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Chapter 3

Genetic Resources of Wild *Arachis* and Genetic Diversity

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Abstract

Genetic resources conserved of wild *Arachis* species doubled in the past decade. The species most represented in the world collection is *A. glabrata*, followed by *A. pintoi*. The largest collections are held at the Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnologia/Empresa Brasileira de Pesquisa Agropecuária (CENARGEN/EMBRAPA), Brazil, and the Texas Agricultural Experiment Station of Texas A&M University, USA. Although the number of available accessions increased significantly, germplasm flow of new material is relatively slow. The main reasons for this are little or no seed production and slow quarantine procedures.

The few studies of genetic diversity emphasizing wild species with forage potential, such as *A. glabrata*, *A. pintoi*, and *A. sylvestris*, showed a high degree of intraspecific variation. Methods that provide satisfactory results include morphological descriptors, isozyme patterns obtained by electrophoresis, and molecular markers.

Introduction

Among the international initiatives focused on genetic resources of the 1980s, the existing genetic resources of legume genera with forage potential, such as *Stylosanthes* and *Centrosema*, were appraised in two workshops (Stace and Edye, 1984; Schultze-Kraft and Clements, 1990). Wild *Arachis* species, however, were looked at mainly in the context of wild relatives of the cultivated groundnut (*A. hypogaea*). This genus was not even mentioned when the International Board for Plant Genetic Resources (IBPGR) prepared its global plan of action for forage genetic resources (Davies, 1984). The revision of existing genetic resources of wild species of *Arachis* (Valls et al., 1985) was thus carried out in the light of a genetic resource for the improvement of *A. hypogaea*.

Some forage researchers, however, have included wild *Arachis* species in their search for better legumes for pasture improvement or for ground cover in the tropics and subtropics since the early 1960s (Table 1) and have identified their potential. Therefore, in the past 2 decades, collecting and preserving wild *Arachis* germplasm has focused on broadening the genetic base of species with forage potential, such as *A. glabrata* and *A. pintoi* (Valls and Pizarro, Chapter 2, this volume). Because of these efforts, the amount of germplasm conserved doubled from 484 accessions of wild *Arachis* species in 1983 (Valls et al., 1985) to over 1000 accessions conserved in 1993 (Table 2).

Agronomic research on forage *Arachis* was also intensified, although few researchers included new germplasm in their evaluation programs (e.g., Pizarro et al., 1993; Quesenberry et al., 1993). Though research concentrates predominantly on two species, *A. glabrata* and *A. pintoi*, a wealth of other, often undescribed species is still awaiting discovery of

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Table 1. Species in the genus *Arachis* being used for forage or ground cover.

Species of *Arachis* (section)[a] Accession no.	Geographic region of study	Cultivars named (year of release)	Reference
A. glabrata (RH)			
PI 118457	Florida, USA	Arb	Prine, 1964, 1972
PI 262839		Arblick	Prine et al., 1981
GS-1		Florigraze (1978)	Prine and French, 1993
PI 262817		Arbrook (1986)	
A. monticola (AR)			
PI 263393[b]	Georgia, USA	-	Beaty et al., 1968
A. repens (CA)			
GKP 10578	Tropical Africa	-	Akobundu and Okigbo, 1984
A. pintoi (CA)			
CPI 58113[c]	Tropical Australia	Amarillo (1989)	Cook et al., 1990
CIAT 17434[c]	Colombia	Maní Forrajero Perenne (1992)	Grof, 1985 Rincón et al., 1992
	Honduras	Pico Bonito (1993)	Sec. Rec. Nat., 1993
Arachis sp. (PR)			
PI 446898	Florida, USA	'Pantanal'	Kretschmer and Wilson, 1988

a. AR = Arachis; CA = Caulorhizae; PR = Procumbensae; RH = Rhizomatosae.

b. = PI 219824.

c. Developed from PI 338314 (= GK 12787).

Table 2. Genetic resources of forage *Arachis* conserved at the main centers of conservation, as of May 1993 (sections in order of importance as a potential forage crop).

