	X	✔
2	Chick peas	X	✔
3	Paddy	✔	X
4	Soybean	X	✔
5	Ground Nut	X	✔
6	Maize	X	✔
7	Sorghum	X	✔

(Source : Deptt. Of Agriculture,Govt. of M.P., Bhopal website)

Cropping pattern of Soybean Area

		Mid June Oct	November March	April - Mid June
1	Soybean/ cotton /Check pea / Maize / Sorghum	✔	X	X
2	Wheat / Gram / Rapeseed	X	✔	X
3	No activity	X	X	✔

Soybean, is mainly sown as a stand alone crop. However, there is an effort now to grow it either with chickpeas or maize. Results with chickpeas have been more encouraging than with maize.

Soybean, being a rainy season crop in India and with an average duration of 90-95 days, is more tolerant to moisture stress (either deficient or excess) and has adapted well to the drylands.

Besides this, soybean in central India, has replaced areas previously covered by groundnut, cotton, maize, sorghum, pulses and coarse grains. (See Table 1).

Table 1. How Soybean substituted other crops.

S.No	Crop Replaced	% of Farmers
1	Sorghum	61
2	Groundnut	22
3	Maize	18
4	Cotton	12
5	Pearl Millet	3
6	Pigeon Pea	4

(Source ; ICAR Project 2000 by Badal Billore, Joshi etc.)

This happened due to the following reasons:
1. General perception that its an easy crop to handle. Grew well in rainfed areas.
2. Soil conditions improve by nitrogen fixation taking place.
3. Lower input costs and yields are better than other Kharif crops. Profitability vis-à-vis other crops is much higher.

4. Post harvest, when crop is transported to the factories market area for sale, transportation costs vis – a -vis other crops like cotton or maize are lower by atleast 20- 25%.
5. Post harvest, the fields can be used for a winter crop like wheat, gram or Rapeseed.
6. Soybean is a cash crop. The installed capacity to process soybeans is very large and the crop size is much smaller. Therefore, there is a ready demand for soybeans and finds enough takers.
Barring cotton, all other Kharif crops do not have enough industrial usage and suffer from severe price falls in case of excessive production.
7. Nitrogen fixation capacity (30 to 60 kgs per hectare) of soybean helps increase the production of the next crop (Bhatnagar, Joshi and Billore , 1995).

Comparative economies

It has been shown that wheat yields with soy wheat rotation gave 2.488 MT/ hectare as against wheat with other crops at 2.246 MT / hectare. (Survey Data).

Further, Indian farmers readily look to growing soybeans because they could clearly see the advantage over other crops which were sown so far:

Table 2. Comparative Cost of Cultivation of the Kharif Crops (Rainy Season Crops) [Figures in INRS.]

	Item Crop	Soybean	Paddy	Cotton	Jower Sorghum	Arhar Chickpeas	Maize
1	Preparation of land and sowing	600	800	1500	500	1000	1000
2	Cost of seed	1500	1000	2500	500	500	500
3	Fertilizer	1000	1500	1500	800	1000	1500
4	Irrigation	200	500	500	-	500	500
5	Plant Protection	800	1000	2000	500	1000	1000
6	Weed Control	500	500	1000	500	800	500
7	Harvesting & Threshing	1000	1000	2000	1000	1000	1000
8	Other Expenditure	500	500	1000	500	500	500
	Total	6100	6800	12000	4300	6300	6500
	Production Yield per / Ha	15	40	10	25	12	20
	Rate Rs / qts	1000/	300/	2000/	300/	1000/	600
	Total Income	15000/	12000/	2000	7500/	12000/	12000/
	Net Profit	8900	5200	8000	3200	5700	5500

(Source : CACP, Ministry of Agriculture, 2001)

Socio economic impact

As Soybean cultivation provided much better returns to the Indian farmers, soybean has changed the Indian cropping system for ever. The effect would be further consolidated and cemented if the technology and information gap amongst the farmers is further narrowed down.

The prosperity level experienced by the soy farmers has resulted into the farmers buying

1. BW / Colour TVs
2. Refrigerators
3. Cooking Gas
4. Housing
5. Motor Cycles
6. Tractors + Implements etc.
7. Mobile phones

A study conducted by MART (Market %\& Research Team), New Delhi and Anugrah Madison, Chennai found that though the penetration of durables was low in hinterland, most of the respondents around cities owned atleast two TV and refrigerator (88% had TVs and 75% had refrigerators). Against this, NCAER study showed that urban families had ownership of aleast 3 durables per family as against rural Indians' one per family.

After mobile phones, cars and white goods, now comes insurance. Rural India seems to have an appetite for it all. A recent study conducted by MART, the rural consumer now is willing to dole out anything from Rs.3500/ - and Rs.2900/- as premium each year. According to the study, while the awareness levels for life insurance is the highest, rural consumers are also aware of motor, accident and cattle insurance.

Another factor which contributed to the prosperity of the Soybean farmers was the very large installed processing capacity of 15 million tonnes as against an average crop size of 5 – 6 million tonnes. (SOPA Data Base 2001)

Then, due to low oilseeds yields and productivity and with a view to support the oilseeds farmers, initially the Govt. of India had restricted the imports of edible oil, and after 1996, allowed free imports but with a sizable duty protection. This also provided a support to the soybean farmers who were insulated from the import of cheap international edible oil prices.

All told, the soy farmers have experienced a very high level of prosperity over the last two decades inspite of a major handicap of low productivity

and a knowledge and information gap on agri practices. The introduction of soybean cultivation came like a manna. Now with the soy processors and stakeholders taking a big interest in helping the soy farmers and initiatives like soy choupals becoming very popular, Soy production in India only has to go up and up.

Here are two more tables which show how soybean cultivation positively impacted the socio economic lives of farmers in Central India

Table 3. Impact of soybean cultivation on Social Well-being as perceived by farmers.

S.No	Particulars	After Adoption of Soybean Cultivation (%)
1	Improvement in the Income Level of the Household	93
2	Improvement in Educational Level of Family Woman	60
3	Improvement in Educational Level of Family Woman	60
	Children	65
4	Better access to medical facilities	61
5	Greater employment to women	53
6	Greater involvement of women in benefit distribution	34
7	Greater Involvement of women in resource allocation	25
8	Greater Social Participation	12

(Source : ICAR Project 2000)

Table 4. This shows the economic surpluses (000 Rs) generated due to soybean cultivation in Central India

Year	Consumers' Surplus		Producers' Surplus			Total Economic Surplus
	Substitution Effect	Expansion Effect	Substitution Effect	Expansion Effect	Residual Effect	
1980-81	708.95	276.87	1095.60	427.9	1006.52	3515.84
1981-82	871.12	-57.80	1346.27	-89.32	658.79	2729.06
1982-83	1023.60	434.73	1581.92	671.86	1515.96	5228.07
1983-84	1183.36	988.72	1828.83	1528.01	1411.45	6940.37
1984-85	1379.22	2840.08	2131.52	4389.22	2205.16	12945.2
1985-86	1588.62	3643.67	2455.14	5631.12	2833.44	16151.99
1986-87	1776.90	3002.44	2746.11	4640.13	3125.31	15290.89
1987-88	1972.87	4039.02	3048.99	6242.12	2385.71	17688.71
1988-89	2258.78	9527.79	3490.84	14724.76	5375.43	35377.6
1989-90	2720.61	13454.98	4204.58	20794.06	6299.55	47473.78
1990-91	2693.00	22744.61	4579.15	35150.76	8032.73	73470.28
1991-92	3356.43	24547.14	5187.21	37936.49	13013.72	84040.99
1992-93	4021.22	37499.42	6214.61	57953.65	16565.17	122254.1
1993-94	4394.84	58452..89	6792.02	90336.28	18479.16	178455.2
1994-95	4681.83	48710.00	7235.55	75279.09	17577.08	153483.6
1995-96	4796.42	69360.41	7412.65	107193.37	22966.25	211729.1
1996-97	4781.84	66605.71	7390.12	102936.09	30533.92	212247.7
1997-98	4938.15	91588.33	7631.69	141545.6	28801.92	274505.7

(Source :ICAR project 2000)

Summary

So far, agriculture, which was a way of life for rural Indians, is gradually changing to be a more organized form of business activity. However, land holdings would continue to be small but accent on modernization and technical upgradation would lead to higher production and productivity.

Incorporation of soybeans in the Indian cropping system has not only increased the profitability and sustainability of the soybean farmers but also have increased their socio economic status, which can be further strengthened by increasing the productivity and production and competitiveness of all the stake holders. With a huge and largely untapped captive market, the Indian Soybean farmers are assured of a reasonably good prosperity in the times to come.

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VII WORLD SOYBEAN RESEARCH CONFERENCE

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Countering the threat to agricultural biosecurity in the Americas

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Abstract

One of the basic tenets of plant biosecurity is that the presence, actual or predicted distribution, intensity, and economic impact of any yield-reducing factor(s) must be known. The development of a real-time, GIS-based (geographic information systems) reporting system for new and emerging agricultural pathogens and pests is extremely relevant in the era of agricultural bioterrorism. The establishment of a real-time, GIS database network can be used to report, monitor, map (temporally and spatially), and predict the spread of new and emerging plant diseases and pests in the Americas. This database network can also be used to geospatially and temporally monitor endemic pathogens/pests. Diagnostic records from Regional and National Diagnostic Centers coupled with remote sensing, GIS, GPS, atmospheric transport models, and weather-based GIS risk prediction models, offers an integrated system of technologies to help ensure the production of a safe and affordable US food supply.

Introduction

Background. Biological warfare has the potential to have severe negative impacts on the political, social, and economic factors of society (1, 4, 8, 11, 13, 22, 25, 61, 67, 74-76). The attack on September 11, 2001 in the US, as well as the use of *Bacillus anthracis* (anthrax) as a weapon in the fall of 2001, are two recent examples that demonstrate that the Americas are vulnerable to attacks by terrorists. Agricultural bioterrorism can be defined as the intentional use of a biological organism as a weapon to strike (with terror) against a target human population by adversely impacting its agricultural biosecurity. The United States National Research Council (NRC) concluded in a 2002 report that US agriculture is vulnerable to bioterrorism directed against agriculture and that the nation has inadequate plans to deal with agricultural bioterrorism (36, 38, 39). It is likely that this is also true for all of the Americas. The use of biological weapons to attack livestock, crops, or ecosystems offers an adversary the means to wage a subtle, yet devastating form of warfare (9, 24, 25). The recent (May 2003)

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detection of bovine spongiform encephalopathy (BSE) in cattle at a single location in Alberta, Canada has had a devastating effect on the agricultural economy of Canada. This single report has brought fear and doubt to Canadian and US citizens regarding the ability of our two nations (and other nations) to produce a safe and affordable food supply (8, 36, 66, 74, 75). It has been estimated that the accidental or intentional introduction of harmful biological agents continues to cost the US economy, public health, and the environment more than $100 billion annually (64).

Needs assessment. The potential for (and consequences of) intentional bioterrorism attacks directed at agricultural targets is now recognized as a serious threat to agriculture in the Americas and their respective economies (1, 8, 14, 66, 74-76). Such attacks could come from foreign or domestic terrorists (13, 24, 25, 30, 37, 75). Although efforts to deter and prevent the introduction of new and emerging agricultural pathogens and pests have significantly increased since September 11, coordination within and among key federal, state, Land Grant Institutions, and private industry for the detection of, response to, and recovery from, potential or confirmed agricultural threats is largely lacking. The recent NRC Report "*Countering Agricultural Bioterrorism*" concluded that "As of spring 2002, no publicly available, in-depth interagency or interdepartmental plan has been formulated for defense against the intentional introduction of biological agents directed at agriculture". We cannot afford to be overconfident that efforts to prevent or deter acts of agricultural bioterrorism will, by themselves, ensure the biosecurity of US agriculture (31, 33, 39, 66, 76, 78). Collectively, we must also have a coordinated and effective detection, monitoring, and response system in place to mitigate terrorists acts aimed at agriculture. This Achilles heel greatly threatens the agricultural biosecurity of the Americas and requires immediate attention. Improved threat surveillance, detection tools, real-time monitoring (mapping) and coordinated information delivery systems need to be developed.

Enhancing the value of the National and Regional Plant Diagnostic Centers. Communication and coordination among Regional and National Plant Diagnostic Centers is vital for determining appropriate and rapid response/mitigation measures (39). The recent establishment of a National Plant Diagnostic Network (NPDN) in the US is a start. This consists of five Regional Diagnostic Centers that provides the infrastructure to: (i) facilitate intra- and inter-regional diagnostic collaboration, (ii) make accurate and rapid diagnoses/identifications, and (iii) gather temporally and geospatially-referenced diagnostic data. The mission of NPDN is to enhance national agricultural biosecurity by quickly detecting new and emerging pathogens and pests that threaten US agriculture. The NPDN has tremendous potential

to facilitate the rapid exchange of critical diagnostic information within and among the five Regional Diagnostic Centers. During crises, the exchange of real-time information concerning the rapid detection of emerging diseases and pests, the documented GPS geographical distribution (i.e., GIS disease/pest prevalence maps), and the predicted distribution and establishment of new and emerging (and endemic) diseases and pests, is paramount to effectively mitigate potential impacts. This can only be accomplished, however, if there is a coordinated effort among the five Regional Diagnostic Centers to develop compatible hardware and software platforms. To date, three of the five Regional Diagnostic Centers, The Great Plains Diagnostic Network (GPDN, Kansas State University), the North Central Plant Diagnostic Network (NCPDN, Michigan State University), and the Northeast Plant Diagnostic Network (NEPDN, Cornell University) have agreed to coordinate hardware and software to communicate and share diagnostic data in real-time. Moreover, these three Regional Diagnostic Centers each have future plans to utilize geographic information systems (GIS) to map, in real-time, the geographical distribution of confirmed occurrences of new and emerging (and endemic) plant pathogens and pests. Our research team is a leader in publishing GIS plant pathogen/disease intensity and impact information (7, 18, 41, 45-47, 52-55, 57, 58, 70). My research group is also currently developing a real-time, GIS disease/pest database that will generate real-time GIS maps to monitor the occurrence and spatial spread of new and emerging pathogens and pests.

Post introduction forensics protocols. Efforts to date to counter agricultural bioterrorism have dealt primarily with preventing the introduction of new and emerging diseases and pests or the development of methods to improve disease/pest detection (36, 38, 39). Little attention has been given to preparedness after the introduction of a new or emerging plant pathogen or pest has been confirmed (39). One of the key post-introduction needs will be to determine if a pathogen/pest was introduced (i) accidentally, (ii) by natural means (long distance atmospheric transport, or (iii) by a deliberate act of agricultural bioterrorism. Plant disease epidemiologists will play a major role in post-introduction forensics just as epidemiologists from the Center for Disease Control and Prevention (in Atlanta) play a major role in post-pathogen introduction forensics and risk assessment for human pathogens. Plant disease epidemiologists trained in sampling and disease assessment can provide valuable quantitative and qualitative information concerning (i) the presence of primary foci, (ii) the spatial pattern of primary foci (random, clustered, regular), (iii) the presence of disease gradients, (iv) the presence or absence of disease foci (gradients) close to access roads, field edges, etc., (v) the presence of disease gradients from point (foci), line, or area sources (which may or may not indicate that pathogen

inoculum was deliberately introduced), (vi) sampling to determine if the introduction consists of a genetically and morphologically homogeneous or a heterogeneous population of isolates/strains, races, etc. and (vii) the presence and spatial pattern of the pathogen/pest in neighboring fields (e.g. by mapping the distance of infected/infested fields from major interstate highways using GPS and GIS technologies).

Generating plant disease epidemics deliberately requires relatively low technologies with the potential for high negative impacts. Geostatistics can be used to determine if the pattern of diseased fields is random or nonrandom (deliberate?). Nutter (45) previously published paper on the theoretical basis for generating plant disease epidemics for the purpose of quantifying the relationships between disease intensity and crop yield and quality. For example, in 1984, Nutter used a remotely piloted aircraft (in collaboration with USDA/ARS scientist Dr. Tim Gottwald) to inoculate a field of wheat with leaf rust (*Puccinia triticina*) (formerly *recondita* f. sp. *tritici*). A population of spores (inoculum) was produced from wheat plants in a greenhouse, mixed with a special oil, and applied as an aerosol to wheat plants from a height just above the wheat canopy. Ten days later, there was a line source of leaf rust in this wheat field. The experiment was a success in that a range of disease intensities was achieved due to the disease gradients that resulted from the establishment of a line source of leaf rust in the center of the field. Although a few cars may have driven by during inoculation, no suspicions were apparently aroused. Moreover, the presence of disease gradients following secondary spread (high disease intensity near the line source with disease intensity decreasing with respect to distance from the line source) would indicate a local, possibly deliberately introduced, source of inoculum (which it was!). The spatial pattern of point sources (foci) along fence lines close to access roads might also indicate that a pathogen was deliberately introduced. Geostatistical tools can be used to confirm or reject these hypotheses. There is also a need to develop sampling protocols as well as chain of evidence protocols once pathogen isolates are obtained (for identification, sequencing, characterization, etc.). Thus, with appropriate sampling and timely disease/pest assessment/detection protocols already in place, a more rapid and accurate determination can be made as to whether the introduction of a plant disease or pest was naturally, accidentally, or deliberately (bioterrorism) introduced.

