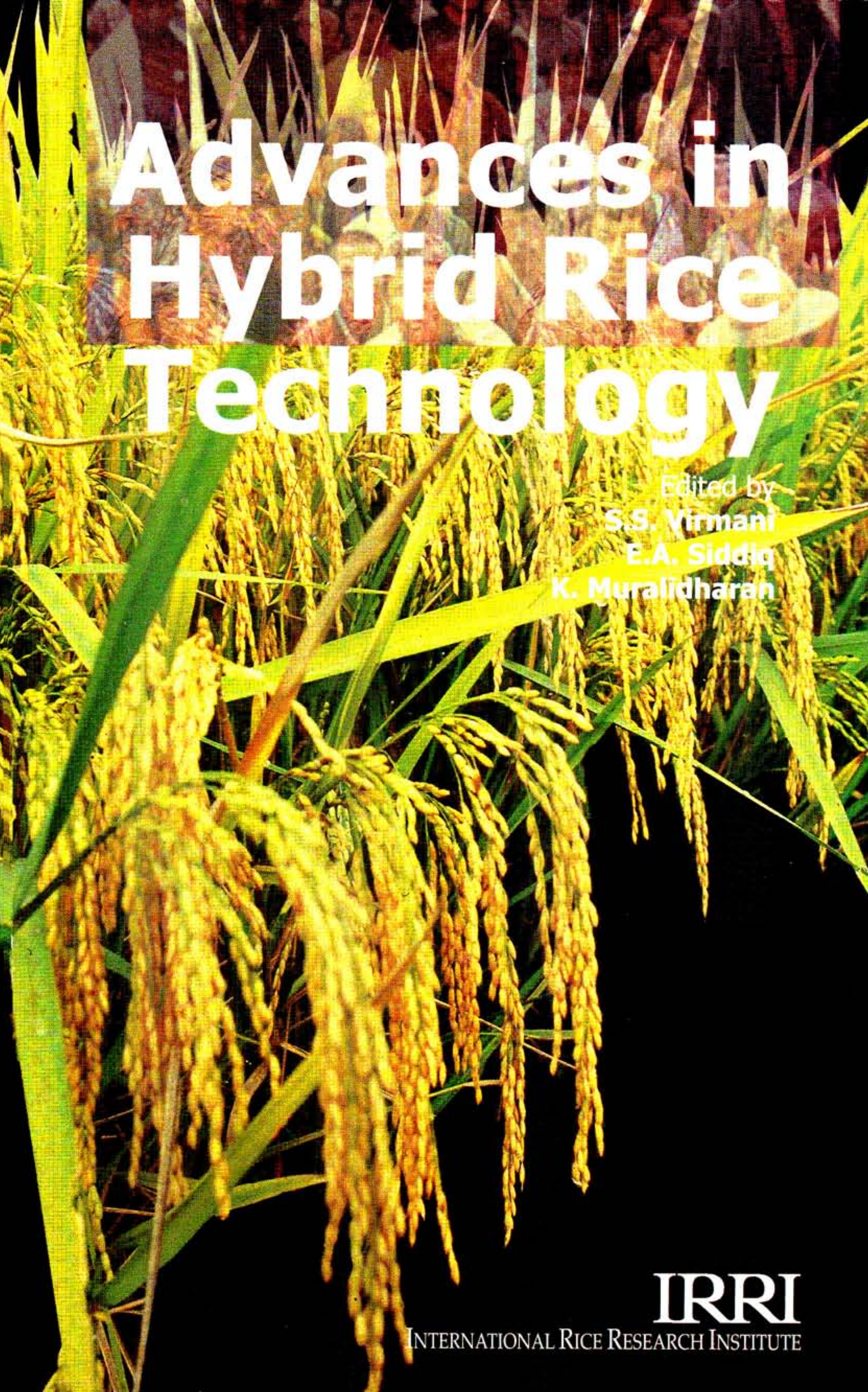

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CHAPTER 31

Developing hybrid rice in Brazil: methodology, highlights, and prospects

E.P. Guimarães, V. dos A. Cutrim, and J.A. Mendonça

EMBRAPA-CNPAF began to explore the prospects for and problems of hybrid rice in 1984 via two areas: the introduction of allogamic traits to develop A and B lines and reciprocal recurrent selection. The methodological steps involve identifying maintainer and restorer lines, introducing allogamic traits into maintainer lines, and transferring the cytoplasmic genetic male sterility system to derived F_4 maintainer lines. Development of line 0461 with allogamic traits and good agronomic behavior and several hybrids is the most significant result. Future plans are directed toward developing an economically viable methodology to produce hybrid seeds, locating partners in the private sector, identifying new cytoplasm sources to produce genetically diverse A lines, and evaluating the economic need to use the allogamic traits.