Section/ Species	Accessions conserved[a]										
	CENARGEN	IAC[b]	INTA/ UNNA[b]	CIAT	TAES[b]	USDA[b]	NCSU[b]	U.Fla.[b]	ICRISAT	CSIRO	Total[b]
Caulorhizae											
A. pintoi	77	2	-	27	40	10	1	1	2	1	80
A. repens	16	1	-	5	3	1	1	-	2	2	16
Rhizomatosae											
A. glabrata	51	22	-	15	200	50	20	110	45	10	320
other spp.	19	-	-	-	6	6	3	-	1	21	19
Procumbensae											
Arachis. sp. 'Pantanal'	1	-	1	1	1	1	1	1	1	1	1
other spp.	29	7	10	-	40	15	8	-	-	9	45
Triseminalae											
A. triseminalis	8	1	2	-	6	2	2	-	-	1	12
Arachis (wild)											
Arachis spp.	138	44	50	1	150	70	80	-	165	18	200
Ambinervosae											
Arachis spp.	110	17	10	-	90	40	12	-	15	2	130
Erectoides											
Arachis spp.	71	20	10	-	50	15	20	-	12	9	71
Extranervosae											
Arachis spp.	134	9	5	-	50	20	15	-	2	1	135
Not identified	-	-	-	1	-	-	-	-	41	3	—[c]
Total	654	123	88	50	636	230	163	112	286	78	1029

a. CENARGEN = Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnologia, Brazil; IAC = Instituto Agronômico de Campinas, Brazil; INTA = Instituto Nacional de Tecnología Agropecuaria, Argentina; UNNA = Universidad Nacional del Nordeste, Argentina; TAES = Texas Agricultural Experiment Station, USA; USDA = United States Department of Agriculture; NCSU = North Carolina State University, USA; ICRISAT = International Crops Research Institute for the Semi-Arid Tropics; CSIRO = Commonwealth Scientific and Industrial Research Organisation, Australia; U.Fla. = University of Florida, Gainesville, FL, USA.

b. Estimated numbers of different accessions.

c. Probably included in other sections of total.

their potential utilization as forage. The purpose of this chapter is (1) to review the existing genetic resources of wild *Arachis* species, (2) to focus on germplasm utilization, and (3) to describe the genetic diversity encountered in wild *Arachis* species with forage potential.

Genetic Resources

Conservation

Numbers for *A. hypogaea* vary from zero in several collections to more than 10,000 at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), including many germplasm collections originally used for forage purposes. In wild *Arachis*, the largest collection of a single species is that of *A. glabrata*, followed by *A. pintoi* (Table 2). Other sections, such as Arachis, Ambinervosae, and Extranervosae, also contain considerable numbers of accessions (sections according to Krapovickas, 1990). The large increase in accessions in the sections Caulorhizae (>550%), Procumbensae (>350%), and Rhizomatosae (>250%), compared with the status of collections in 1983 (Valls et al., 1985), reflects the intensive search to broaden the genetic base of potential forage species (Valls and Pizarro, Chapter 2, this volume).

With more than 600 accessions each, the largest germplasm collections of wild *Arachis* species are held at CENARGEN/EMBRAPA, Brazil, and the Texas Agricultural Experiment Station (TAES) of Texas A&M University, Stephenville, Texas, USA (Table 2). The latter helps substantially to increase the accessions introduced into the United States Department of Agriculture (USDA) collection at Griffin, Georgia, USA. Other significant collections are maintained at ICRISAT, India, and North Carolina State University (NCSU), Raleigh, North Carolina, USA, in order of collection size. A large part of the collected material is conserved. Particularly, collections acquired prior to 1976 have been maintained and duplicated in several institutions. Some portion of the overall collection is duplicated in a few institutions, according to the use of the species.

Historically, collections at TAES, USDA, NCSU, and ICRISAT were intended to provide wild relatives for groundnut breeding programs. Those in Australia, the University of Florida (Fort Pierce and Gainesville), and CIAT were assembled for forage use. The large collection at CENARGEN and those at the Instituto Agronômico de Campinas (IAC) and in Argentina served conservation and characterization purposes. All activities related to germplasm maintenance in Brazil do not depend only on CENARGEN, but also involve other EMBRAPA centers (such as the Centro de Pesquisa Agropecuária dos Cerrados—CPAC).

Most institutions maintain plants in the field or in glasshouse collections that are complemented by seed conservation. Conditions for plant maintenance were described by Valls et al. (1985). Seed conservation in cold rooms is not always satisfactory because after some storage time, seeds may show dormancy or may lose their viability. Research on seed physiology to improve long-term storage of wild *Arachis* is urgently needed (Ferguson, Chapter 11, this volume).