Coupling GIS, GPS, and remote sensing technologies. The coupling of remote sensing data with GPS and GIS-referenced disease/pest data offers a potentially powerful monitoring and risk assessment tool for plant biosecurity in the post-September 11 era. Quantitative, geospatially-referenced data concerning the impact of new and emerging plant diseases and pests on

crop (plant) health and productivity can provide powerful (quantitative data to document impact. Our laboratory presently works with remote sensing images (data) obtained from three platform levels (ground, aircraft, and satellite) (6, 19, 53, 58). Assessments of plant health and productivity are currently conducted using visual ground-based observations for disease/pest intensity (20, 41-43, 50, 51, 56) and/or the use of hand-held multi-spectral radiometers (6, 18, 19, 41, 45, 47, 51, 53, 54, 56, 58). Although these methods are appropriate for within-field temporal and spatial assessments, they are both time- and labor-intensive (41, 48). If the crop production area to be monitored and assessed is large, then aircraft and satellite remote sensing platforms will be required to detect and quantify disease/pest-induced crop stress. Integrating remote sensing technologies into the day-to-day management of cropping systems (precision agriculture) offers tremendous new opportunities for agricultural producers to cost-effectively narrow the yield gaps (35, 40, 52) that presently constrain, or threaten to constrain, agricultural cropping systems. Thus, remote sensing, GPS, and GIS technologies can be integrated to detect, monitor, and quantify the probable impacts of plant pathogens and pests on crop yield and quality.

Atmospheric transport models-or which way did they go? Once a new or emerging disease or migratory endemic disease or pest is found (diagnosed), it is highly critical to be able to predict the local, regional and long-distance dispersal (transport) potential of the newly introduced pathogen/pest. Meteorological conditions influence, and in many cases enable, the dispersion of plant pathogens and pests. The process of dispersion (release, transport, deposition) is paramount to the development of disease epidemics and pest outbreaks – without this process, there would be no epidemic or outbreak (5). Pathogen/pest atmospheric transport models provide a link between meteorological conditions combined with the biological and physical properties of the pathogen or pest (3, 5, 10, 12, 28, 59, 60, 62, 69, 71). A number of pathogen, pest and crop-specific models have been developed to predict the spread of pathogens and pests (Klein, Paulitz, Fernando), however, a number of general short, meso- and long-distance models have been proposed (21, 62, 63). These include the NOAA Air Resources Laboratory Hybrid Single-Particle Lagrangian Integrated Trajectory Model (HYSPLIT) and the MM5 Community Model that was developed at The Pennsylvania State University (http://www.mmm.ucar.edu/mm5/mm5-home.html) and the National Center for Atmospheric Research (http://www.ncar.ucar.edu/ncar/). An advantage of the HYSPLIT model is that it can be used to model back trajectories (source of inoculum) which would be very helpful to determine if the pathogens/pests were disseminated by natural meteorological processes or were deliberately introduced.

Weather-based GIS risk assessment maps. Once atmospheric transport models predict where a pathogen or pest is likely to have been dispersed beyond the point of introduction, weather-based GIS data coupled with disease/pest warning models can be used to predict the probability of infection/establishment (2, 32, 72). Disease-warning systems are management decision aids that may provide economic and environmental benefits (40, 73) by increasing the efficiency of pesticide use (2, 15, 29, 32, 49, 55, 58, 68). Leaf wetness duration (LWD) is an important input to many disease-warning systems because the risk of epidemics for many foliar diseases is related to the duration of periods when free water is present on crop surfaces (23). Measuring LWD with electronic sensors is challenging, however, not only because of the inconvenience of installation and management of sensors and data loggers, but also because of the spatial variability of LWD within and among crops (15-17, 65).

A reliable forecast for leaf wetness duration (LWD), at least 24 hr in advance, could enhance the effectiveness of a disease-warning system by enabling growers to take protective actions before infection occurs or rainy weather prevents access to fields. For example, Shtienberg and Elad (68) reported that forecasted weather information was more effective than real-time measurements for control of *Botrytis cinerea* in vegetables. Private companies, (e.g., SkyBit, Inc., Bellefonte, PA), provide hourly, site-specific LWD forecasts using models that input forecasted air temperature, relative humidity (RH), and wind speed, for periods up to 72 h into the future. Despite the potential advantages of using forecast data, large inherent errors may hinder implementation of LWD forecasts, especially for physical models, whose accuracy is highly dependent upon accuracy of input data (34). Magarey (1999) reported that energy balance model based on hourly weather estimates resulted in large errors in LWD estimation. It may be possible to forecast LWD more reliably when empirical LWD models are used. Kim et al. (26, 27) showed that an empirical model incorporating physical principles into inputs of wind speed was able to estimate LWD within 1 h/day, using the same kind of site-specific weather estimates as Magarey (34). A LWD model based on fuzzy logic may be also useful to predict wetness occurrence, because fuzzy logic is known for its tolerance to imprecise input and allows adjustment of the outcome of model using a weight value (26, 77).

Disease/pest management, extension, and education component. The development of "Train the Trainer" workshops for "First Detectors" and "First Responders" and other stakeholders regarding disease/pest sampling, disease/pest assessment, sample processing, and record keeping, need to be developed and implemented. In addition, Web-based outcomes (GIS

maps for disease/pest prevalence, predicted spread, weather-based disease/pest risk maps) need to be developed and easily accessed from National, NPDN and Regional Plant Diagnostic Networks Web-sites. Finally, timely disease/pest alerts and disease/pest management recommendations need to be quickly disseminated to growers to mitigate the impacts of new and emerging (and endemic) plant disease s and pests.

Conclusions

One of the basic tenets of post introduction plant biosecurity (and precision agriculture) is that the presence, distribution, intensity, and economic impact of any yield-reducing factor(s) must be known (40, 48, 53, 55). The development of a coordinated real-time, GIS-based reporting system for new and emerging (and endemic) agricultural pathogens and pests coupled with remote sensing, atmospheric transport models, and GIS weather-based disease/pest warning systems are extremely relevant in the era of agricultural bioterrorism. Such tools will help researchers, extension personnel, private industry, crop consultants, and most importantly, agricultural producers, to implement disease/pest management strategies and mitigation tactics to better safeguard agriculture in the Americas from introduced and endemic plant diseases and pests.

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Actions and infrastructure for biosecurity in agriculture

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Abstract

An attack on a nation's crops, rangelands or forests could reduce crop yield and quality, consumer confidence, economic health, and possibly human nutrition. Most countries do not currently have adequate resources to quickly detect new plant pathogens or to unambiguously identify and trace them. Strategies for securing our plant resources must include prevention and preparedness. A strong national security plan should include steps for: (a) early detection and diagnostic systems, (b) microbial forensic technology , (c) epidemiological data for modeling, (d) effective and reasonable policies and guidelines, (e) a distributed physical and administrative infrastructure, (f) a national coordination plan and infrastructure, and (g) linkage to a strong, cooperative international initiative to coordinate security efforts at a global level.

Introduction

Many of the world's crops, rangelands and forests are vulnerable to the introduction of plant pathogens, whether by nature, accident, or nefarious intent. In wealthier countries such an event could cause negative impacts on crop yield and quality, consumer confidence in the food supply and the economies of rural and agricultural sectors. In less-fortunate countries it could lead to human hunger and suffering. In all nations the practice and communication of scientific endeavors may be affected by the implementation of new regulations and restrictions designed to enhance security.

Plant pathogens are easy to obtain, move around, and deploy. Most countries, including the U.S., do not currently have adequate resources to quickly detect new pathogens, particularly those from outside the nation's borders, or to unambiguously identify them and to trace their origin. A significant incident could quickly challenge our response and recovery systems beyond capacity.

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National and international crop biosecurity issues

Strategies for securing our plant resources (crops, range and forests) must address both prevention and preparedness (1). Efforts and resources have so far been targeted largely towards prevention, with strategies aimed at eliminating the introduction and movement of pathogens and pests. Elements of effective prevention may be different from those needed to respond to and mitigate an attack should one occur. New investments in preparedness will be very critical in our overall national security programs (1, 2, 3, 4). A strong national security plan should include steps for:

A. Early detection and diagnostic systems, needed to recognize a potential epidemic before its damage becomes significant and to positively identify the pathogen to subspecies levels

B. Microbial forensic technology to determine the source of the pathogen and to provide evidence related to attribution

C. Epidemiological data for modeling and predicting pathogen movement and impact, and novel disease management strategies

D. Effective and reasonable policies and guidelines for crop biosecurity

E. A distributed physical and administrative infrastructure within which all activities are coordinated and processed

F. A national coordination plan and infrastructure

However, no national plan can be optimally effective on its own. Many are calling for a strong, cooperative international initiative to coordinate security efforts at a global level.

Early detection and diagnostic systems

Research

We must support new research initiatives in genomic sequencing and functional genomics of threatening plant pathogens. There is significant potential for rapid and reliable pathogen detection, and absolute identification, using pathogen-unique sequences. We must be able to recognize exotic pathogens that may be new to a location, differentiate among taxa (at the sub-species, pathovar, and isolate levels), and detect genetically altered forms of familiar pathogens. Unfortunately, genomic information is currently available for only a few plant-associated microbes, most of which are not among the organisms that pose the greatest risks to crop security. To optimally exploit genomics for biosecurity there is an urgent need for more aggressive sequencing programs targeted at select agents.

People

Current personnel trained to detect and diagnose new diseases and pathogens are inadequate for the proposed intensification of biosecurity efforts. Enhanced training opportunities are needed for those currently in

diagnostic, extension, and crop consultant positions. In addition, more personnel must be trained to achieve the speed and accuracy necessary for a stong biosecurity response.

Microbial forensic technology

The science of forensics is aimed at tracing pathogen origin and the timing of initial infection, with the ultimate goal, from a criminal perspective, of absolute attribution. Such efforts are necessary for, and linked to, the activities of both security and law enforcement communities.

Epidemiology and disease management

1. Modeling of the global movement and impact of select agents is essential for prediction of natural introductions and epidemics. Effective models require extensive knowledge of pathogen biology, host-pathogen interactions, dissemination mechanisms including alternate hosts and/or vectors, and current geographical distribution of threatening pathogens. Electronic disease reporting systems must be developed for rapid and coordinated communication and response. Increased international cooperation will be critical, as many diseases occur in inaccessible areas where they may escape notice.
2. Because resources in funding and personnel are limited, it is also important to gather and model information related to risk-assessment, to guide in the prioritization of needs.
3. Novel disease management strategies also are needed to prevent, or to quickly and effectively limit, damage from an introduced pathogen or pest. Traditional approaches must be optimized, and new technologies exploited, specifically, for each priority agent. Response cascades must be put in place for each priority pathogen and for categories of unknown pathogens.

Effective and reasonable policies and guidelines

Biological scientists are not used to being subjected to rigorous regulation of experiments, movement of organisms or publication of research results. However, any data may be used for both benefit and for harm, a phenomenon called the "dual use dilemma" (5). How can we foster the aggressive and creative scientific research that is critical, not only for human health and welfare, but also for international security, while also limiting the potential for mis-use of that same research? In the U.S., the findings of research supported by federal funding are placed into the public domain unless designated 'classified.' However, an un-official new category, 'sensitive,' has arisen recently. Unfortunately, the term is very difficult to define. Policy-makers and the security community have different views of what may be sensitive than scientists do. Many believe it should be the scientific community, rather than the security community, that defines the standards

and policies for sensitive research and its findings. Such policies are likely to be more reasonable, effective and accepted by the scientific community if they arise from within its own members.
In the U.S., several pieces of regulatory legislation are already in effect, including the Patriot Act of 2001 and the Bioterrorism Preparedness and Response Act of 2002. Scientists' concerns include:
• Stricter regulation of the acquisition, handling, and security of high threat pathogens and toxins
• Requirements for higher biocontainment levels (resulting in costly remodeling and upgrading)
• Loss of investigator privacy due to background checks
• Restrictions on who can have access to and handle certain agents
• Scaled-up monitoring and permitting requirements for experiments involving "select agents"
• Delays in bureaucratic processes
• More reporting and accountability
• Difficulties for international student/scholar visas
• Restrictions on publication of "sensitive" scientific results

The U.S.'s National Academy of Sciences recently charged its National Research Council (NRC) to consider these issues, and in November 2003, the NRC published its "Proposed Strategy for Oversight of "Sensitive" Scientific Inquiry" (4). The report included a 7-step process for responsible and effective oversight of potentially sensitive experimentation:
1. Educate the scientific community about dual-use research and scientists' responsibily to mitigate its risks.
2. Establish a review process for seven categories of experiments relating to threatening microorganisms. These include studies that:
• Render a vaccine ineffective
• Confer resistance to antibiotics or antivirals
• Enhance or enable virulence
• Increase transmissibility
• Alter host range
• Enable evasion of detection or diagnosis
• Enable weaponization

The review would be done by the existing Recombinant DNA Advisory Committee (RAC), a unit of the National Institutes of Health formed several years ago to self-regulate experiments involving recombinant DNA.
3. Review of research at the time of publication, to be a self-governance process by scientific publishers.
4. Create a National Science Advisory Board for Biodefense (NSABB), administered through the U.S. Department of Health and Human Services, to provide case-specific advice and to assist the self-governance process.

5. Rely upon existing legislation and regulation, with review by the NSABB, to provide protection of bio-materials, physical containment and personnel supervision.
6. Establish more effective communication channels between the scientific community and those charged with national security.
7. Convene an International Forum on Biosecurity, to develop and promote harmonized national, regional and international measures as a counterpart to our national systems.

A distributed physical and administrative infrastructure

The existing physical infrastructure in most nations for plant pathogen detection, diagnosis, forensics, and research, is inadequate to handle the many activities described. We must find funds and mechanisms to support new infrastructure needs.
1. In the U.S., the USDA's new National Plant Diagnositic Network (NPDN), a distributed set of plant disease and pest diagnostic laboratories, was based on an existing system of diagnostic laboratories at Land Grant Universities in each state. Each state laboratory reports new or unusual occurrences to a regional "hub" laboratory. Data are entered into a national database for analysis and action. This system has great promise, but much is still in development, and additional national coordination is needed.
2. We need more trained field personnel. The USDA's Cooperative Agricultural Extension Service has long served as a critical link between the universities and their diagnostic labs with ag producers. However, recent financial limitations have led to severe understaffing in Extension. Greater support of these programs will help, but we have a valuable resource in our crop consultants, industry representatives, and amateur groups such as "Master Gardeners."
3. High-level containment (BL-3 and BL-4) facilities are very expensive to build and to secure. Thus, the number of locations able to support research on, or activities related to, select agents is very small. A coordinated plan for increasing our physical capacities for containment activities is crucial. It might include the placement of new laboratories and/or the upgrading of existing facilities in key locations.
4. The Centers for Disease Control (CDC), acts as a national clearinghouse for information and activity related to human health, but there is no national coordinating body for plant pathogens and crop health. Recently, the American Phytopathological Society (1.2.3) proposed the development of a National Center for Crop Biosecurity (NCCB) to build on, enhance, and provide leadership and coordination of national efforts for documenting, monitoring, and protecting crops, forests, and rangelands against new or emerging plant diseases. This Center could assure that the following are addressed:
• Program review, vulnerability assessments, and strategic plans;

- Coordination of plant biosecurity activities;
- Research needs related to plant biosecurity;
- Real-time database on new and emerging plant diseases and pests;
- Standards for diagnostics and lab certification;
- Pathogen and pest collections;
- Database of national and international expertise;
- Education and training;
- Complementing the National Plant Diagnostic Network; and
- Public relations.

A national coordination plan and infrastructure

Many of the strategies and concerns that exist for plant health and security are relevant also to human and animal security, and in fact, may already be effectively addressed in one of these related disciplines. Databases, tools and technologies, culture collections, and scientist expertise cross disciplinary boundaries. A national coordination plan is needed to link and coordinate biosecurity efforts among these communities. It would likely include government entitites (local, state, national and international), universities, private industry, professional societies, grower groups, and others.

Government

In the U.S., the recently formed Department of Homeland Security (DHS) has established the National Biodefense Analysis and Countermeasures Center (NBACC) to integrate all of the nation's efforts for homeland security, drawing on resources from public health, veterinary and plant pathology groups, law enforcement, and national security. NBACC's mission is to provide a unique, interdisciplinary capability to better defend against the full range of human, animal, and plant BSL-3 and BSL-4 biothreat agents.

Scientific Societies

Professional societies, independently or as coalitions, can help federal administrators and policy-makers create effective, mission-consistent policies. A respected society that represents the collective education, experience and perspectives of the nation in a discipline, and that can serve as a reliable, and unbiased information provider, can be a welcome resource. The American Phytopathological Society's National Public Policy Board began even before September 11, 2001, to call for new investments and preparedness against threatening plant pathogens. Although it isn't easy to measure progress in public policy, APS has seen resulting benefits to plant pathology such as more emphasis on plant pathogens in federal funding programs, positive changes in lists of pathogens for genomics and biosecurity, and the development of new funding programs.