The hybrid rice program at the Brazilian Enterprise for Agricultural Research–National Research Center for Rice and Beans (EMBRAPA–CNPAF) began in 1984 as a joint project with the Institute for Research in Tropical Agriculture (IRAT, today CIRAD–CA, Centre de coopération internationale en recherche agronomique pour le développement, departement des cultures annuelles). Because the yield plateau experienced with conventional varieties in the irrigated environment turned out to be the main issue, farmers pressured researchers to generate new technology. Farmers expressed their willingness to invest in the rice crop to obtain higher yields. A detailed study indicated that the possibility of success was in developing hybrid rice for the irrigated environment and not for the upland ecosystem.

This hybrid rice project followed Chinese technology. The three-line breeding method involved cytoplasmic genic male sterile lines (A lines). maintainer lines (B lines) to pollinate and produce seeds on A lines carrying the male sterile gene, and restorer lines (R lines) carrying genes to restore fertility in A lines and produce hybrid seed. The two-line method (thermosensitive or photoperiod-sensitive genic male sterility) currently pursued vigorously to develop hybrids may be one alternative that will make hybrid rice more attractive to countries with little or no investment in this technology.

The prospects for developing three-line breeding to produce hybrids and the problems encountered in achieving this goal have been described already (Lin and Yuan 1980, Yuan and Virmani 1988). In countries with an agricultural system different from that of the Chinese, the key point for this technology is the hybrid seed production technique as described by Virmani and Sharma (1993). An important component for obtaining more than 4 t ha^{-1} of hybrid seed is the amount of hand labor required in this process.

As in other countries (Guok 1994, Moon et al 1994), labor-intensive hybrid seed production is a major constraint to the development of the seed industry in Brazil. If practical solutions are not found for this component, the hybrid seed cost will increase to levels that are commercially unaffordable to farmers. Experience in the Philippines demonstrated that if proper adjustments are made in seed production technology, it can provide incentives to seed growers and make hybrid seeds available at a price that farmers can afford (Lara et al 1994).

Thus, the basic idea of this project was to incorporate in hybrid rice development a factor that would reduce hand labor requirements. The way found was to introduce in the cultivated species *Oryza sativa* allogamic traits from the wild species *O. longistaminata;* the targeted trait was large stigma. The project drew inspiration from the French experience (Taillebois 1983). A few lines with the genetic background of cultivated species and allogamic traits were developed and tested in Brazil. The results observed by Taillebois and Guimarães (1988) and Breseghello and Neves (1995) indicated that lines with large stigma ensured higher outcrossing rates than normal lines.

This chapter attempts to describe the strategy adopted by the EMBRAPA–CNPAF/CIRAD–CA project (from 1994 onward EMBRAPA–CNPAF took full responsibility for the project). We also present some results obtained during the period and discuss future prospects.

Methodology

Identifying germplasm with the restoration gene

To identify B lines and R lines, the first step is to introduce or develop locally adapted cytoplasmic male sterile lines (CMS lines). The next step is to evaluate and identify lines that maintain the sterility of the CMS line or restore the fertility in the F_1s. To achieve this goal, it is necessary to screen germplasm from the target area for these genes.

It is important to have an ongoing conventional program in association with the hybrid development project. Advanced breeding lines or lines ready to be released, with resistance genes for diseases and pests, and genes for good grain quality, can be found easily. This will help identify restoration and maintenance ability in test crosses made with the best breeding lines available and the CMS line.

Guimarães et al (unpublished) tested 1,046 lines in this project during 1984 to 1995. Of these, 378 (36%) showed strong restoring ability and 43 (4%) possessed good maintaining capacity. The results clearly demonstrate the difficulty in finding maintainer lines; of the 43 identified, 18 came from EMBRAPA–CNPAF's germplasm screened in 1984. Most of these lines have no agronomically desirable characteristics. In the past three years, the best available breeding lines in the country coming from the National Rice Breeding Network (CTArroz) as an observation nursery have also been evaluated for this purpose.