Documentation

Origin of accessions. Documentation of the origins of accessions is an important part of germplasm management. The so-called passport data are maintained in computerized databases at all major institutions. The documentation of "old" germplasm accessions, however, is often erratic and information on the history and origin may be lost. Germplasm collected from 1976 to 1983 was documented in a catalog by Simpson and Higgins (1984). The passport information on more recently collected germplasm, in particular that collected in Brazil, is well documented in CENARGEN's *Arachis* database. A world catalog of wild *Arachis* germplasm would be a useful tool to make this information available to researchers.

Identification of accessions. Material may be identified by institutions and by collectors. There are institutional numbers of national character, such as BRA, CPI, and PI (Annex 1). Other institutional numbers are of local character, such as CIAT, CPAC, ICG, IRFL, and IRI, and assist internal control. Many accessions are also identified by the collectors' abbreviations, with letters such as G, K, P, S, V standing out (Annex 2). As collection of wild *Arachis* species has been an example of true international collaboration, most accessions are identified in the most important collections by the abbreviations of participating collectors.

The same accession has often received a number from each institution in which it is held. This is probably illustrated best by the first accession of *Arachis pintoi* collected by Geraldo Pinto in 1954. From the plot he planted at the Instituto de Pesquisa Agronômica de Leste (IPEAL) in Cruz das Almas, Bahia, several researchers received material to be incorporated into germplasm banks (Valls, 1992). The accession was thus registered under many different numbers, such as GK 12787, PI 337361, PI 338314, PI 338447, CPI 58113, CIAT 17434, IRI 2270, IRFL 4222, and I 44457 (Figure 1). Several of these denominations were ephemeral. To avoid unwanted duplication of