International cooperation and initiatives

Because scientific capabilities and knowledge cross all national boundaries, no national security plan can be optimally effective on its own. Plans adopted and monitored at a global level will be most effective. The NRC's last recommendation (see above) (4), is harmonized international oversight of biosecurity. They propose a meeting of nations in an International Forum on Biological Security, sponsored by governmental and non-governmental entities. Suggested topics include:

- Education of the global scientific community about potential security threats, ways to minimize risk, and related ethical issues for scientists
- Designing mechanisms for international jurisdiction related to breaches of biosecurity
- Developing globally applied standards and policies for regulation of threatening agents among laboratories and nations
- Designing mechanisms for international management/oversight of research involving select agents
- Developing an international understanding for the dissemination of "sensitive" scientific information

Such a Forum would be a very positive step towards re-focusing the global community of scientists on the positive applications of our research and teaching. Smaller international cooperations are also instrumental in developing common understanding. Scientist exchanges, collaborative research projects, and international congresses such as the World Soybean Research Congress are good examples. The good will and common goals for the benefit of humankind, evidenced in such international meetings, will provide a strong underpinning for an effective global biosecurity plan.

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Economic importance of and control strategies for the major soybean diseases in Argentina

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Abstract

Diseases are among the biotic factors that reduce soybean production in Argentina. Since the introduction of this crop in the country several decades ago, the number of infectious diseases has increased steadily, especially since the early 1990s. Approximately 40 infectious diseases affect soybean in Argentina. Severe epidemics occurred within the last 8 years (Sclerotinia stem rot in 1995/96 and 1997/98, stem canker in 1996/97 and 1997/98, frogeye leaf spot in 1999/2000, and charcoal rot in 2000/01 and 2002/03). In addition, endemic diseases cause considerable losses every year. At present, there is great concern because of the presence of soybean rust in the northeastern part of Argentina and in neighboring countries (Paraguay, Brazil, and Bolivia), where it has caused high yield losses. Several factors have contributed to the increase in the number and severity of soybean diseases in Argentina including continued monocropping, no-tillage systems, susceptible cultivars, poor-quality seed, poor soils, and environmental conditions favorable for the development of most diseases. Integrated management programs are recommended which combine cultural, chemical, and biological methods, with special attention to the economic aspects of crop production. However, the implementation of these programs is still incomplete, and soybean production continues to suffer significant losses due to diseases, with the constant risk of the occurrence of new epidemics.

Introduction

The importance of soybean [*Glycine max* (L.) Merr.] in Argentina has increased considerably in the last two decades. Data on production, industrialization, and exports of soybean grain, oil, and meal indicate the growth and significance of the soybean industry in the country. In the 2002/03 growing season, 35.5 x 10^6 t were produced from 12.7 x 10^6 ha, with an average yield of 2.8 t.ha^{-1}. Argentina is the leading world exporter of soybean oil and soybean meal, exporting 9 x 10^6 t (43.3% of the world market in 2002) and 15.8 x 10^6 t (35.1%), respectively.

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In the 2002/03 season, 82.7% of the soybeans were produced in the northern Pampean region (provinces of Córdoba, central and southern Santa Fe, northern Buenos Aires and northeastern La Pampa). The northeastern region (Entre Ríos, Chaco, Corrientes, Formosa, Misiones, northern Santa Fe, and eastern Santiago del Estero) produced 11.8%. An additional 5.5% were produced in the northwestern region (Tucumán, Salta, Jujuy, northwestern Santiago del Estero and southeastern Catamarca). Soybean is planted as a single annual crop or as a second crop following wheat.

Infectious diseases are important limiting factors, as crops in most regions are grown under conditions that favor the survival and development of many of the numerous pathogens that affect soybean. The number and severity of infectious diseases have increased steadily, particularly since the early 1990s (Vallone and Giorda, 1997; Ploper, 1997, 1999). New diseases were reported in recent years causing major losses to growers.

This paper reviews the current status of soybean diseases in Argentina and the different strategies recommended in the country to manage the most important disease problems.

Evolution of soybean diseases in Argentina

In Argentina, soybean has been produced in large scale since the early 1960s. At first, crops were characterized by their excellent health. Diseases started to appear a few years later and some of them became major constrains to production. Seedborne diseases were the first to be detected. They had been introduced with seed from other countries and gradually were spread to nearly all regions due to poor seed production techniques. As a result of successive years with soybean and the expansion of the crops into new areas, other pathogens became adapted to soybean, thus increasing the number of diseases present in the crop.

Several factors have contributed to the escalation of disease problems in the country. In addition to problems with the seed, at first related to inadequate seed production techniques but in latter years to the lack of use of certified seed, other crop management practices that became popular during the 1990s have favored the survival of pathogens and the development of diseases. Continued monocropping and no-tillage systems, which are practices widely used at present in soybean production, have been related with many of the disease problems occurred in recent years.

Currently, approximately 40 infectious diseases are reported on soybean in Argentina (Tables 1 and 2). Yield losses for the 1997/98 growing season

were estimated at 8%, which at that time represented close to 1.4 million t (Wrather et al., 2001). A recent report mentions that soybean diseases in Argentina can cause 8-10% yield loss (2.8 to 3.5 million t) (Vallone, 2002).

The impact of these diseases is variable. Some of them are endemic and usually do not produce heavy losses, whereas others can cause severe epidemics under favorable conditions. Most disease problems have been solved using adequate management techniques, including the use of resistant cultivars and fungicide applications. However, the threat of new diseases or new races of known pathogens is continuous and requires permanent attention.

Prevalent soybean diseases in Argentina

The first diseases to be detected during the season are seed rots and pre- and postemergence **damping-off** caused by various pathogens, such as *Fusarium* spp., *Pythium* spp., *Rhizoctonia solani*, and *Phytophthora sojae*. However, these problems are only important in early plantings in the northern Pampean region.

Brown spot (Septoria glycines)

Is the most prevalent soybean disease during the vegetative stages and is endemic throughout all soybean-growing areas of Argentina. It can first be detected in the unifoliate leaves but symptoms progress to the upper leaves as the plants grow. Under favorable conditions it can cause significant defoliation. It is also important in the late reproductive stages, causing premature senescence as part of the late season disease complex.

Other foliar diseases start to appear towards the end of the vegetative stages. The most common ones are **bacterial blight** (*Pseudomonas syringae* pv. *glycinea*) and **downy mildew** (*Peronospora manshurica*).

Phytophthora rot (Phytophthora sojae)

Can be found in the vegetative stages as well as in reproductive stages. It was first reported in 1978, but only became important as growers started to adopt conservation tillage systems, particularly no-tillage. Its incidence in some fields in the northern Pampean region has been close to 100%. Only races 1 and 4 have been reported, although unpublished data indicate the existence of additional pathogenic variability.

Numerous diseases affect soybean in Argentina during the reproductive stages. In addition to bacterial blight and downy mildew, several other diseases can be found on the aerial parts of the plant. **Frogeye leaf spot**

(FLS) (*Cercospora sojina*) is one of the most destructive foliage diseases of soybean. It caused an important epidemic in northwestern Argentina during the 1999/2000 growing season (Ploper et al., 2001a). Yield losses up to 48% were reported in fields planted with susceptible cultivars such as A 6445 RG and Coker 6738. The importance of the disease decreased with the use of resistant cultivars; however, during the 2002/03 season, FLS was reported causing significant yield losses in some fields of central and southern Córdoba (northern Pampean region), where susceptible cultivars are still being grown.

A foliar disease that is increasing in importance, particularly in northwestern Argentina, is target spot (*Corynespora cassiicola*). In some seasons and locations symptoms can also be seen in stems and pods. On the other hand, *Alternaria leaf spot (Alternaria spp.)* and powdery mildew (Microsphaera diffusa) have remained at low levels so far.

Table 1. Occurrence of soybean fungal diseases in Argentina.

Common Name	Causal Agent
Alternaria leaf spot	*Alternaria* spp.
Anthracnose	*Colletotrichum truncatum*
Brown spot	*Septoria glycines*
Brown stem rot	*Phialophora gregata*
Cercospora blight and leaf spot	*Cercospora kikuchii*
Charcoal rot	*Macrophomina phaseolina*
Downy mildew	*Peronospora manshurica*
Frogeye leaf spot	*Cercospora sojina*
Fusarium wilt and root rot	*Fusarium* spp.
Phomopsis seed decay	*Phomopsis* spp.
Phyllosticta leaf spot	*Phyllosticta sojicola*
Phytophthora rot	*Phytophthora sojae*
Pod and stem blight	*Diaporthe phaseolorum* var. *sojae*
Powdery mildew	*Microsphaera diffusa*
Purple seed stain	*Cercospora kikuchii*
Pythium rot	*Pythium* spp.
Rhizoctonia root rot	*Rhizoctonia solani*
Sclerotinia stem rot	*Sclerotinia sclerotiorum*
Sclerotium blight	*Sclerotium rolfsii*
Seed diseases	*Fusarium* spp., *Alternaria* spp., etc.
Soybean rust	*Phakopsora pachyrhizi*
Stem canker	*Diaporthe phaseolorum* var. *caulivora*
	Diaporthe phaseolorum var. *meridionalis*
Sudden death syndrome	*Fusarium solani* f. sp. *glycines*
Target spot	*Corynespora cassiicola*
Violet root and lower stem rot	*Rhizoctonia croccorum*
Zonate leaf spot	*Gonatophragmium mori*

Table 2. Occurrence of soybean diseases caused by bacteria, virus, and nematodes in Argentina.

Common Name	Causal Agent
Bacterial blight	*Pseudomonas syringae* pv. *glycinea*
Bactterial pustule	*Xanthomonas campestris* pv. *glycines*
Wildfire	*Pseudomonas syringae* pv. *tabaci*
Alfalfa mosaic	*Alfalfa mosaic virus (AMV)*
Brazilian bud blight	*Tobacco streak virus (TSV)*
Peanut mottle	*Peanut mottle virus (PMV)*
Soybean mosaic	*Soybean mosaic virus (SMV)*
Geminivirus	*Begomovirus* (under study)
Cucumovirus	*Cucumovirus* (under study)
Lesion nematodes	*Pratylenchus* spp.
Root-knot nematodes	*Meloidogyne* spp.
Soybean cyst nematode	*Heterodera glycines*

Among diseases that affect roots and lower stems, *Sclerotinia* stem rot (SSR) (*Sclerotinia sclerotiorum*) was the most important disease until the mid 1990s, with yield losses that in some fields reached 55%. Adequate management techniques, based primarily on early maturing cultivars and early planting dates, have reduced the impact of this disease.

Sudden death syndrome (SDS) *(Fusarium solani f.* sp. *glycines)*

Was first identified in the northern Pampean region in the 1991/92 season and in the northwestern region in the 1992/93 season. Since its appearance, SDS has gradually increased in importance to become one of the most destructive soybean diseases in Argentina. Yield losses of up to 15% and 90% have been reported in the central and northern regions, respectively. Greenhouse and field evaluations have revealed that the cultivars differ in their reaction to this disease. In addition to SDS, there are other root and lower stem rots caused by different species of *Fusarium*.

Stem canker *(Diaporthe phaseolorum* var. *meridionalis)*

Became a very important disease in the mid to late 1990s. Severe epidemics occurred in the central and northern regions during the 1996/97 and 1997/98 growing seasons, with 100% yield loss in many fields as only a third of the cultivars available at that time had genetic resistance to stem canker (Ploper et al., 1997). The use of resistant cultivars the following seasons rapidly decreased the significance of this disease. In recent years, the causal

agent of the "northern stem canker" (*Diaporthe phaseolorum* var. *caulivora*) was identified in the provinces of Córdoba, Santa Fe, and Buenos Aires (Pioli et al., 2001). This has raised some concerns since the genes that confer resistance to *D. phaseolorum* var. *meridionalis* do not necessarily protect against *D. phaseolorum* var. *caulivora*.

One of the most prevalent diseases during the 2000/01 growing season was charcoal rot (*Macrophomina phaseolina*). Warm and dry conditions prevailed in several provinces, including Catamarca, Chaco, Córdoba, Entre Ríos, Santa Fe, Salta, Santiago del Estero, and Tucumán, resulting in heavy losses caused by this disease in soybean crops (Ploper et al., 2001b). Yield losses close to 100% were reported in some locations of the province of Santa Fe and Chaco, in fields with poor soils and little water accumulation capacity. Damage by charcoal rot also occurred in the 2002/03 season, although restricted to Tucumán, Catamarca, and Santiago del Estero, provinces which suffered a persistent drought coupled with very high temperatures.

The "late season disease complex", a combination of various diseases that becomes more noticeable in the mid to late reproductive stages, affects stems, leaves, pods, and seeds, causing a premature senescence and lowering yield and seed quality. The main diseases included in this complex are brown spot, Cercospora blight and leaf spot (*Cercospora kikuchii*), target spot, FLS, Alternaria leaf spot, anthracnose (*Colletotrichum truncatum*), and pod and stem blight (*Diaporthe phaseolorum* var. *sojae*). Occurrence of this group of diseases has gradually increased in most soybean-growing regions of Argentina since the mid 1990s, probably favored by successive years of continuous monocropping and no-tillage, as well as environmental conditions favorable for disease. Yield losses caused by this complex have been reported to be as high as 40%.

The incidence of Phomopsis seed rot (*Phomopsis* spp.) and purple seed stain (*Cercospora kikuchii*) is variable, primarily influenced by environmental conditions during the late reproductive stages. Other pathogens usually isolated from soybean seed are *Alternaria* spp., *Fusarium* spp., and *Colletotrichum* spp. Less frequently are detected species of *Aspergillus*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Epicoccum*, *Nigrospora*, *Monilia*, *Penicillium*, *Phoma*, *Rhizoctonia*, *Rhizopus*, and *Verticillium*. The presence of fungi in the seed is more prevalent in early-maturing cultivars and in early-planted fields.

The most recent fungal disease reported on soybean in Argentina is soybean rust (*Phakopsora pachyrhizi*). It was first detected in the province of Misiones in the 2001/02 growing season (Rossi, 2003). The following season it was

found in the provinces of Corrientes and Formosa, but still in areas distant from the main soybean producing regions. Major concern exists among soybean producers, consultants, and government personnel because of the presence of this disease in the northeastern part of the country and the reports about the damage caused by the disease to soybean crops in Paraguay, Brazil, and Bolivia in recent years.

Soybean mosaic (*Soybean mosaic virus*) is the most prevalent viral disease in Argentina. Less frequently detected are alfalfa mosaic (*Alfalfa mosaic virus*), peanut mottle (*Peanut mottle virus*), and Brazilian bud blight (*Tobacco streak virus*). In the northwestern region the most important viral disease has been attributed to a geminivirus transmitted by whiteflies (*Bemisia tabaci*). Molecular characterization of the virus has suggested that it is a new geminivirus species in the genus *Begomovirus*. A new viral disease, caused by a *Cucumovirus*, was detected during the 2000/01 growing season also in soybean crops in the northwestern region.

Soybean cyst nematode (SCN) (*Heterodera glycines*) and root-knot nematodes (especially *Meloidogyne incognita* and *M. javanica*) are the most important nematodes that affect soybean in Argentina. SCN was reported causing yield losses of up to 65% during the 1997/98 season in the provinces of Córdoba and Santa Fe. Field surveys indicated the occurrence of races 1, 3, and 14 in the northern Pampean region; and races 3, 5, and 6 in the northwestern region. In northwestern Argentina, the highest levels of SCN infestation were recorded in northeastern Tucumán, with up to 25 viable cysts per 100 cm^3 of soil. Root-knot nematodes are frequently detected in the provinces of central and northern Argentina.

Soybean disease management in Argentina

As a result of the different epidemics that occurred in Argentina in the last 10 years, special attention is now given to disease management strategies. Integrated management programs have been recommended, which combine cultural, chemical, and biological methods, with special attention to the economic aspects of crop production (Ploper, 1999). However, the implementation of these programs is still incomplete and soybean production continues to suffer significant losses due to diseases, with the constant risk of the occurrence of new epidemics.

Crop rotation, one of the most efficient cultural methods to manage many soybean diseases, is only practiced in a small proportion of the grain production area of Argentina. Increased demands for soybean in recent years and low production costs have resulted in more land devoted to soybean production and consequently less rotation with other crops. A

successful management of foliage diseases has been observed in those fields rotated with maize.

Although destruction of crop residues by tillage is an effective way to lower the survival of most pathogens that infect the aerial parts of the soybean plant, this practice is not generally recommended for soybean production in the country. Conservation tillage systems, such as no-tillage, are preferred to manage soil erosion, increase physical and chemical soil fertility, and favor the water balance of the system. Several million hectares could not have been incorporated to soybean production in Argentina if it not were for the adoption of these conservation tillage systems. Nevertheless, these systems favor the overseasoning of inocula of many pathogens.

Another important practice recommended to manage soybean diseases is the use of high-quality, pathogen-free seed. However, most soybean growers in Argentina plant seed that they produce, not always with the proper technology. This has contributed to the spread of diseases into new areas and fields.

Other cultural practices suggested in management programs are oriented to prevent or reduce soil compaction and improve soil drainage (for charcoal rot, Phytophthora rot, SDS, and other diseases caused by *Fusarium* spp.), and maintain good soil fertility (for charcoal rot, seed diseases, and SCN). Other aspects that should be considered are planting date (for charcoal rot, late season diseases, and soybean mosaic), use of early-maturing cultivars (for SSR, SDS, and soybean rust), appropriate row spacing and seeding rates (for charcoal rot and SSR), weed control (for SSR), irrigation (for charcoal rot), and timely harvest (for seed diseases).