The major constraint in breeding hybrid rice for Brazil is the low frequency of maintainer lines among Brazilian rice cultivars evaluated. So far, progress has been made in transferring the allogamic trait to some lines and some cytoplasmic male sterile lines have been developed. It may be difficult to find good combinations from the narrow genetic base available in the Brazilian (Rangel et al 1996) and Latin American varieties (Cuevas-Pérez et al 1992) to produce higher heterosis. Therefore, we need to increase the number of maintainer lines to make it possible to develop numerous good A lines. Because hybrids are a product of A and R lines, a larger genetic distance between these lines would result in higher heterosis. The nonavailability of genetically diverse germplasm in Brazil may limit the identification of heterotic rice hybrids.

The two-line hybrid breeding approach may open up new possibilities for achieving the desired heterosis.

Steps to develop A and B lines

Figure 1 shows the germplasm flow chart followed to develop A and B lines in Brazil. Selected maintainer lines were crossed with an allogamous line (#24Z) developed by EMBRAPA–CNPAF and CIRAD–CA from a cross between *O. longistaminata* A. Chev. and *O. sativa.* The most commonly used allogamous line was #2RI (032G-98-1-5-1-1-1), which was obtained from the cross IR13540-56-3-2/#24Z.

Initially, we planned to backcross (BC) the F_1 plants twice with the maintainer. Because only a limited number of plants with allogamic traits were observed in the segregating generations, it was necessary to backcross only once. Besides, linkage drag also resulted in the transfer of several undesirable wild traits with the targeted allogamic characteristics in the segregating populations, which always presented a high degree of grain shattering, lodging, and poor grain type.

The best maintainers were crossed with the allogamous donor and one backcross was made. The BC_1F_1 plants were selfed and the BC_1F_2 seeds were planted at the Palmital experiment station, under irrigated conditions, to exercise selection for allogamic traits. The BC_1F_3 seeds were also planted at the Palmital experiment station, but under rainfed lowland conditions to expose plants to increased disease pressure. In the F_3 generation, selection was targeted not only for allogamic traits but also for other desired agronomic traits.

The BC_1F_4 generation was also planted at the Palmital experiment station under irrigated conditions. At this stage, all plants already exhibited large stigma and selection pressure basically targeted agronomic traits. The best plants were selected to

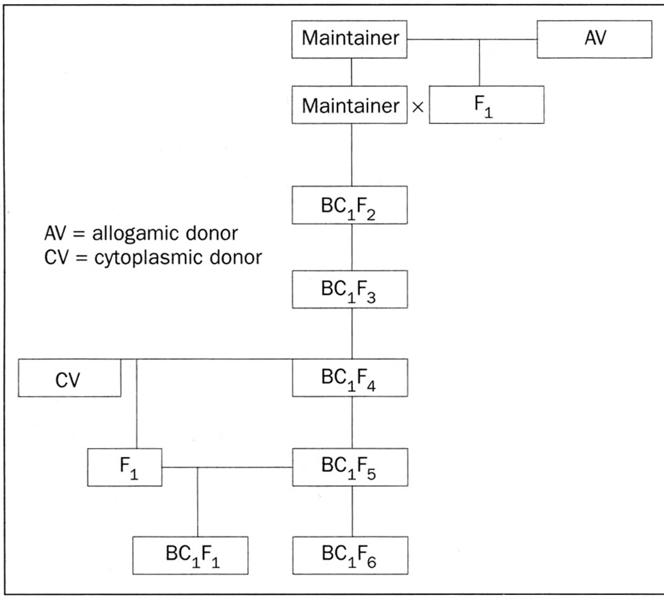

Fig. 1. Flow chart to develop A and B lines to produce hybrid rice in Brazil.

test-cross with a cytoplasmic male sterile donor to begin sterilization of the best maintainer lines. Between 20 and 50 BC_1F_4 plants were chosen and their panicles were clipped at the blooming stage and taken to the Capivara experiment station for hand crossing to cytoplasmic male sterile line Zhen Shan 97A. Recent emphasis, however, has shifted to 046IA, an improved CMS line developed locally.

The procedure involved a large number of backcrosses in each generation to incorporate the male sterile cytoplasm and to develop maintainer lines. The entire process was conducted at the Capivara experiment station under screenhouse conditions. The F_1 plants that originated from the combination of BC_1F_4 and the male sterile cytoplasm source were backcrossed to the BC_1F_5 (Fig. 1). The last backcross expression used in reality differed from the textbook meaning as the F_1s were combined to select BC_1F_5 plants. These plants were chosen and selfed when the BC_1F_4 generation was used to combine with the cytoplasmic donor, resulting in a similar genetic content. This backcross procedure was repeated several times (F_1s were crossed to BC_1F_6, the product was crossed to BC_1F_7, etc.). This step was repeated five or six times. Completely sterile plants were obtained from the backcrosses (A line) and plants with the capacity to maintain sterility were produced from the selfed generations (B line).