Among biological methods of control, the use of genetic resistance constitutes the only available strategy in Argentina. Currently there are cultivars with high levels of resistance to stem canker, FLS, Phytophthora rot, and SCN, and with moderate levels of resistance to SSR, SDS, downy mildew, and target spot. The use of resistant cultivars has been the key to overcome the stem canker and FLS epidemics of the 1990s.

Chemical control recommendations include both seed-treatment fungicides and foliage-applied fungicides. Seed treatments are used extensively to increase seed germination when poor-quality seed is used and when planting conditions are not favorable for germination and emergence. The use of foliage-applied fungicides has increased steadily in the past 5 years. Data from field experiments in the different soybean-growing regions have shown the benefits of these applications in terms of reducing late season diseases,

increasing yields, and improving seed quality (Ploper et al., 2002, 2003). Yield increases resulting from fungicide use generally have been higher in the northern regions than in the northern Pampean regions. Fungicides used, alone or in combinations, belong to the following chemical groups: carbendazim (benzimidazol), thiophanate-methyl (thiophanate), epoxiconazol (triazol), difenoconazol (triazol), propiconazol (triazol), cyproconazol (triazol), azoxystrobin (strobilurin), pyraclostrobin (strobilurin), and trifloxistrobin (strobilurin).

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Four *Fusarium* species cause soybean sudden death syndrome

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Abstract

North and South American isolates that cause soybean sudden death syndrome (SDS) were examined systematically and phylogenetically together with closely related dry bean root-rot pathogens isolated in the United States and Japan. Maximum parsimony analysis of multilocus DNA sequence data, including sequences from the nuclear ribosomal intergenic spacer region and the single copy nuclear gene translation elongation factor 1-a, together with detailed morphological comparisons of conidial features, indicate that SDS of soybean in North and South America is caused by four distinct species: *Fusarium virguliforme* (previously known as *F. solani* f. sp. *glycines*) in North America, and *F. tucumaniae* and two undescribed species of *Fusarium* in South America. In addition to *F. phaseoli* (previously known as *F. solani* f. sp. *phaseoli*), one of the two undescribed Brazilian soybean SDS pathogens was also identified as a dry bean root-rot pathogen in the United States and Japan.

Soybean sudden death syndrome has been reported from all major growing regions in North and South America (Rupe et al., 2001; Ploper, 1993; Nakajima et al., 1993) and this disease has recently become a serious constraint to the commercial production of this crop. Since its discovery in Arkansas in 1972, the etiological agent of this disease has been reported as *F. solani* (Mart.) Sacc. or its forma specialis, f. sp. *glycines* Roy (Roy et al., 1997). Recent molecular phylogenetic analyses of DNA sequences, however, revealed that the *F. solani* species complex or section *Martiella* comprises at least 26 phylogenetically distinct species (O'Donnell, 2000), many of which are undescribed. North American isolates of the SDS pathogen, *F. virguliforme*, have been shown to be genetically homogeneous and closely related to a root-rot pathogen of *Phaseolus vulgaris* L., *F. solani* f. sp. *phaseoli* (Burkh.) W. C. Snyder & H. N. Hansen (O'Donnell, 2000, Li et al., 2000, Rupe et al., 2001). In 2000 and 2001, field surveys were conducted in Argentina for the soybean SDS pathogen as part of a collaboration between INTA-EEA, Marcos Juárez, Argentina and JIRCAS,

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Japan. Twenty strains of *Fusarium* were recovered from soybean plants exhibiting typical symptoms of the disease. In addition to the newly isolated Argentinean pathogen, strains of *F. solani* f. sp. *glycines* responsible for soybean SDS in the United States and in Brazil preserved at NRRL, NCAUR, USDA/ARS, U. S. A. and at Embrapa Soybean, Londrina, Brazil were studied systematically and phylogenetically. Strains of the dry bean root-rot pathogen, *F. solani* f. sp. *phaseoli* isolated in the United States and Japan and stored at NRRL, FRC (Pennsylvania State University, U. S. A.) and MAFF (NIAS, Tsukuba, Japan) were also included in the study because of their close phylogenetic relationship (O'Donnell, 2000). Typical strains of *F. solani*, including biological species within the *F. solani* species complex comprising mating populations I-VII, preserved at NRRL were also examined for comparison. The objective of this study was to compare North and South American isolates of the soybean SDS pathogen and their allies morphologically and molecularly using genealogical concordance phylogenetic species recognition (Taylor et al., 2000), in an effort to understand their genetic diversity and species limits.

Detailed procedures for investigating morphological characters were described in Aoki et al. (2003). *Fusarium* strains were incubated on potato dextrose agar (PDA) and SNA (Nirenberg, 1990) in 9-cm plastic Petri dishes at 20 C in complete darkness, under continuous fluorescent light or under daylight to study morphological and cultural characteristics. Average and standard deviation (SD) in the size of conidia were based on the measurement of 50 randomly selected conidia, based on the number of septa and cultural conditions. Molecular phylogenetic analyses of DNA sequences from the strains followed O'Donnell (2000). DNA sequences from the entire nuclear ribosomal intergenic spacer (IGS) region, the nuclear ribosomal internal transcribed spacer (ITS) region and domains D1 and D2 at the 5' end of the nuclear large subunit rDNA (28S), and a portion of the translation elongation factor (1-á) gene were obtained using an Applied Biosystems-Hitachi Prism 3100 or 3730 genetic analyzer. Sequence data was edited with Sequencher version 4.1.2 (Gene Codes, Ann Arbor, MI) and then the aligned sequences were analyzed phylogenetically in PAUP* version 4.0b10 (Swofford, 2002), using maximum parsimony.

Parsimony analysis of the combined DNA sequence data (4560 bp) indicates that SDS of soybean is caused by four phylogenetically distinct species while strains causing dry bean root-rot in the United States and in Japan were resolved as two phylogenetically distinct species. One of these latter two species also contains a strain that is responsible for soybean SDS in Brazil.

Detailed comparisons of conidial morphology revealed that the soybean SDS and dry bean root-rot pathogens share unique, aerial conidial features in that they all form septate conidia with a foot-cell on tall and mostly simple aerial conidiophores and nonseptate minute ellipsoidal conidia (so-

called microconidia) on short aerial conidiophores. These features were not observed in typical strains of the *F. solani* species complex represented by mating populations I-VII. The Argentinean soybean SDS pathogen produces diagnostic septate sporodochial conidia (so-called macroconidia) that are longer than those of the other soybean SDS pathogens in the United States and Brazil and those of the dry bean root-rot pathogens in the United States and Japan. The Argentinean pathogen, described as *F. tucumaniae* T. Aoki et al. (Aoki, et al., 2003), was discovered also in Brazil causing soybean SDS. The soybean SDS pathogen in the United States, described as *F. virguliforme* O'Donnell & T. Aoki (Aoki, et al., 2003), produces unique comma-shaped sporodochial conidia together with septate sporodochial conidia (macroconidia). A paper containing formal descriptions of the two undescribed soybean SDS pathogens in Brazil is in preparation. One of these species, *Fusarium* sp. 3, produces septate sporodochial conidia (macroconidia) with rounded ends that differentiate this species. The other species, *Fusarium* sp. 4, causes soybean SDS in Brazil and dry bean root-rot in the United States and Japan. This species is diagnosed by the production of septate sporodochial conidia (macroconidia) with a rostrate apical cell like a wedge-shaped beak and a distinct basal foot cell. In addition, a dry bean root-rot pathogen, previously known as *F. solani* f. sp. *phaseoli*, was recombined as *F. phaseoli* (Burkh.) T. Aoki & O'Donnell at the rank of species (Aoki, et al., 2003). It is characterized by shorter septate sporodochial conidia (macroconidia) with an acuate apical cell and a distinct basal foot cell that is typically hooked ventrally. Distinguishing diagnostic morphological features of the four *Fusarium* species responsible for soybean SDS and the two species that cause dry bean root-rot are summarized in Table 1.

Table 1. Diagnostic morphology of *Fusarium* species responsible for soybean SDS and dry bean root-rot.

Species	*F. tucumaniae*	*F. virguliforme*	*Fusarium* sp. 3	*Fusarium* sp. 4	*F. phaseoli*
Aerial conidia					
1) Multiseptate	+	+	+	+	+
2) Minute nonseptate	+	+	+	+	+
Sporodochial conidia					
3) Multiseptate					
Average length	> 60 µm	50-60 µm	45-60 µm	50-65 µm	< 55 µm
width	< 5 µm	5-5,5 µm	5-5,5 µm	5-5,5 µm	5-5,5 µm
Apical cell	Acuate	acuate	typically rounded	wedge-shaped beak	acuate, hooked
Basal foot cell	Distinct	typically indistinct	typically rounded	distinct	distinct
4) Non(-2)septate	+/-, short-clavate	+, comma-shaped	+/-, short-clavate	+, short-clavate	+, short-clavate
Host	Soybean	soybean	soybean	soybean, dry bean	dry bean
Distribution	Argentina, Brazil	USA, Argentina[a]	Brazil, USA	Brazil, USA, Japan	USA, (cosmo-politan?)

[a] M. Scandiani (pers. comm.).

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Approaches to soybean rust management in Argentina

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Soybean is the most important crop in Argentina accounting for an important percentage of the gross national product. Over 12.6 million hectares of soybean were planted in the 2002/2003 growing season producing 36.0 million of tons of soybean. There has been a tremendous growth in soybean production since 1965 with the greatest growth occurring in the last 10 years (Table 1). Most of the production (91%) is in the area called the Pampa Húmeda (Humid Pampa), with 5% produced in the Northwest and 4% in the Northeast region of the country.

Table 1. Soybean production in Argentina

Year	Production (Tn)
1965/70	21.860
1975/79	1.756.000
1995/96	12.600.000
1997/98	18.800.000
1999/00	20.600.000
2000/01	24.000.000
2001/02	30.000.000
2002/03	36.000.000

Not only has the total area planted to soybean increased, but so have the yield with average yields increasing from 1624 to 2670 kg/ha from 1970 to 2003. A major constraint on soybean production in Argentina is disease. It is estimated that between 8 and 10% of the crop is lost to disease each year. These diseases losses are caused by a number of diseases. While some of these diseases can be quite destructive (eg. sudden death syndrome and sclerotinia stem rot), but the overall damage regionally is usually limited due to the nature of the disease.

Recently, the soybean crop has been threatened by a disease that has the potential for severe widespread damage that could seriously limit crop production in Argentina (Ivancovich and Botta, 2002). The disease is soybean rust caused by *Phakopsora pachyrhizi*. Soybean rust is new to

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South America, first being found in Brazil and Paraguay in the 2001/2002 growing season and then in Argentina, in the northern province of Chaco in the 2001/2002 growing season (personal communication from G. de Alvarez, Universidad del Noreste, Corrientes, Argentina), and in 2002 in the province of Misiones in the 2002/2003 growing season (Rossi, 2002).

During 2003 soybean rust was detected by INTA's researchers (National Institute for Agricultural Technology), in field trials at the Experimental Station of INTA Cerro Azul (province of Misiones*), and in two field plots at Gobernador Virasoro (province of Corrientes*). The pathogen was confirmed as *Phakopsora pachyrhizi* by molecular studies (PCR). Besides soybean, *Phakopsora pachyrhizi* was found on several alternative hosts from these areas, in particular cajan (*Cajanus cajan*), kudzu (*Pueraria lobata*) and mucuna (*Mucuna cochinchinesis*).

* While the provinces of Misiones and Corrientes are not currently considered as soybean productive areas in Argentina. The occurrence of soybean rust in these provinces is of concern since it is likely that the pathogen could travel the 1000 km to the main soybean area of Argentina and cause major yield losses.

Symptoms

Symptoms of soybean rust may be observed anytime during the growing season, but they are more common after flowering. The disease progresses from lower to upper leaves with pustules mainly observed on the adaxial soybean leaf surface, but can be seen on the abaxial soybean leaf surface as well.

Symptoms are observed as small chlorotic areas on the leaves that increase in size and change color from gray to reddish brown or tan on the adaxial leaf surface. Rust symptoms and signs consist of uredinia that release urediniospores. The color of the pustule is important. The reddish brown lesions produce less uredinia and urediniospores on the leaves and are associated with necrotic areas with few urediniospores.

The symptoms of soybean rust may be confused with those produced by brown spot (*Septoria glycines*) and bacterial pustule (*Xanthomonas axonopodis* pv. *glycines*), but are easily distinguished by the presence of urediniospores.

Epidemiology

Phakopsora pachyrhizi is a biothrophic pathogen that does not survive on debris, but in living plants (either soybean or alternative hosts). It is spread

primarily by wind-borne urediniospores that can be transported over long distances, possibly between continents. This pathogen has a wide host range including kudzu (*Pueraria lobata*), clover (*Melilotus* spp.), lupine (*Lupinus hirsutus*), beans (*Phaseolus vulgaris*), *Mucuna cochinchinesis*, cowpea (*Vigna unguiculata*), *Cajanus cajan*, *Crotalaria*, *Dolichos*, *Lablab*, *Medicago*, *Pachyrhizus*, *Rhynchosia*, and *Vicia*.

Spore germination requires high humidity (75-80%), with a minimum of 6 hours of dew and temperatures between 14 and 30°C (60 and 86°F, respectively). After infection, pustules are produced within 5 to 7 days, and a new generation of spores in 10-20 days (Yang, 2002).
Under ideal conditions (cool and humid weather) it is possible for the disease to increase from the initial infections to 90% of the leaf area affected in three weeks. These conditions are not uncommon in the major soybean growing areas of Argentina. In the 2002/2003 growing season, weather conditions in the northern part of Buenos Aires province (one of the main soybean growing areas of the country) had weather conditions that would have been favorable for disease.

Losses due to soybean rust are related to the phenological stage of the plant and the severity of the symptoms. Rust causes the plants to senescence early resulting in smaller and fewer seeds.

Disease management

The primary management options for soybean rust are the use of tolerant/ resistant cultivars, and foliar fungicides. There are four major genes for resistance: Rpp_1, Rpp_2, Rpp_3 and Rpp_4, identified in PI 200692, PI 230970, PI 462312 (Ankur) and PI 459025, respectively.

Since available commercial varieties are susceptible the only effective tool to reduce yield losses is the application of fungicides. Among the fungicides used to control soybean rust are: Mancozeb, Triazol (cyproconazole, difenoconazol, epoxiconazol, tebuconazol) and Strobilurines (azoxistrobin, pyraclostrobin, y trifloxystrobin) (Vello et al. 2002).

National project on soybean rust of INTA (National Institute of Agricultural Technology) in Argentina

In response to the threat of rust to soybean production in Argentina INTA's researchers initiated the National Project on Soybean Rust during the soybean growing season of 2002/2003.

The objectives of the Project are:
1- Survey soybean diseases in the northwest and northeast regions of Argentina with emphasis on soybean rust.
2- Review of available information on rust around the world and in South America.
3- Begin breeding programs for rust resistance.
4- Identify cultural management practices that reduce disease impact.

Objective 1: Survey for the occurrence of soybean rust.
Soybean in grower and experimental plots in the northwest and northeast regions of Argentina are being carried out for the occurrence of soybean rust. Survey sites include the National Net of Soybean Cultivar Evaluation, six additional locations in the Northeast Region, various cultivar tests and planting date studies, farmer fields and twenty other locations around the country. Besides soybean, alternative hosts are being examined at these locations.

In the 2002/2003 season, rust was not found at any location in Chaco or Formosa provinces even though rust had been reported in Chaco province the year before. In Corrientes province, rust was not detected in any experimental plots, but was observed in two grower fields near Gobernador Virasoro. These are the first reports of rust in Corrientes province. Rust was found in a field trials at Cerro Azul, Misiones. Rust samples from all locations were identified molecularly as *Phakopsora pachyrhizi* at INTA Marcos Juárez by Eduardo Guillín.

Rust was not found on any known alternative host in Chaco, Formosa or Corrientes, but was observed on Kudzu (*Pueraria lobata*), Cajanus (Cajanus cajan) and Mucuna (*Styzolobium niveum*) at the Experimental Station of INTA Cerro Azul, Misiones.

Objective 2: Information on Soybean Rust
Workshops were held at INTA Cerro Azul, Misiones and at INTA Banda Rio Salí, Tucumán, involving professionals from the provinces of Misiones, Corrientes, Entre Ríos, Chaco, Formosa, Salta, Tucumán, Córdoba, Santa Fe and Buenos Aires. Results of the surveys and information from the literature were presented.

Objective 3: Genetic Breeding
Under this objective, possible sources of resistant germplasm available in Argentina are to be identified and resistant cultivars will be developed using resistant germplasm from around the world. This objective is just beginning.

Objective 4: Cultural Management
Under this objective, the efficacy of various fungicides and fungicide schedules will be evaluated along with other management practices like

planting date and cultivar growth habit and maturity. Combinations of these practices will also be evaluated such as cultivar response or fungicide effectiveness in late planted soybean.

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Major soybean diseases in India: economic impact and control strategies adopted

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Abstract

About thirty-five diseases are commonly occurring in different parts of India. Twenty out of them are considered as major based on the magnitude of yield losses they are causing. Annual yield losses from diseases in soybean are in the tune of 12% of the total production. Eleven diseases viz. rust, anthracnose, Cercospora blight, powdery mildew, charcoal rot, sclerotial blight, bacterial pustule, yellow mosaic, soybean mosaic, bud blight like malady and no-podding syndrome are distributed throughout the soybean areas of the county while others like Myrothecium leaf spot, frogeye leaf spot, target leaf spot, brown spot, Fusarium root rot, pod & stem blight, Rhizoctonia root rot and aerial blight, bacterial blight etc are restricted to some regions only. In India, during 1994, a loss of 4.2 lakh metric tones was estimated on account of anthracnose, Fusarium root rot, pod & stem blight, Rhizoctonia aerial blight, bacterial and viral diseases. For the management of diseases, a general package and some disease specific packages of recommendations are being developed and endeavours are on the way to transfer them to farmers *in toto*. But at large, chemical control is more practiced by farmers, as it is usually effective. At times it becomes highly uneconomical and impractical to the farmers. Therefore, farmers are now being convinced to pursue integrated approach for soybean disease management. The main integrated disease management practices followed by farmers are deep summer ploughing, crop rotation, intercropping, use of balanced fertilizers, cultivation of disease resistant/tolerant varieties with varietal cafeteria approach, avoiding deep sowing, optimum seed rate and plant population, seed treatment, sanitation and roguing of infected plant parts and plants and chemical sprays. For chemical management of diseases, pre-planting seed treatment with thiram + carbendazim 2:1 at 3g/kg for the control of seed borne fungal diseases, with thiamethoxam 70 WS at 3 g/kg seed for yellow mosaic is recommended. Seed treatment with *Trichoderma viride* at 4-5 g/kg seed is also recommended and practiced by the farmers. For the management of (i) foliar and pod diseases, two sprays of carbendazim 50 WP or thiophanate methyl 70 WP at 0.05% (ii) bacterial pustule, streptocycline 0.02% + COC 0.2%, (iii) soybean rust, sprays of

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hexaconazole 5EC at 0.1% or propiconazole 25EC at 0.1% or triadimefon 25EC at 0.1% (iv) for yellow mosaic and soybean mosaic spray of thiamethoxam 25 WS at 100 g/ha 7 days after germination or ethafenprox or methyl demeton at 1l/ha are recommended. A computerized symptom-based disease diagnosis system for correct identification of diseases and their appropriate management has been developed at this center

Economically important diseases of soybean in India

Thirty-five diseases are commonly occurring in India (Gupta, 2001), out of them 20 are economically important. Kind of diseases vary region-wise depending on the variety of the crop and on the prevailing environment. In recent years rust, charcoal rot, yellow mosaic, fungal complex, bud blight like syndrome, no-podding have assumed importance (Gupta and Karmakar, 1999; Ansari and Gupta, 1997). Rust which was earlier considered as minor and restricted in few pockets of North Eastern States of India, all of sudden became major from rainy season of 1994 and since then incidences have been reported from the states of M.P., Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu, Kerala, Rajasthan (Gupta et al., 1999; Sarbhoy and Mahendra Pal, 1997). The major diseases of soybean in India are listed in Table 1.

Table 1. Major diseases of national and regional importance of soybean and losses caused

S. No.	Disease	Pathogen	Yield loss under optimum conditions
1	Rust	*Phakopsora pachyrhizi*	30-100%
2	Yellow mosaic	*Mungbean yellow mosaic virus*	15-75%
3	Soybean mosaic	*Soybean mosaic virus*	25-50%
4	Collar rot or Sclerotium blight	*Sclerotium rolfsii*	30-40%
5	Charcoal rot	*Macrophomina phaseolina (Rhizoctonia bataticola)*	77%
6	Rhizoctonia root rot and aerial blight	*Rhizoctonia solani (thanatephorus cucumeris)*	35%
7	Bacterial pustule	*Xanthomonas campestris pv. Glycines*	20%
8	Anthracnose	*Colletotrichum dematium* f. sp. *truncatum* or *Colletotrichum truncatum*	16-25%
9	Powdery mildew	*Microsphaera diffusa*	10-35%
10	Myrothecium leaf spot	*Myrothecium roridum*	20-40%
11	Alternaria Leaf Spot	*Alternaria alternata & A. tenuissima*	15%
12	Bud Blight Like Syndrome	A strain of *groundnut bud necrosis virus*	22%
13	Bacterial Blight	*Pseudomonas savastanoi* pv.*glycinea*	5-18%
14	Purple Seed Stain	*Cercospora kikuchii*	15-30%
15	Brown Spot	*Septoria glycine*	8-15%
16	Frog eye Leaf Spot	*Cercospora sojina*	22%
17	Target Leaf Spot	*Corynespora cassiicola*	18-32%
18	No-podding Phyllody/Bud proliferation/	Confirmed etiology is still to be worked out	20-30%
19	Fusarium seedling/collar rot	*Fusarium equiseti*	64%

Depending on the cultivar, disease and weather the variation in yield loss potential can vary from 5 to 100%. In India, during 1994, a loss of 4.2 lakh metric tones was estimated on account of anthracnose, Fusarium root

rot, pod & stem blight, Rhizoctonia aerial blight, bacterial and viral diseases (Wrather et al.,1997). It amounts to approximately 11 million $ at the current rate of Rs. 1300/ton.

A computerized disease diagnosis system and their appropriate management have been developed at this centre (Savita et al., 2003). Disease identification is based on symptoms observed visually, by magnifying lens, by microscope and by laboratory tests as well. Other factors such as weather temperature, humidity, rainfall, soil moisture, temperature, pH etc. are also being taken into account. The system also provides information on pathogen, economic loss, distribution etc. Confirmation of the diagnosed disease is done through colored pictures. To start with, the system is designed to cover 25 major soybean diseases of India but it has provision to include more disease if they appear in future.

Control strategy adopted:

Long-term management of any disease can hardly be achieved by employing only one method therefore, major emphasis is on the integrated disease management (IDM) strategies. Disease specific management strategies for yellow mosaic, soybean mosaic, rust, no-podding syndrome, charcoal rot are also developed and currently being used by farmers of this region. The various management practices that form the integral part of the IDM in India are described below

Cultural practices

Deep summer plowing in recent years is becoming popular among farmers for reducing sclerotial rot, charcoal rot, anthracnose, Phyllosticta leaf spot, bacterial blight and bacterial pustule. Use of balanced fertilizer makes plant to contact low levels of diseases. Adequate phosphorous and potash nutrition manage bacterial blight and pustule, charcoal rot, Fusarium blight, pod and stem blight, Rhizoctonia root rot. Potassium and nitrogen fertilization reduces the disease incidence of sclerotial rot (Deb and Dutta, 1992; Gupta et al., 2003). Rust was observed to be more severe at nitrogen doze of 60 kg/ha as compared to 0 level (Sharma et al., 1996). Use of clean and healthy seeds save soybean crop from many seed borne diseases like anthracnose, purple seed stain, frogeye leaf spot, bacterial blight, bacterial pustule, soybean mosaic etc. Early sowing help the crop to escape the damage from charcoal rot, soybean mosaic and rust (Anahosur and Patil, 1998), and other seed borne disease like anthracnose (*Colletotrichum truncatum*), purple seed stain (*Cercospora kikuchii*), *Alternaria alternata*, Rhizoctonia aerial blight and soybean mosaic. Normal or late sown crop suffers more from rust infection as pod initiation to bean beginning (R4 to

R5) stage will coincide with the rust congenial climate generally occur in August to early September months. Farmers are always advised not to use excessive seeding rate and not to sow seed deep to minimize damage from pre and post emergence mortality from *Rhizoctonia*, *Sclerotium* and *Fusarium*. Similarly, high population also increases Septoria brown spot and pod and stem blight Myrothecium leaf spot. Roguing and destruction of infected crop/plant parts helps in containing the incidence and secondary spread of sclerotial rot, rust, Yellow mosaic, bacterial pustule, bacterial blight and leaf spots caused by Myrothecium, Alternaria, Phyllosticta, Corynespora, Septoria etc. Regular scouting facilitate such mechanical removal of infected plant parts/plants. Crop rotation with sorghum, maize and cotton have been very effective for reducing the incidences of charcoal rot, sclerotial rot, fusarium root and stem rot in soybean. Taking a single crop of soybean in a year (in rainy season only) is found very effective for the management of rust. Recommendation is also passed to the farmers to cultivate more than one variety at a time.

Chemical control

In India chemical control of soybean diseases is accomplished by (i) seed treatment, and (ii) spray.

Seed treatment

Use of seed treatment for the control of seed borne diseases is the most common in India as it is cheap, effective and easy method. For the control of seed borne fungal diseases it is recommended to treat the seed prior to planting with thiram + carbendazim 2:1 @ 3g/kg or thiram alone @ 3-4g/kg seed or with a combination product of thiram 37% + carboxin 37% @ 2 g/kg. However, captan @ 3g/kg seed is also used specially for the control of anthracnose, purple seed stain and Rhizoctonia aerial blight. Seed treatment of Thiamethoxam 70 WS is found extremely effective for the management of yellow mosaic.

Spray treatment

Fungicidal sprays are recommended for the control of foliar, pod and stem diseases and also to improve seed quality. In general, fungicides applied during growth stages R2-R5 provide maximal yield increase and improves seed quality by reducing fungal infection..

For the management of foliar, pod and stem diseases the recommended fungicides are given in the table 2. Both protective and systemic fungicides have been registered for use on soybean. However, systemic fungicides, such as carbendazim and thiophanate-methyl have performed better than

protective fungicides such as chlorothalonil and mancozeb. From the trials conducted at the various centres of All India Coordinated Research Project on Soybean indicated that, in general, two sprays of carbendazim (0.05%) or thiophanate methyl (0.05%) control most of the foliar and pod diseases to a great extent with high cost-benefit ratio. To improve the seed vigour and germination for seed production purpose, two sprays of carbendazim (0.1%) or mancozeb (0.2%) at R2 and R6 stages of the crop is recommended. If only one spray is to be given then it should be taken up at R6 stage. For spray the spray volume should not be less than 700-800 litre/ha

Table 2. List of fungicides used for the management of important foliar, stem and pod diseases.

Fungicide/insecticide/ Antibiotic	Doze (%)	Disease
Benomyl	0.1	Cercospora blight (purple seed stain), pod and stem blight, powdery mildew
carbendazim or thiophanate methyl	0.05	Pod & stem blight, Myrothecium & Alternaria leaf spot, aerial blight, brown spot, powdery mildew
Hexaconazole or propiconazole or triadimefon or oxycarboxin	0.1	Rust
Copper-oxy chloride	0.2	Choanephora leaf blight
copper-oxy chloride + streptocycline/ COC + kasugamycin	0.2 + 0.02	Bacterial pustule
	both 0.2	
Ethofenprox or methyl demeton	0.1	yellow mosaic and soybean mosaic
Mancozeb	0.25	anthracnose, Myrothecium & Alternaria leaf spot, aerial blight, brown spot
Streptocycline	0.02	bacterial pustule
thiamethoxam 25 WG	100g/ha	yellow mosaic and soybean mosaic

Biological control

Though, biological management of soybean diseases is recommended and also practiced by some farmers but not popular and still to get momentum Seed treatment with *Trichoderma viride* @ 4-5 g/kg seed is recommended for the control of charcoal rot and sclerotial blight. Use of *Pseudomonas fluorescens* as seed treatment (10 g/kg), soil treatment (2.5 kg/ha) and spray (0.1%) is under testing for the management of seed and seedling diseases of soybean, initial results are encouraging.

Immunization or disease control through breeding and use of resistant varieties

For all kinds of plant diseases, use of resistant varieties is the most economical, environment friendly, easy and effective method and most preferred by the farmers for disease management. Some of the resistant, moderately resistant and tolerant varieties are: Bragg, Himso 1563, JS 80-21, NRC 7, PK 472 for anthracnose, NRC 37 for sclerotial blight, JS 71-

05, JS 79-81, MAUS 47, NRC 37 for Myrothecium leaf spot, PK 564. SL 295 for Rhizoctonia aerial blight, Indira soya 9, JS 80-21, PK 1024, PK 1029, MAUS 61-2 for rust, Bragg, Himso 1563, Indira soya 9, JS 71-05, MACS 450, NRC 37, PK 327, PK 472, SL 96 for bacterial pustule, JS 71-05, PK 262, PK 327, PK 472, Punjab 1 for soybean mosaic and PK 327, PK 472, PK 564, PK 1024, PK 1029, PK 1042, SL 259 for yellow mosaic.

There are, however, certain constraints in implementation of IDM recommendations and farmers' adoption to the desired extent. In the entire production technology, a high yielding variety is the only component adopted by the farmers religiously. Other components of IDM are often either ignored or given least importance. The farmers, mainly because of slow effect on target pathogen, viability problem of bioagent used and relatively higher cost, take use of bio-fungicides as IDM component with reluctance. On the contrary, farmers are more enchanted with chemical fungicides that are apparently handier, economic and have quick knock-down effect. It is hoped that farmers' education on biology and damage symptoms of diseases, their natural antagonists and IDM *per se,* through training and on-farm demonstrations would bring needed awareness towards its long-term benefits and sustainability of soybean based cropping system.

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Exchanging, preserving, characterizing, and utilizing soybean germplasm in the USA

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Abstract

Current soybean production in the U.S. was made possible by germplasm exchanges in the early part of the last century. The first soybean introductions in the U.S. were used as breeding populations from which to make selections. The concept of germplasm changed to include intentional preservation and then intentional collection. Germplasm evaluation has also evolved from the collection of minimal information for immediate use to the extensive accumulation of data that is critical for germplasm management. Germplasm utilization began with the creation of the initial genetic base from which the first commercial cultivars were derived. Incorporation of new sources of disease resistance has been a critical part of the expansion of the soybean production. In the future, fully integrating the diversity available in germplasm collections to address all aspects of cultivar improvement will be needed to address the needs of producers and consumers. The USDA has a policy of free exchange of germplasm. As *ex situ* collections become the only source of germplasm in future, exchange among collections will become more important. As technology allows a greater understanding and more utilization of germplasm, the challenges created by the enormous diversity available will make cooperation more critical.

Soybean production in the United States was founded on and developed with germplasm exchange. This should not be a surprise since, as we all know, the soybean originated in China. Certainly views of germplasm have changed over the past 200 years and are still evolving, but for many U.S. soybean scientists, exotic germplasm is part of the gene pool that is quite distantly removed from most of their scientific activity. In this presentation, I will discuss the changes in perceptions about soybean germplasm in the U.S. over the past 100 years: where we have been, where we are, and where we may be going.

The soybean first arrived in the United States in 1765 (Hymowitz and Harlan, 1983) but neither that introduction nor others that occurred over the next 100 years permanently established soybean in the U.S. The oldest

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extant accession in the USDA Soybean Germplasm Collection (hereafter referred to as the USDA Collection) was introduced into the U.S. prior to 1880 but the exact origin and circumstances of introduction have been lost (Bernard et al., 1987). Formal record keeping of plant introductions began in the U.S. in 1898. The first soybean recorded in the system (PI 480) came from Siberia in 1898. These first introductions provide a clue about how early germplasm was viewed. At this time, soybean was not a crop but a scientific curiosity. New introductions were saved if they were perceived to have value and the others were discarded. Between 1898 and 1948, 8,225 soybean introductions were brought into the U.S. but only 1,803 were still available when the USDA Collection was established in 1949 (Bernard et al., 1987). With the advent of the USDA Collection there was both a mechanism for recording introductions and a repository for preserving the seeds. This advanced the concept that germplasm should be preserved even when the immediate value was uncertain. Beginning in 1949 all soybean germplasm introduced was carefully preserved, but for the next 25 years there was little active effort to expand the collection. A major turning point for germplasm, in general, in the U.S. was the southern leaf blight epidemic (caused by *Helminthosporium maydis,* Nisikado & Miyake) of 1970 in maize. This publicly highlighted the perils of genetic uniformity of crops in modern agriculture and increased the visibility of and support for germplasm programs (Committee on Genetic Vulnerability, Nat. Acad. of Sci., 1972; Nat. Acad. of Sci., 1978). This period also coincided with an impetus to intentionally increase the holdings of the USDA Collection with a particular focus on Asia. From 1949 to 1974, an average of 72 accessions were added to the USDA Collection each year. From 1975 to 2000, this number increased to over 500 annually. Some of these accessions were collected by U.S. scientists in cooperation with host countries but nearly 90% of the new additions were obtained through exchanges with other collections. Worldwide, the era of soybean collection is coming to an end. There are still a few places in Asia where primitive varieties, not currently in any germplasm collection, are being grown by farmers, but these isolated pockets are rapidly disappearing as cultivars from new and expanding scientific breeding programs are being widely distributed and grown. This loss of genetic diversity that has been managed by farmers increases the responsibility of *ex situ* collections and the importance of germplasm exchange among these collections.