All this work was quite tedious. During each backcross generation, a large number of backcross progenies and plants in the selected progenies were discarded on the basis of absence of allogamic traits, a continuous high level of segregation for fertility in A line development, instability of sterility under different environmental conditions, and poor agronomic traits.

Following this methodology, we have been able to generate one cytoplasmic male sterile line (046IA) and 13 promising lines in advanced stages. Virmani et al (1991) reported that from 1980 to 1988, the International Rice Research Institute (IRRI) could generate 40 A lines, but only 3 had the required characteristics for commercially hybrid production. This experience has also helped to develop a well-trained staff and create satisfactory working conditions, including infrastructure.

Reciprocal recurrent selection

This reciprocal recurrent selection was targeted for mid- and long-term goals, as presented by Neves et al (1994). The strategy was to develop two heterotic populations for grain yield. One population was used as a source of A lines with male sterile wild abortive (WA) cytoplasm and from the same population attempts were made to obtain B lines for the cytoplasm. This population also carried allogamic traits. The other population was used as a source for R lines to restore fertility and produce hybrid seeds.

In the reciprocal recurrent selection method, the first step was to identify parents with a wide genetic base and to create two contrasting populations. Within the project, CNA 2M (source of A and B lines) and CNA 3R (source of R lines) were developed. To facilitate the pollination process, the large stigma trait was introduced in the first population.

In 1993-94, population CNA 3R/0/2 was planted and 700 S_0 male sterile plants were identified. From these, 300 were further selected based on seed production and plant height. During the 1994-95 cropping season, these S_1 lines were planted to select within and between rows; 150 lines were picked and two plants were chosen within each. A similar procedure was used with the CNA 2M/1/0 population. The selection criteria differed only in plant height. In this population, the plants had to be shorter to facilitate pollination.

In 1995-96, 300 S_2 plants were taken to the field. The male sterile plants in each line were marked, and at the blooming stage they were pollinated using pollen samples from the reciprocal population. A similar process was used with the reciprocal population also. This work resulted in the production of 300 half-sib families from each population.

The plan for 1996-97 includes evaluation and selection of all these half-sib families. General combining ability will be measured and the lines that show the best combinations will be selected to generate the base population for the next recurrent cycle. The remnant S_1 seeds will be used to recombine and generate new populations.

The hybrids will be produced through the combination of A and R lines coming from the two populations, CNA 2M and CNA 3R, respectively. Therefore, during each recurrent cycle, the best lines from each population will be extracted and advanced through pedigree. The large stigma trait will be taken into account in each generation and restorer capacity will be tested when the lines are fixed.

Results

Developing line 046IA

There are at least three ways to obtain A and B lines for a hybrid rice program: (1) introducing lines from another program, (2) transferring cytoplasmic male sterility to new materials, and (3) developing a line with new male sterile cytoplasm. EMBRAPA–CNPAF concentrated on the first two ways. Since 1985, it has introduced 18 lines with WA cytoplasm, mainly through IRRI (Table 1).

In 1988, line IR13540-56-3-2 was identified as a maintainer. In 1989, this line was crossed to the initial allogamous line (#24Z) and the combination was coded as 032G. The cross underwent selection for large stigma and agronomic traits from F_2 to F_4 (pedigree 032G-85-1-5). As mentioned earlier, we began to test-cross in the F_4 generation to incorporate cytoplasmic male sterility from Zhen Shan 97A. The F_1 was crossed to self-pollinated F_5 plants from the cross 032G. Sterile plants with allogamic and desirable agronomic traits were selected. The process was repeated five times and the 046IA and its maintainer *2RF were obtained in 1994.

In the initial stages of development, selection was for sterile plants. During the early generations, there was a wide range of segregation for sterility from fully fertile to completely sterile plants. As selection progressed, the percentage of completely sterile plants increased. In most crosses, most lines were discarded in this selection process because of a lack of stability, poor agronomic traits, and unsuitable grain type.

The efficiency of this work was improved by the check made for sterile pollen grains using a microscope. At the early stages, however, this check was done only in the field and, because of environmental conditions, this introduced an error compo-

Table 1. A and B lines introduced in the hybrid rice project by EMBRAPA–CNPAF.