The distinction between evaluation and characterization may be somewhat arbitrary but the difference is important for germplasm management. Evaluation is the research that identifies variation for useful or potentially useful economic traits. Many of these are quantitative traits that require replication over time and space to be accurate. Agronomic traits such as

time of maturity, height and seed yield are good examples. Disease resistance can be either mono- or polygenic but all would be the products of germplasm evaluation as I have defined it. Characterization largely involves qualitative traits that are generally much less important economically but are critical for the management of a germplasm collection. Pigmentation and plant morphological traits are examples of such traits. It is vitally important for a collection to be accurately characterized for a selected number of qualitative traits that can be used to define each accession. Without this internal identification it is nearly impossible to maintain accession identity over the long term. The USDA Collection has nine traits that are routinely described for all accessions and three other categories that are used to describe more uncommon characteristics of the seed, plant, or leaf (Bernard et al., 1998, Hill et al., 2001). Mathematically there are over 1 billion unique combinations of these descriptors. Some characters are quite rare and some combinations are genetically impossible, so only approximately 5,100 unique combinations actually exist within the USDA Collection with more than 160 accessions sharing one common set of descriptors. Even with this level of redundancy, these descriptors are a very useful management tool for maintaining accession integrity.

Accurately characterizing germplasm assumes that each accession has a describable phenotype. Heterogeneous accessions make this process very difficult if not impossible. Nearly 50 years ago the decision was made to maintain accessions in the USDA Collection as pure lines, lines that are both homozygous and homogeneous. Since soybean is nearly exclusively self-pollinated achieving homozygousity is a natural process. Approximately 80% of the accessions in the USDA Collection were homozygous and homogeneous when they were received but as many as 10 pure lines have been extracted from new introductions. Separating the homogeneous lines from the mixed samples requires human intervention. The process of pure lining germplasm accessions runs counter to traditional germplasm management methods, but our experience strongly indicates that for self-pollinated species pure lining is a superior strategy for maintaining, evaluating, and utilizing genetic diversity. Natural selection and genetic drift will alter heterogeneous accessions unless rigorous control is exerted over population size and harvesting procedures each time the accession is grown. Implementation of such procedures would greatly increase the cost of germplasm maintenance. It is very difficult to precisely describe heterogeneous accessions, so it is more likely that accidental mixtures by seeds of other accessions will not be noticed and the genetic composition of the accession will be permanently changed with each contamination. Evaluation of heterogeneous accessions will be, at best, an average of all of the genotypes present and thus will not identify the extreme values that

are most likely to be useful. If accessions are not accurately described and maintained, it will challenging to exchange known genotypes. Accurate description, long-term preservation, effective utilization, and precise exchanges are all facilitated with pure line accessions.

Germplasm evaluation is the critical first step in effective germplasm utilization. The cost of evaluation is generally much higher than for characterization but the benefits to plant breeding are enormous. For the USDA Collection, we have decided that having an estimate of yield potential for all germplasm accessions is important. Evaluation plots that provide the yield data also produce data on the other common agronomic traits used by plant breeders. These data are available through the internet (USDA-ARS, 2003) and in a series of technical bulletins available from the curator. Evaluations for responses to biotic and abiotic stresses are often too costly to be done on all accessions but are too critical to be ignored. To make the best use of available resources and meet the needs of plant breeders, intentional strategies are required for each disease, insect or stress. Soybean cyst nematode is probably the most important disease in the U.S. and the resources have been committed to evaluate all accessions for resistance to at least one strain of this nematode. For other diseases, not all accessions have been evaluated and the intensity of ongoing screening efforts is relative to the importance of the disease, the level of the resistance already identified in exotic germplasm or in use in commercial cultivars, and the efficacy of the screening procedures available. There has been less research to identify tolerance to abiotic stresses than to insects and pathogens, but some evaluations for tolerance to drought, heat, flooding, salt, high or low soil pH, aluminum, and ozone have been done. When selective evaluation is done within a collection, making informed choices about the accessions to be evaluated will increase the effectiveness of the research. Core collections (Brown, 1989) are a formalize method of determining a subset of a collection that provides a good representative of the whole. We have not yet established a core collection for the USDA Collection but have used the concept to select groups of accessions based on origin, maturity, and descriptive information for specific evaluations.

DNA markers are a powerful tool in germplasm management. Resources available to germplasm collections will have to be greatly increased before it will be feasible to even consider characterizing entire collections at a significant number of marker loci. Even if it were possible, it may not be the highest priority to gather these data. Selective use of DNA markers can establish genetic relationships where pedigrees are unknown (Thompson and Nelson, 1998), predict genetic relationships based on origin (Li and Nelson, 2002), provide assessments of genetic diversity (Li and Nelson,

2001) and identify quantitative trait loci in exotic germplasm that can improve economically important characteristics such as yield (Kabelka et al., 2004).

Germplasm utilization was not explicitly included in the scope of this symposium but because I believe that it will have such a profound impact on the future of both germplasm collections and cultivar development programs, any discussion of germplasm preservation needs to also include germplasm utilization. In the U.S., germplasm utilization began with the assembling of the initial genetic base of the commercial crop. A review of the first 15 years of the USDA sponsored regional soybean tests that began in 1939 showed that about 150 soybean introductions were regionally evaluated. Of those tested, 86 were used in crosses that produced progeny that were also evaluated in these regional tests. After years of testing and crossing, 37 of these 86 lines became part of the genetic base of North American soybean cultivars. These 37 lines include 17 of the 36 ancestral lines that contribute 95% of the genes to the current U.S. genetic base (Gizlice et al., 1994). Most other members of the genetic base were selected from the USDA Collection as sources of disease resistance (Gizlice et al., 1994). Approximately 85% of the current North American genetic base was complete by 1954, and 95% by 1970 (Thompson and Nelson, 1998). The second stage of germplasm utilization in the U.S. was and still is the selective inclusion of accessions based primarily on disease resistance. Without this infusion of new disease resistance, current U.S. soybean production would not be possible but because these resistances were often backcrossed into existing cultivars the genetic base remained relatively unchanged. As the national soybean cultivar development program in the U.S. matures with a combination of state, federal, and proprietary programs, we have the opportunity to move into the third stage of germplasm utilization, full integration of germplasm into breeding programs to address all major issues of cultivar improvement, including yield. In this stage, a substantial part of the national soybean breeding capacity would be devoted to the extraction of useful diversity from the germplasm. Part of this process would include making this diversity available in well-described and enhanced forms so that it could be immediately incorporated into cultivar development programs. Soybean cultivars in China, Japan and North America developed through scientific breeding methods have been derived from approximately 700 ancestral lines (Carter et al., 2004). Even with a conservative estimate of approximately 47,000 unique soybean germplasm accessions in collections world wide (Carter et al., 2004), less than 1.5% of the available germplasm has been used for cultivar development in these three national programs that are among the oldest in the world. Clearly we have only begun to exploit the genetic diversity that is available in the soybean.

There are increasing trends to restrict the exchange of genetic resources within the U.S. and internationally, but the USDA has maintained a policy of free exchange for all germplasm within the National Plant Germplasm System. As *ex situ* collections become the only source of soybean germplasm in future, exchange among collections will increase in importance. It is imperative that these collections are well maintained and accurately described if this critically important diversity is to be preserved and the integrity of each accession is retained. This high level of quality control is necessary for the maximum usefulness of germplasm exchange. It will allow for accurate data as well as correct genotypes to be moved between collections and from collections to soybean scientists. As new technology allows a greater understanding of the genetic diversity in germplasm collections, the challenges created by the enormous diversity that will be available will likewise increase. The great genetic challenge of the future will be to identify the allelic diversity within the species, and perhaps related species, for the loci affecting economically important traits of soybean. Accomplishing such a task will take more and not less cooperation. Reaching this goal may not be possible during the lifetime of anyone at this meeting but I am optimistic that I will see this process begun during my career. The future of soybean breeding is in our germplasm collections and new genetic technologies will continue to increase the value of these collections.

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Exchanging, characterizing and preserving soybean germplasm in Brazil

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Abstract

The availability of genetic variability is essential for developing of new cultivars with good productivity and resistance to pest and diseases. In 1973, the Brazilian Agriculture Research Corporation (Embrapa) initiated a cooperative program based on two research centers – Embrapa Soybean and Embrapa Genetic Resources and Biotechnology – to the development of important genetic resources and soybean breeding. These two research centers work together to increase genetic variability of soybean through germplasm introduction, exchanging and breeding. Also, they promote characterization and evaluation in order to have the available germplasm ready for use. Efforts are being developed to conserve for long-term this important reserve of variability in adequate chambers at –18°C, with relevant data monitored by the Brazilian Genetic Resources Information System (Sibrargen).

Introduction

Genetic diversity is a natural resource ever since the origin of agriculture in the Neolithic time in a number of nuclear centres – the fertile crescent of the Near-East, Meso-America, the Andes and China. The expansion of civilizations and the migrations of human cultures had meant that man has moved his domesticated plants and animals from place to place. All countries in the world, however rich in genetic diversity, are interdependent in genetic resources and share the responsibility for the management and the privilege of using these resources. At present time, many agricultural economies are based on crops that were introduced from other regions of the world. As the result of these world-wide crop dispersions, their genepools has been regarded as a heritage of all (IBPGR, 1984). Individual institutes, universities and private organizations can contribute to the maintenance of germplasm, but this responsibility must be through a national, government-supported and centrally managed program (CGIAR, 1993).

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From 1945 until mid-1980's, the access to plant genetic resources was largely characterized by free exchange. The principle of free exchange was reafirmed on the International Undertaking on Plant Genetic Resources by Food Agriculture Organization – FAO, in the 1983, which held that germplasm was a "common heritage of mankind" and should be made available without restriction (IPGRI, 1999). Although free exchange was reinforced by the 1992 Convention on Biological Diversity (CBD), several countries are developing or have adopted restriction legislation that regulate the access to their genetic resources. After several meetings, the International Undertaking was approved in November 3, 2001. It is a framework for a formal establishment of a multilateral system for exchange of plant genetic resources used in agriculture (with a crop list of 35 species to facilitate the exchanging process) (Esquinas-Alcazar, 1999; IPGRI, 1999; IPGRI, 2002).

Exchanging

Embrapa coordinates through the Embrapa Genetic Resources and Biotechnology the introduction and exchange of plant genetic resources in Brazil. According to Bonato (1982), soybean was first introduced in Brazil through Bahia State, in 1882. Since then, it was introduced in the States of São Paulo in 1892, Minas Gerais in 1902, and Rio Grande do Sul in 1914. In 1960 it began to be a cultivated crop in the Southern States at latitudes around 30°S. Nowadays, the soybean is the most important crop grown in the Central and Northern States up to latitude 4° N.

The Brazilian Soybean Collection has around 7,000 seed accessions introduced from several countries, mainly from the American Soybean Collection, Department of Agriculture of the United States – USDA/ARS, in Illinois. Some wild perennials and related soybean species has been introduced, mainly from Australia. According to Hymowitz (1999) they are rich sources for resistance to major pests. The Table 1 shows the countries involved in exchanging soybeans through Embrapa Genetic Resources and Biotechnology, during the period of 1989 – 2003 (Batista et al., 1998).

The post-entry quarantine is made on all introduced genetic material from abroad through the Quarantine Service of Embrapa Genetic Resources and Biotechnology. The results of inspections indicated mainly *Cercospora kikuchii, Fusarium equiseti, Phomopsis phaseoli* f. sp. *meridionalis, Colletotrichum dematium* var. *truncata, Fusarium pallidoroseum, Macrophomina phaseolina, Phomopsis longicolla, Pseudomonas savastanoi* pv. *glycinea, Xanthomonas axonopodis* pv. *glycines, Ditylenchus dipsaci and soybean mosaic vírus* (Marinho, 2004).

Some research results indicated a limited genetic base in the Brazilian soybean cultivars (Hiromoto & Vello, 1986; Vello et al, 1988). It is necessary to continue the introduction of new accessions in the country, and use them in breeding programs. The wild soybean (*Glycine soja*) needs to be introduced from eastern Asia (Siberia, China, Japan, and Korean). In addition, more accessions of soybean related perennial species could be obtained from Africa, Asia and Australia.

Table 1. Exchange of soybeans seed germplasm in Brazil in the period 1989 - 2003

Origin	Destination
Brazil (Embrapa Genetic Resources and Biotecnology)	Argentina, Australia, Bangladesh, Belize, Bolivia, China, Colombia, Cuba, Ecuador, England, Guatemala, Guyana, Hawaii, India, Italy, Japan, Kenya; Lesotho, Mozambique; Nigeria, Paraguay, Pakistan, Peru, Portugal; Russia, Scotland, Spain, Trinidad and Tobago, Uruguay, Zimbabwe, USA, Venezuela, Zaire and Zambia.
Argentina, Australia (CSIRO), Bolivia, Ivory Coast, China (CAAS), France, Japan, Mexico, Nigeria, North Korea, Poland, South Korea, Taiwan (AVRDC), USA (USDA/ARS/ American Soybean Collection).	Brazil (Embrapa Genetic Resources and Biotecnology)

Germplasm characterization and evaluation

The plant genetic resources variability only will be available to the plant breeders if the accessions are characterized and evaluated correctly in their potentiality (IPGRI, 2003). Passport data provide the basic documentation, including taxonomic designation and information about where an accession was collected. These data are provided by the collector or donor institution. Unfortunately, such minimal data are not yet readily available for many of the germplasm accessions.

Characterization involves the assessment of a varying number of descriptions ranging from morphological, cytological, biochemical, molecular, and others. In 1984, the International Board for Plant Genetic Resources - IBPGR published the descriptors for soybeans. The descriptor list was prepared in a standard format following advice from crop experts. It is important the passport data (accession identifiers), characterization (traits highly heritable)

and preliminary evaluation (additional traits). Evaluation is a lengthy, often repetitive process of examining accessions for traits of significance or potential contribution to the users, like disease resistance for example.

The Embrapa Soybean has spent efforts to characterize and evaluate the soybean collection to improve the use of germplasm in breeding and research programs. These works require a good knowledge of the crop and correct methodology (IPGRI, 2003). From 1975 to 1978, the Embrapa Soybean carried out characterization process in a collection of 1,600 accessions, in Londrina, Parana State. Since then, characterization and evaluation processes have been developed in 5,000 accessions in the Active Collection. Among other traits, flowering and maturity time, cotyledon, flower, pubescence and pod colors, seedcoat and hilum colors, pubescence type, growth type/habit, first-pod height, pod dehiscence, plant lodging, seed size and seed form data were collected. Part of the collection was evaluated for reactions to soybean mosaic virus, *Cercospora sojina*, *C. kikuchii*, *Diaporthe phaseolorum* f.sp. *meridionalis*, *Peronospora manshurica*, *Septoria glycines*, *Microsphaera diffusa*, *Pseudomonas savastanoi* pv. *glycinea*, *Xanthomonas axonopodis* pv. *glycines*, *Pseudomonas savastonoi* pv. *glycinea* and *P. syringae* pv. *tabaci* (Embrapa, 1982).

Nowadays, the collection is comprised of nearly 7,000 accessions as a result of new introduction of germplasm. A Core Collection will be set in such a manner so as to represent the larger base collection, with the available data. Measuring genetic diversity at the DNA level is desirable, and new genes needs to be found for pest resistance. It is also desirable to locate DNA markers closely linked with pest resistance to facilitate transferring genes from primitive accessions to modern cultivars.

Preserving

Harrington (1973) concluded that the cheapest and most convenient way to maintain genetic resources of crop plants is by seed storage. The precise relationship among moisture content, temperature and seed-viability controls the longevity of stored seeds. However, appropriate and efficient policies for training scientists and technicians in germplasm management should be developed and implemented at plant genetic resources systems.

The Soybean Germplasm Collections are maintained following the rules established in the Brazilian system of genetic resources (Embrapa, 1998; Wetzel, 1999). In this sense, two collection types were established. The Soybean Active Collection, comprised of 7.000 accessions, is maintained for short- or medium-term storage at the Embrapa Soybean, which is also

responsible for its characterization and evaluation. The Active Collection is stored in chambers with relative humidity around 45% and temperature about 10°C. The seed-stock for each access is large enough to attend to the breeders and researchers. The Soybean Base Collection is comprised of *Glycine max* (6,969 accessions), *G. clandestina* (2), *G. latrobeana* (2), *G. tabacina* (3), *G. tomentella* (5), *G. canescens* (3), *G. falcata* (2) and *G. latifolia* (2), and conserve for long-term all the genetic variability available in Brazil. This collection is maintained at Embrapa Genetic Resources and Biotechnology in chambers at –18°C, without humidity control, in aluminum foil bags hermetically closed. Before storage, the seeds are previously dried at 6% to 7%, but is required that the seed accession has germination higher than 80% and stock of 1,500 or more seeds for long-term conservation (IPGRI 1994).

Seed viability tests are essential to seed banks as a way to control genetic erosion during long period of storage (ISTA, 1985). The tests are performed at the beginning of the storage, and monitoring tests are accomplished each ten years. In 1980, the Embrapa Soybean sent 394 accessions to the Base Collection for long-term conservation. The initial evaluations were performed in samples of 200 seeds and twenty two years latter other tests were performed in samples of 100 seeds taken from each accession. In the first evaluation, 83.8% of the accessions had over 80% of germination rate. After twenty two years of long-term storage, the results (Figure 1) indicated that germination rate did not decrease significantly when the initial germination rate was higher than 80% (Wetzel at al., 2002).

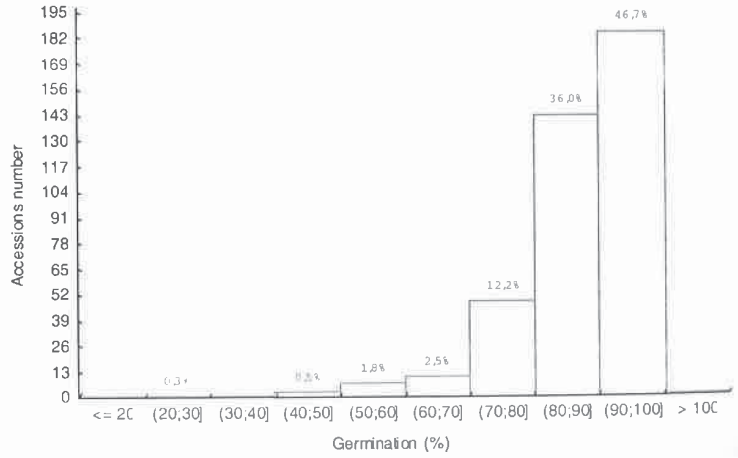


Figure 1. Freqüency distribution of seed germination rate of 394 soybean genotypes after 22 years of long-term storage.

Efficient germplasm conservation is fundamental to safeguard collections during storage in order to assure the distribution and use of materials with high sanitary quality. Pathogen longevity in stored seeds is possible because the storage conditions to maintain seed viability are also beneficial to the survival of most of the plant pathogens. A batch of 394 soybean accessions, stored for 22 years, was analysed by the standard blotter test method to detect fungi incidence in the seedstock. A significant amount of saprophytic and pathogenic fungi was detected, mainly *Alternaria* sp., *Cercospora kikuchii*, *Colletotrichum* sp., *Drechslera* sp., *Fusarium* sp., *Pestalotia* sp., *Phoma* sp. and *Phomopsis* sp.. It was also observed that germination rate was not affected after the long storage period. The fungi longevity in long-term stored seeds is evident, and for this reason more detailed studies about penetration, colonization and location of inoculum in the seeds are relevant to improve germplasm management.

Germplasm documentation

All information regarding the soybean germplasm is monitored by the Brazilian Genetic Resources Information System-SIBRARGEN (Cajueiro et al., 2001). The Sibrargen System manages all of the data associated with introduction, collection, quarantine, characterization, testing, evaluation, conservation, multiplication, regeneration, monitoring, distributions, health, and others. The principal element in the maintenance of germplasm is the accurate and up-to-date information about all accessions. The information process requires be included the passport data (taxonomy, country and origin and other special attributes), characterization data, evaluation data, and inventory data from the active and base collection. The Sibrargen System permits to monitor all the germplasm information and data from the two soybean collections, and will be available for direct on-line linkage through satellite communication between all Centers in the Embrapa Network.

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Exchanging, preserving and characterizing germplasm in China

L. Qiu and R. Chang[1]

Abstract

China reserves the most soybean germplasm in the world because of extensively exchanging germplasm in the national wide and among the countries of the world. The majority of these soybean germplasm originated in China and only 0.4% of them were introduced from the other countries. All of the accessions have been evaluated for the morphological traits and listed in the Catalog. Most of the accessions are analyzed for the seed protein contents and oil contents, and about 35% or less of the total accessions were evaluated for the other quality traits and resistances. The genetic diversities were estimated at both morphological traits for all Chinese accessions and molecular level for primary core collection. The results were used to determine the best strategy of sampling and evaluating for establishment of the Chinese soybean core collection. The core collection consists of 384 wild soybeans and 1055 cultivated soybean, taking 5% of the total accessions and representing over 85% genetic diversity of the whole collection. It will play a very important role in characterization and utilization of Chinese soybean germplasm.

Keywords: Soybean, China, exchange, preserve, character, core collection

Exchanging and utilizing soybean germplasm

Utilization of germplasm in the national wide

The exchanging soybean germplasm has been mainly used as parents and developed more than 700 new soybean cultivars for soybean production in the whole country. Chinese soybean breeding program started in 1950. Some elite landraces were screened out and used in the production such as yaojiaohuang in Shandong province, niumaohuang in Henan province, houzimao and aijiaozao in Hubei province and Taixingheidou in Jiangsu province, were screened out and used in the soybean production for a long period of time. Among these landracesÿAijiaozao originated at Hubei had been widely planted with accumulating area up to 20 mha in Hubei, Hunan,

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Jiangsu and Zhejiang; and both of Houzimao and Taixingheidou had been used as checking cultivars of the regional tests in both spring sowing and summer sowing soybeans in Changjiang region until 1980. Some farmers in the southern China still utilize some of these landraces in the production even today. During 1923 ~ 1995, a total of 651 cultivars were developed, which were traced back to 348 ancestral lines. These ancestors included 250 landraces, 46 introductions (major U.S. or Japan) and 52 unknown sources of accessions. Almost no landrace has been directly used as parents to develop cultivars since 1970 but more and more breeding lines or cultivars were put into soybean breeding program instead. A number of elite cultivars, including Zihua no.4 in Heilongjiang province, Qihong no.1 in Shandong, 58-161 in Jiangsu, Xiangdou no.3 in Hunan, derived a big number of modern cultivars, such as 129 cultivars derived from Zihua no.4, 58 cultivars derived from Qihuang no.1, 61 cultivars from 58-161. Entering 1970, more and more elite breeding lines including Zheng 77249, Tie 5621 and Ke 4430-20 etc, became the major sources of crossing parents. Soybean cultivars have been replaced for 3 ~ 4 time in various region because of application of new cultivars. Most of these cultivars and some elite breeding lines is extensively exchanged, especially within the ecotype region.

For introduced soybean germplasm, L83-544 from United States has been directly used in the production, because it has characteristics of high yield, resistant lodging and adapting intercropping system. Except this line, some introduced accessions were used as parents and develop a number of new cultivars. By crossing Swedean Logbeaw and Dongnong 47-1D, the cultivar Dongnong 36 with extreme early maturity was developed and extended soybean production 100 km to the northern China. Based on the statistics (Qiu and Chang, 1993), 121introduced accessions were used to develop new cultivars. The mostly used accessions includes Shishengchangye from Japan, L83-4387(*titi*) and Century (Ix2.3)ÿAmsoy, Williams, Clark, Beeson, Wilkin from United States.

However, the exchanging germplasm focused on a small amount of accessions, especially for the breeding lines or cultivars. The efficient rate of the germplasm was relatively lower compared to the accessions reserved in the national Genebank.

Exchanging germplasm in the world

Based on the records of the accessions listed in the catalogues, a total of 2154 soybean accessions have been introduced from more than 22 countries in the world. Among them, 1473 accessions were introduced from U.S.A, 114 accessions from Japan, 97 accessions from German and less than 50 accessions from the rest 19 countries (Table 1).

Table 1. Soybean accessions of introduced abroad and providing exchanging between countries

Country	Acc. No.	Country	Acc. No.	Country	Acc. No.
Austria	1	Canada	33	Pre-Soviet Union	35
Australia	2	Roumania	4	Thailand	42
Poland	16	U.S.A	1473	Hungary	21
North Korea	16	Yugoslavia	22	Italy	2
South Korea	26	Nigeria	3	Brirain	28
Danmark	10	Japan	114	Brazil	2
German	97	Sweden	8	Unknown	161
France	41	Srilanka	1	Total	2156

The more than 4079 Chinese soybean accessions have been providing to 54 countries during 1973 ~ 2002 just based on the limited records. Among these countries, the Untied States ranked the first, which is over 50% of the total accessions.

Preserving soybean germplasm

During four five-year-plan, the total of 32415 Chinese soybean accessions and 2156 introduced soybean accessions were evaluated for agronomic traits and listed in the publication of Chinese Soybean Germplasm Catalog. Among the Chinese soybean accessions, there are 23587 cultivated soybeans and 6672 wild soybeans. For the cultivated soybean, more than 93% of them are landraces and less than 7% are modern cultivars or breeding lines. About 400 cultivated soybeans originated in Guizhou province were accidentally lost because of natural disaster. In addition, 45 soybean cultivars developed during the eighth five-year-plan were did not put into long time storage. Therefore, the number of accessions in the long-term storage is 31970, which has 445 shortage compared to the records in the catalogs. Except national long-term storage, there has duplicated accessions reserved in the midterm storage for exchanging and utilization (Table 2).

Table 2. Varieties for catalogue and conservation in long-term and midterm storage in various period

Items	6th-five-year	7th-five-year	8th-five-year	9th-five-year	Total
Catalogue	6814	16134	7807	1660	32415
China	6814	16134	5861	1450	30259
Cultivated	6814	10853	4970	950	23587
Wild	0	5281	891	500	6672
Introduction	0	0	1946	210	2156
Long-term storage	0	22548	7762	1660	31970

Characterizing

Quality Evaluation

Seed protein content: There are 170 Chinese soybean accessions with protein content over more than 50%. Among them, Jing783 in Hubei province ranks the first and its protein content is 55.15%, following with 52.90% for Liyang Qinggang Huangdou in Jiangsu province, 52.70% for Jingan Dahuangdou in Jiangxi province, 52.16% Changning Wuzhuadou and 51.97% for Jinyang Dabaidou.

Amino acid content: A total of 2341 soybean germplasm from northeast China were analyzed. The average contents of glutamic acid and aspartic acid are highest in all 18 different amino acids, which are 18.80% and 11.43% respectively. Lysine content is 6.19%, cystine content is about 1.45% and methonin content is only 1.29% (Lv, 1988). The lower content of sulphur containing amino acids becomes a limiting factor to improve soybean quality. By screening the wild soybean, five accessions with the higher sulphur containing amino acid content were found, which were 1.46~1.55% for methione content and 1.67~1.71% for cystine content and 3.03~3.25% for the total content of both methione and cystine (Xu et all., 1993).

Seed fat content: There are a total of 38 soybean accessions with seed fat content over 23%. The highest fat content accession (24.2%) is Wuzhai Huangdou in Shanxi province. The fat content of Qihuang 21 in Shandong province is 24.0%, Fenju Huangdou is 23.7%, Mancangjin is 23.6%, Teifeng 16 is 23.1%. There are also many high fat content varieties in Huanghuai region and south region in China, such as Niumaohuang in Jiangsu province which its content is 22.5%, 22.5% for Gaoma Shizi and 22.5% for Yuanyan Bayuehuang in Sichuan.

Fatty acid content: Soybean fatty acid is composed of steric acid(16:0), palmitic acid(18:0), oleic acid (18:1), linoleic acid(18:2), linolenic acid(18:3) and a small quantity of arachidonic acid. Unstaturated acids account for more than 80% of the fatty acids. Analyzing 2314 soybean accessions in northeast region, the average palmitic acid content is 11.33%, steric acid content is 3.20%, oleic acid content is 20.78%, linoleic acid content is 55.28%, linolenic acid content is 9.18%. There are 38 soybean accessions in Huanghuai region in China which linoleic acid content is more than 60%. Among all the linoleic acid content of them, Longyao Heidou is highest accounting for 63.4%, 7651-2 in Zhangjiakou is 63.2%, Xiaoheidou in Shanxin is 62.9%. There are 29 soybean germplasm which its linolenic acid content is lower than 5% in 7000 soybean germplasm in Huanghuai region and South region. The linolenic acid content of Tongren Eshuizao in

Guizhou province is lowest accounting for 4.2%. The linolenic acid contents for both Zaoshu Heidou and Longan in Guangxi province are both 4.3%.

Isoflavone: Some types of bean-products are more tasty because they are made of the varieties with lower isoflavone content. 50 cultivated soybeans were evaluated and observed three highest ones (547.9~655.8 mg/100g) and lowest ones: 45.6~82.4 mg/100g.

Lipoxygenase: The lipoxygenase were analyzed for 1636 Chinese soybean accessions from 26 provinces. The 4 types of Lox lacking mutants were observed, including 1 accession lacking Lox-1, 12 accessions lacking Lox-2, 80 accessions lacking Lox-3, 6 accessions lacking Lox-2.3. Among these mutants, 97 accessions distributed in the southern region, 2 accessions from Huanghuaihai summer soybean and no accession from Northeast spring soybean. No accession lacking Lox1.2.3 were found.

Kunitz proteinase inhibitor: There were 19116 accessions (14067 cultivars ,5049 wildness) evaluated for alleles of Kunitz proteinase inhibitor. The majority of them is Ti^a accounting 18773. There are 255 accession with Ti^b and 35 accessions with Ti^c, but none is lack SBTI-A2 (titi). The SBTI-A_2 activity in soybean is range 20.3~26.3TIU /g, which is higher than common bean (2.93-6.41 TIU/g), broad bean (2.01-2.87 TIU/g) and garden pea (1.66-2.38 TIU/g).

Oligo sacchaide: The oligo sacchaide content range from 2.7% to 6.4% in 181 accessions in Jinlin province. The result show that there are obviously variation of oligo sacchaide contents in different accessions, and it is accessible to screen out some accessions with low oligo sacchaide contents.

Protein subunit: 1757 Chinese soybean were analyzed for protein subunits. The variation for the ratio of 11S/7S ranged 0.72~2.99 with the average of 1.885(Wang et al., 2003). The accessions lacking ², ±'and 28S were found (Guan et al., 2003).

Evaluation of resistances

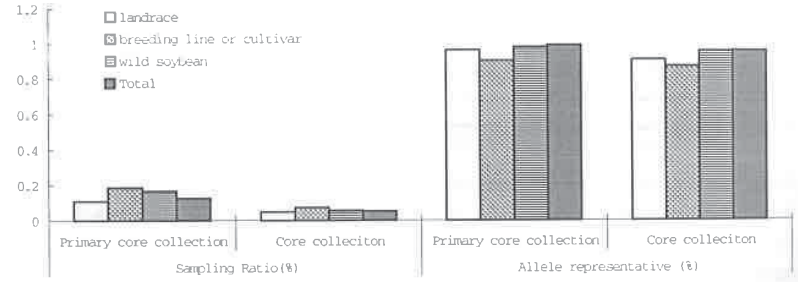
Chinese soybean accessions have been identified for the characteristics of stress resistances, disease resistances and insect resistance (Table 3). However, the average identifying rate for 15 characters were less than 35%. A total of 3929 accessions with immunity or high resistance were identified for all 15 characters except for soybean rust. In addition, the accessions resistance to sour rain and sour soil were identified. Recently, the molecular markers associated with the resistances were used to analyzed

for salt tolerance accessions (Guo et al., 2000), SMV resistance accessions (Zheng et al., 2001), and SCN resistance accessions (Wang et al., 2003; Qiu et al., 2003). The allele variations were observed and found some new alleles which can be utilized in the breeding program.

Table 3. Characterization of soybean germplasm and identification results

Characterization		Total no.	Rate (%)	Accessions with immunity or high resistance
Stress resistance	Drought resistance of the plant	6023	25.53	463
	Drought resistance at germination stage	6013	25.49	874
	Salt tolerance at germination stage	10298	43.70	1101
	Salt tolerance at seedling stage	10298	43.70	464
	Cold tolerance	5126	21.70	260
	Resistance to acid rain at seedling	1000	4.20	68
Disease resistance	SMV resistance	16969	71.90	46
	SCN1 Race 1 resistance	14350	60.84	142
	SCN2 Race 2 resistance	4202	17.81	142
	SCN3 Race 3 resistance	11167	47.34	309
	SCN4 Race 4 resistance	14039	59.52	20
	SCN5 Race 5 resistance	4309	18.27	20
	Resistance to soybean rust	7654	32.45	0
Insect resistance	Resistance to leaf insect	6724	28.51	20

Evaluation of genetic diversity



The genetic diversity centers of phenotypes based on the agronomic traits were identified for both cultivated soybean (Zhou et al., 1998) and wild soybean (Dong et al., 2002). Most of them are coincidences with molecular markers revealed by analyzing primary core collections with the 45 SSR loci for cultivated soybean (Qiu et al., 2003) and wild soybean (Dong et al., 2003; Li et al., 2003). It was a stripe region from southwest to northeast, including Sichun, Shaanxi, Henan, Shanxi, Shandong and Hebei provinces (Zhou et al., 1998). The exception is that northeast spring soybean has the higher genetic diversity at molecular level compared to the phenotypic

level for cultivated soybean. In addition, The genetic diversity was evaluated for soybean accessions with high resistance to SMV using 61 SSR primers (Mi et al., 2003) , high resistance to SCN (Wang et al., 2003) and salt tolerance (Guo et al., 2001) .

Establishment of core collection

In order to characterize and utilize Chinese soybean germplasm efficiently, the best sampling methods were selected by comparing 20 sampling methods with whole collection for variety classification, mean and variance of 5 quantitative characters, mean and variance of 14 characters genetic diversity, coincidence of 14 characters, mean of variety distance. The soybean primary core collection was set up by grouping accessions with variety classification; determining sample size with proportion; and selecting accession after cluster (Qiu et al., 2003). For the wild soybean, the best sampling strategy was to select individuals by genetic diversity index based on the clustering result for each ecotypes (Dong et al., 2003). A core set of SSR loci were identified with 80 autumn soybean accessions, which distributed on 20 integrated linkage groups with an average of 50 cM genetic distances between two adjacent loci and showed a higher level of polymorphism with an average of 8.8 alleles and 0.773 value of polymorphism informative content (PIC) per locus (Xie et al., 2003). This core set loci was confirmed by analyzing 190 random soybean accessions from the whole country (Wang et al., 2003). By analyzing over 3635 primary core collection with a core set of SSR loci, 1439 accessoins (384 wild soybeans and 1055 cultivated soybean) accessions taking of the 5% of the total accessions, was selected as core collection, which represent over 85% genetic diversity of the whole collection. When the sample size decreased to 220 accessions (1% of the total cultivated soybeans), the average genetic similarity among accessions was about 0.28, which could represent over 70% genetic diversity of the total collection in cultivated soybean.