Year	A lines	B lines	Origin
1985	Zhen Shan 97A	Zhen Shan 97B	China
1985	Er-Chiu-Nan 1A	Er-Chiu-Nan 1B	China
1985	V41A	V41B	China
1985	WU10A	WU10B	China
1985	Yar Ai Zhao A	Yar Ai Zhao B	China
1985	MS577A	MS577B	Korea
1985	MS519A	MS519B	Korea
1985	Pankari 203A	Pankari 203B	IRRI
1992	IR58025A	IR58025B	IRRI
1992	IR62829A	IR62829B	IRRI
1992	IR64608A	IR64608B	IRRI
1995	IR68886A	IR68886B	IRRI
1995	IR68888A	IR68888B	IRRI
1995	IR68891A	IR68891B	IRRI
1995	IR68887A	IR68887B	IRRI
1995	IR68275A	IR68275B	IRRI
1995	IR68890A	IR68890B	IRRI
1995	IR68281A	IR68281B	IRRI

Table 2. Yield (kg ha^{-1}) of rice hybrids during the 1995–96 cropping season at Goiás, Tocantins, and Rio Grande do Sul.

Treatment[a]	Goiás	Tocantins	Rio Grande do Sul	Mean
H348	6,668	11,000	6,044	7,904
H40	7,021	11,083	5,593	7,899
H29	6,399	10,500	5,517	7,472
H38	5,752	10,917	5,746	7,471
H37	6,258	9,833	5,968	7,353
H16	5,965	9,917	5,352	7,078
H349	5,794	9,583	5,815	7,064
H329	6,755	7,583	6,152	6,830
H39	5,479	8,583	6,361	6,807
H200	6,340	7,500	6,419	6,753
H518	5,935	9,250	4,825	6,670
BR-IRGA 419	5,976	8,500	4,419	6,298
Metica 1	6,763	9,583	3,421	6,589
H35	6,884	8,167	4,672	6,574
H347	6,406	9,000	4,146	6,517
Javaé	6,281	6,500	6,495	6,425
H512	6,065	5,833	5,149	5,682
H34	4,758	5,750	3,739	4,749
Mean	6,250	8,838	5,324	6,785
CV(%)	13.0	15.0	12.6	14.3

[a]Metica 1 and Javaé were check varieties in Goiás and Tocantins and BR-IRGA 419 was used as a check in Rio Grande do Sul.

nent that increased the amount of work. Later, this was corrected by bringing materials to the laboratory for a complete check on pollen sterility.

Evaluating hybrids

In 1994, the EMBRAPA–CNPAF project produced 660 hybrid combinations by using 10 A lines and 88 R lines. In the 1994-95 cropping season, these hybrids, their parents, and local checks were planted under irrigated conditions at the Palmital experiment station. From this effort, 30 high-yielding combinations were selected.

In the 1995 cropping season, only the best 15 hybrids were evaluated simultaneously under tropical (Tocantins and Goiás) and subtropical (Rio Grande do Sul) conditions. Yield data (Table 2) showed a marginal superiority of the hybrids (4%) in relation to the local checks at Goiás and Tocantins. But these hybrids outperformed the check at Rio Grande do Sul. The results demonstrated a potential for hybrid production, mainly in the subtropical region. The challenge was to develop a suitable and economically viable hybrid seed production technique. The project had already directed its efforts and resources to develop such a technique. During the 1996 winter season, massive seed production of CMS line 046IA was launched. Simultaneously, seed multiplication of several restorer lines for use as combiners with the cytoplasmic male sterile line was also being carried out.

Future plans

Future plans for hybrid rice in Brazil include the following:

- Develop a suitable and economically viable hybrid seed production technique for Brazilian conditions.
- Evaluate yield gains from hybrid seed production caused by the introduction of allogamic traits in male sterile lines and decide on the need to continue using this approach in the hybrid rice breeding program.
- Develop and deploy genetically diverse CMS sources.
- Once the hybrid rice technology is packaged, EMBRAPA will open financial participation to the private sector. EMBRAPA–CNPAF has already invested more than US$4 million in hybrid rice research since 1984. The proposal to develop hybrids that yield 30% more than commercial varieties would rely on strong participation by the private sector, with an annual investment of $300,000-400,000.

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Notes

Author's address: National Research Center for Rice and Beans (CNPAF), Brazilian Enterprise for Agricultural Research (EMBRAPA). Caixa Postal 179, 74001-970 Goiânia, Coils. Brazil.
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