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Progress in soybean transformation using *Agrobacterium*

D. A. Somers; P.M. Olhoft[1] and T.-S. Ko

Abstract

Soybean transformation methods based on DNA delivery via *Agrobacterium tumefaciens* use proliferating apical meristems, cotyledons of immature embryos and seedling cotyledonary nodes as sources of regenerating cells for the production transgenic plants. Improvements in T-DNA delivery, *Agrobacterium* strain, tissue culture conditions, and selection of transgenic plants have increased the efficiency of these transformation systems. Further improvements are required to expand the range of genotypes that can be transformed using these procedures and to shorten the time to produce transgenic plants. Characterization of T-DNA loci produced using the cotyledonary-node method indicate that on average about one simple locus is produced per transgenic plant indicating that *Agrobacterium*-based soybean transformation systems produce plants with a high proportion of simple transgene loci.

Introduction

Transformation-based genetic modifications of soybean offer the opportunity to augment conventional breeding efforts by both altering the expression of soybean genes and introducing genes from other organisms, neither of which are achievable in a directed manner via Mendelian genetics, wide crosses or mutation breeding. Expression of endogenous soybean genes can be silenced, upregulated or directed to different tissues. Examples include high oleic acid oil (Buhr et al., 2002) and enhancing or decreasing isoflavone content (Yu et al., 2003). Genes from any organism may be used to improve or modify soybean as demonstrated by the Roundup Ready® trait conferred by a bacterial gene for Roundup resistance (Padgette et al., 1995). Numerous opportunities exist for future development of transgenic traits in soybean. However, achieving these goals requires that simple efficient soybean transformation methods are available to both private and public soybean researchers. Recent progress in improving soybean transformation has been impressive. The purpose of this symposium paper is to review the status of *Agrobacterium*-mediated soybean transformation.

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Soybean transformation

Transgenic soybean plants are produced using various DNA delivery methods and plant tissues including microprojectile bombardment of shoot meristems (McCabe et al., 1988) and embryogenic suspension cultures (Finer and McMullen, 1991) and *Agrobacterium tumefaciens*-mediated T-DNA delivery into axillary meristematic tissue located in seedling cotyledonary-nodes (Hinchee et al., 1988), immature cotyledons (Parrott et al., 1989; Yan et al., 2000; Ko et al., 2003a), and proliferating shoot meristems (Martinell et al., 2002). While these different methods provide researchers with numerous options for producing transgenic soybean plants, soybean transformation remains constrained by inefficient production of transgenic plants from a limited range of genotypes. Thus continued efforts to improve soybean transformation are required to establish a robust method (Trick et al., 1997).

An ideal soybean transformation method should (1) be simple, inexpensive, efficient and rapid for production of transgenic plants; (2) be successful with a range of cultivars; and (3) produce simple (nonrearranged) transgene loci that will be most amenable to regulatory characterization and eventual commercial development. In our opinion, *Agrobacterium*-mediated transformation methods come closest to these criteria. Although *Agrobacterium*-mediated methods require incorporation of genes of interest between the T-DNA borders of binary plasmids and transformation of the finished binary plasmid into the appropriate disarmed *Agrobacterium* strain, improvements in binary vectors have rendered this step no more complicated than construction of plasmids for direct DNA delivery. Moreover, *Agrobacterium*-mediated transformation results in a higher proportion of simple loci compared to microprojectile bombardment. Based on these attributes, further research into improving *Agrobacterium*-mediated transformation is justified.

Proliferating meristem method

Transgenic plants have been produced by targeting cells in proliferating meristems of freshly germinated seeds for T-DNA delivery (Martinell et al., 2002). This method is cultivar independent and presumably rapid because the explant does not enter a callus or proliferative phase. There are few other reports on this method.

Immature cotyledonary explant method

Agrobacterium infection of immature zygotic cotyledons and induction of plant development through somatic embryogenesis was first reported by Yan et al. (2000). This transformation method has a relatively short culture

time of four to five months and results in phenotypically normal and fertile transgenic plants. Ko et al. (2003a) investigated modifications of this method including co-cultivation o f immature cotyledons with the virulence helper strain KYRT1, increasing *Agrobacterium* concentration and duration of co-cultivation, and incubating the explant with the adaxial side oriented towards the selection medium (Ko et al., 2003a). These modifications resulted in production of transgenic somatic embryos at high frequency from immature zygotic cotyledons followed by recovery of transgenic plants from a range of cultivars with transformation frequencies ranging from 1.1 to 1.7 % transgenic plants produce per immature cotyledon. Multiple transgenic somatic embryos exclusively arise from cells located in the margins of the immature cotyledon suggesting that this method can potentially produce large numbers of independent transgenic plants from a single immature cotyledon.

Efficient induction of somatic embryos from immature cotyledons depends on using the *Agrobacterium* strain KYRT1 carrying the partially disarmed virulence helper plasmid, pKYRT1 (Ko et al., 2003b). No transgenic somatic embryos were recovered from immature cotyledons co-cultivated with *Agrobacterium* strains carrying fully disarmed virulence helper plasmids including pKPSF2, the fully disarmed version of pKYRT1. The utilization of partially disarmed strains of *A. tumefaciens* may provide a useful approach for the transformation and regeneration of legumes or other recalcitrant plant species. Further studies must be conducted to confirm this hypothesis. Further refinements of co-cultivation conditions, tissue culture, and selection of regenerated transgenic plants will likely continue to improve this method.

Cotyledonary-node method

Our research is focused on the cotyledonary-node transformation method originally reported by Hinchee et al. (1988) and recently improved by Zhang et al. (1999) and Olhoft et al. (2003). This method is simple and inexpensive because the explant is derived form 5-d-old seedlings eliminating the labor, time and expense of growing plants for the production of immature embryos or maintaining cultures of somatic embryos. The main remaining drawbacks of this method are that substantial skill is required to properly wound and treat the explants for efficient *Agrobacterium* infection and, although improving, the method is somewhat slow for producing transgenic plants taking on the order of about six months. We have investigated factors for improving the production of transgenic plants via this method. Inclusion of thiol-containing compounds such as cysteine and dithiothreitol in the co-cultivation medium significantly increased T-DNA transfer into cotyledonary-node cells as determined by transient assays of the reporter gene *gusA* (Olhoft and Somers, 2001; Olhoft et al., 2001). Moreover, thiols increased

transient GUS expression in a range of cultivars, and *Agrobacterium* strains and binary plasmids. Production of transgenic plants was increased from 0.9 % transformation efficiency (independent Southern-confirmed plants produced per cotyledonary-node explant) for non-thiol treated explants followed by phosphinothricin (PPT) selection to a transformation efficiency of 2.1 % for explants treated with L-cysteine (Olhoft and Somers, 2001).

Thiol compounds appear to improve T-DNA delivery by inhibiting the activity of plant pathogen- and wound-response enzymes, such as peroxidases and polyphenol oxidases (Olhoft et al., 2001). In our work with PPT-selection we noted production of a large proportion of nontransgenic selection escapes. Selection efficacy can be improved by adjusting the glufosinate selection regime for the genotype under investigation (Zeng et al., 2003). However, we found that tighter selection of L-cysteine-treated explants afforded by hygromycin B-containing media increased production of transgenic plants to 7.7% (Olhoft et al., 2003). We also observed that co-cultivation of explants on medium containing mixtures of L-cysteine, sodium thiosulfate, and dithiothreitol combined with hygromycin selection further increased transformation efficiency to an average of 16.4%. This improved transformation method has been used to produce transgenic plants with multiple *Agrobacterium* strains and binary plasmids. However, the cotyledonary-node method still does not meet all characteristics of an ideal transformation system. Although we have been able to produce transgenics from different soybean genotypes, doing so requires empirical adjustments of selection regime and other system components.

Characterization of T-DNA loci in soybean

Designing transformation experiments for either functional genomics or crop improvement requires knowledge of transgene locus structure, number, transmission and expression. To evaluate transgene loci resulting from the *Agrobacterium*-mediated cotyledonary node method, we characterized 270 independent T_0 plants and 95 randomly selected T_1 progenies for T-DNA locus complexity using Southern analysis. The lines were transformed with *A. tumefaciens* strains LBA4404 or EHA105 carrying the binary plasmids pGPTV, pTOK233, pCAMBIA1303, or pCAMBIA1309 in the presence of thiol compounds and regenerated in medium supplemented with or without silver nitrate ($AgNO_3$). Analysis in the T_0 generation showed that the *hpt*-hybridizing fragments per plant ranged from one to 15 and 31.5% of the lines had a single *hpt*-hybridizing fragment. Each primary soybean transformant had on average 2.0 unlinked transgene loci for which half of the segregating loci in the T_1 progenies were single, simple T-DNA insertions. Of the loci containing multiple T-DNA fragments, a low frequency had

tandem and inverted repeat T-DNA structures. Integration of binary plasmid backbone sequences occurred in 37% of primary transformants. *Agrobacterium* strain, binary plasmid, and thiol treatment had no significant effect on transgene locus structure, numbers or expression. Exposure of soybean explants to $AgNO_3$ during shoot induction and elongation decreased silencing of *gusA* expression in the T_1 generation but appeared to increase transgene locus complexity.

Conclusions

Agrobacterium-based soybean transformation systems have been substantially improved over the past five years resulting in significant increases in the production of transgenic soybean plants. In the cotyledonary node method tighter selection regimes using PPT selection (Zeng et al., 2003), glyphosate selection (Clemente et al., 2000), and hygromycin selection (Olhoft et al., 2003) have overcome earlier difficulties including the production of phenotypically abnormal plants, chimeric plants and problems with sterility have been successfully minimized. In addition, improvements in *Agrobacterium* and vector construction and the use of thiol compounds during co-cultivation have overcome poor infection and T-DNA delivery to target soybean cells. To reduce the time and labor needed to produce large numbers of transgenic plants, adoption more efficient and less damaging selection technologies, such as reef-coral fluorescent proteins (Wenck et al., 2003) or GFP (Finer and Finer, 2000), may allow for earlier detection of transgenic shoots and disposal of nontransformed explants early on in the tissue culture process. More detailed studies also need to be done in determining factors that affect the complexity of transgene insertions. For example, we found that the incorporation of silver nitrate into shoot initiation and elongation media increased the frequency of plants with complex transgene loci. These results suggest that tissue culture conditions and other factors can be manipulated to increase the frequency of plants with simple transgene loci further increasing the efficiency of *Agrobacterium*-mediated transformation of soybeans.

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IV INTERNATIONAL SOYBEAN PROCESSING AND UTILIZATION CONFERENCE

MARCH 1

Overview of research on the relationship between soya intake and breast cancer and potential mechanisms by which soya affects breast cancer risk

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Abstract

Soyafoods have attracted the attention of the research community for their purported health benefits. One reason for the interest in soya is because the soyabean is essentially a unique naturally dietary source of isoflavones. Isoflavones are often referred to as phytoestrogens although they differ from estrogen and are frequently classified as selective estrogen receptor modulators (SERMs), such as the breast cancer drug tamoxifen and the osteoporosis drug raloxifene. Furthermore, isoflavones have important non-hormonal properties. The relationship between soya intake and breast cancer risk has been actively investigated for approximately 15 years. Certainly, the low breast cancer rates in Asia helped fuel initial enthusiasm in soya. Additionally, the knowledge that estrogen-like compounds such as isoflavones have the potential to exert antiestrogenic effects under some experimental conditions provided a possible mechanism by which soya could reduce breast cancer risk. However, beginning in the late 1980s, focus began to shift to the effects of genistein, the main isoflavone in soyabeans, on signal transduction. In vitro, genistein inhibits the growth of both estrogen-dependent and independent breast cancer cells although high concentrations are required. Furthermore, at low concentrations, genistein stimulates the growth of estrogen-receptor positive breast cancer cells. Animal studies generally indicate that isoflavone-rich soya protein and isolated isoflavones inhibit chemically induced mammary cancer and inhibit the growth of tumors in mice implanted with mammary cancer cells. Although again, in some experimental models growth is enhanced. In contrast to the animal studies, there is relatively little epidemiologic support for the hypothesis that adult soya consumption is protective against breast cancer. Results from clinical studies involving markers of breast cancer risk such as serum estrogen levels and estrogen metabolism, cell proliferation, and breast tissue density are, mixed. An emerging and intriguing hypothesis that has both animal and epidemiologic support is that early soya intake is protective against the later development of breast

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cancer. Concerns have arisen about the use of soya consumption by women at high risk of developing breast cancer, breast cancer patients, and women taking tamoxifen. Although controversial, most evidence indicates soya consumption isn't contraindicated for such women.

Introduction

Research into the anticancer effects of soya has been actively underway for more approximately 15 years. A key early development in this field was a National Cancer Institute (NCI) sponsored workshop held in 1990 – the charge of the workshop participants was to evaluate the role of soya in reducing cancer risk (Messina and Barnes, 1991). On the basis of the workshop recommendations in 1991 the NCI issued a request for applications (RFA) worth $2.9 million calling for study of soya.

Initial interest in the relationship between soya intake and breast cancer risk can be attributed to three primary observations. These are the low breast cancer mortality rates in soyafood consuming countries (Pisani et al., 1999), a study published in 1990 showing that the addition of soya protein to a typical laboratory diet reduced the incidence of mammary cancer in rats (Barnes et al., 1990), and the possible antiestrogenic effects of isoflavones, for which the soyabean is the only nutritionally relevant natural dietary source (Murphy et al., 1999). The primary isoflavones in soyabeans are genistein (4' 5, 7-trihydroxyisoflavone) and daidzein (4', 7-dihydroxyisoflavone) and their respective ß-glycosides. Typically, there is somewhat more genist(e)in than daidz(e)in in soyabeans and soyafoods (Murphy et al., 1999). There are also small amounts of a third isoflavone in soyabeans, glycitein (7,4'-dihydroxy-6-methoxyisoflavone) and its glycoside, glycitin.

Research by Folman and Pope conducted nearly 40 years ago first demonstrated the antiestrogenic effects of genistein (Folman and Pope, 1966). In their study injected genistein inhibited estrone-induced stimulation of uterine growth in female mice. Similarly, three years in 1969, they showed that when genistein was injected along with estradiol, estradiol uptake by the mouse uterus and vagina was substantially decreased (Folman and Pope, 1969). It was not until 1984, that Setchell et al began to popularize the notion that isoflavones, because of their weak estrogenic, and therefore possibly antiestrogenic effects, might be beneficial for breast cancer (Setchell et al., 1984). Interestingly however, it was the effects of genistein on signal transduction, in particular the identification of genistein as a specific inhibitor of tyrosine protein kinase in 1987 by Akiyama et al that really first drew widespread attention to the possible anticancer effects of isoflavones

and soya (Akiyama et al., 1987; Ogawara et al., 1986). The 1991 NCI workshop did identify several possible anticarcinogens in the soyabean (Messina and Barnes, 1991) but without question evidence points toward the isoflavones as the putative anticarcinogens (Messina and Flickinger, 2002).

Background on isoflavones

Isoflavones are similar in chemical structure to mammalian estrogens so it is not surprising they bind to estrogen receptors and affect estrogen-regulated gene products (Markiewicz et al., 1993; Mayr et al., 1992). Hence, isoflavones are often referred to as phytoestrogens. Although isoflavones are regarded as weak estrogens they have long been thought to have the potential to exert physiological effects in vivo in people who eat soyafoods since serum isoflavone levels can be 10,000 fold higher than endogenous estrogen levels (King and Bursill, 1998). Furthermore, with the recent identification of a second estrogen receptor called estrogen receptor beta and the finding that isoflavones bind more tightly to this receptor than to estrogen receptor alpha, older estimates of the "estrogenicity" of isoflavones are now viewed as outdated.

Of critical importance however is the recognition that isoflavones are considered to be selective estrogen receptor modulators (SERMs). SERMs like the drugs tamoxifen and raloxifene have tissue selective effects, exerting estrogen-like effects in some tissues but either no effects or antiestrogenic effects in other tissues. Research indicates for example that isoflavones exert estrogen-like effects on the bones (Arjmandi and Smith, 2002; Messina et al., 2001), but unlike estrogen do not increase endometrial cell proliferation (Duncan et al., 1999; Upmalis et al., 2000) or serum triglyceride levels (Anderson et al., 1995). A recent animal study provides a good example of the SERM-like activity of genistein and how it contrasts with estrogen. Although both compounds increased bone density in ovariectomized mice only the former increased uterine weight (Wu et al., 2001). Consequently, it is inappropriate to draw conclusions – favorable or unfavorable – about the health effects of isoflavones based on the effects of estrogen. For the same reason, it is more accurate to refer to the estrogen-like, rather than to the estrogenic, properties of isoflavones.

Finally, although the potential antiestrogenic effects of weak estrogen-like compounds such as isoflavones, which as previously noted were first observed in mice nearly 40 years ago (Folman and Pope, 1966) certainly provide a possible mechanism for the hypothesized protective effects of soya consumption against breast cancer it is by no means the only