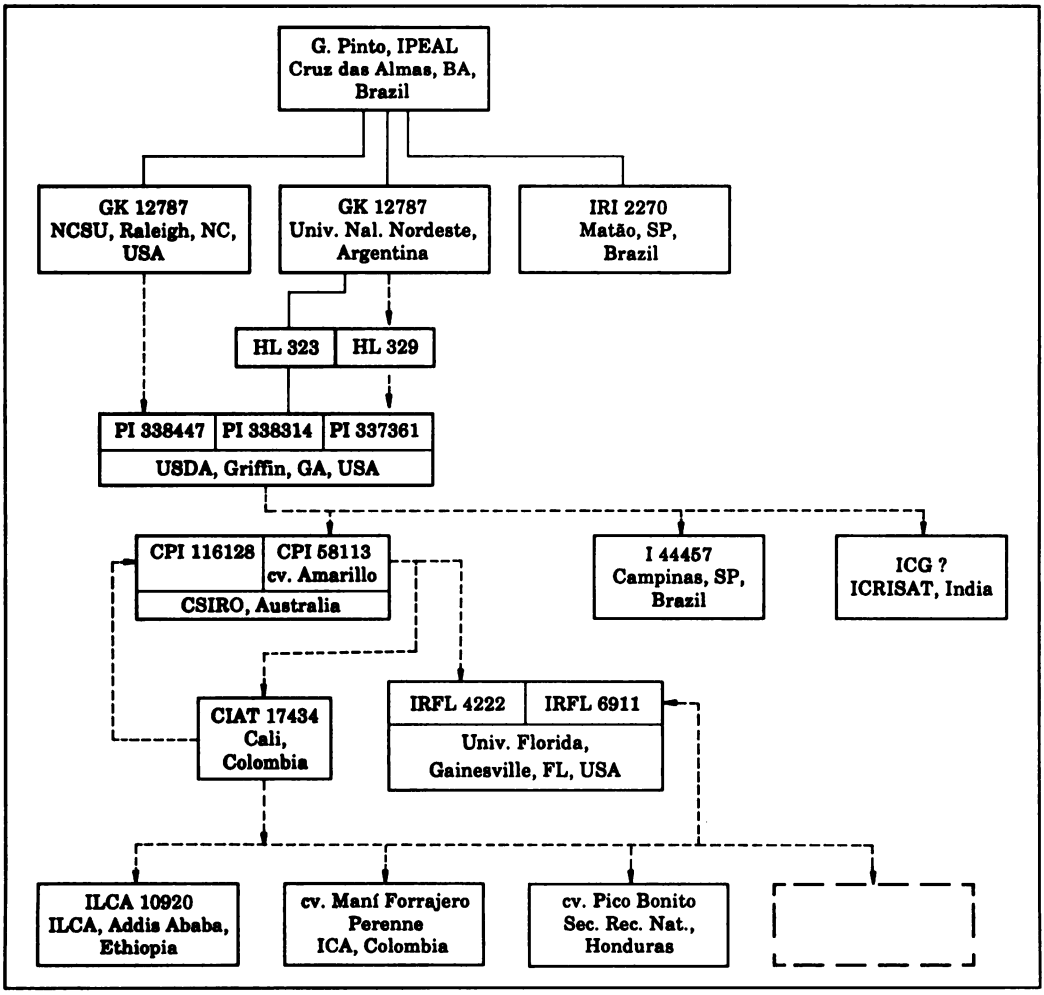

Figure 1. Multiple numbering and naming of one accession of *Arachis pintoi* (germplasm flow by seed ----➔; by vegetative material ——).

accessions, the collector's abbreviation and number should always be stated when germplasm is moved between institutions.

Incorrect naming may occur when an accession is identified with a released cultivar, where this particular material was not part of the basic population or its descendants—as cultivars are defined. For example, CIAT 17434 should not be identified with cv. Amarillo because the CIAT accession is not from the basic population from which the cultivar was derived, or is just a progeny of that cultivar.

In the present situation of unclear taxonomic nomenclature, forage *Arachis* researchers tend to not be much concerned with identifying the species, but rather the particular accession they are working with. For example, the same accession of *A. glabrata* may be referred to in different publications as "*A. hagenbeckii*" and "*Arachis* sp. section Rhizomatosae."

Distribution

Germplasm accessions collected prior to 1976 have been distributed widely. From 1980 to 1992, CIAT's Genetic Resources Unit distributed 17 samples of *A. pintoi* accession CIAT 17434 to 14 countries in tropical America, Asia, Africa, and Europe, and only in 1990 began to distribute other accessions of *A. pintoi* collected in the early 1980s. "New" accessions of *A. pintoi* were introduced to CIAT from CENARGEN in late 1992 and still require initial increase before they will be available for distribution. ICRISAT supplied samples of 648 accessions of wild *Arachis* species to 52 users in different countries, after 1988 (M.H. Mengesha, personal communication).

Introduction of *Arachis* germplasm is usually a slow process because many accessions or species produce little seed, particularly under glasshouse conditions (Cook et al., Chapter 14, this volume). Thus, postentry quarantine and phytosanitary observations may delay the introduction process. Safe international shipments, however, are of high priority, and alternative methods of germplasm movement should be explored. For example, tissue culture may be used to handle and clean up material. Protocols are in place for several species of the genus *Arachis* (Valls and Simpson, Chapter 1, this volume; Simpson et al., Chapter 4, this volume).

Germplasm Utilization of Species with Forage Potential

Species of known value

Germplasm acquired prior to 1976 was the common base from which most characterization data were obtained. Evidence of the potential of the main species was often obtained from the first available accession of each. Thus, the first accession of *A. repens* Handro (GKP 10578) collected by Otero at Jequitaí, Minas Gerais, in 1941 has shown great potential as a ground cover, green manure, or ornamental plant. The first accession of *A. pintoi* (GK 12787) collected by Pinto at Boca do Córrego, Bahia, in 1954 (G.C. Pinto, personal communication, 1993), is now an international success as a forage and cover crop. Cultivar Florigraze originated from a seedling germinated next to a plot of *A. glabrata* (PI 118457), this one collected by Archer and Gehrt at Campo Grande, Mato Grosso do Sul, in 1936 (Prine, 1964; Prine et al., 1981). The 'Pantanal' peanut resulted from the direct adoption of a new germplasm accession, the first available in this species, collected by Kretschmer and Rayman in the lower Pantanal area of Mato Grosso, Brazil, in 1976 (Kretschmer and Wilson, 1988).

Data on the characterization and agronomic performance of *A. pintoi* and *A. glabrata*, respectively, are now being generated, in Florida, USA (Quesenberry et al., 1993), and from a nursery established at CPAC/EMBRAPA of recently collected material in Brazil (Pizarro et al., 1993). Given the strong

increase in the available germplasm base, it is obvious that the genetic potential of recognized forage species is not known at this stage. There is a risk that researchers will continue to use the most popular accessions because of availability of seed or vegetative propagules for *A. glabrata* and *A. repens* and convenience, as has happened with practically all tropical forage legumes. Other species, such as the 'Pantanal' peanut, do not yet have sufficient germplasm available to begin studies of intraspecific variability.

Agronomically "new" species

Besides the two species, *A. glabrata* and *A. pintoi*, on which forage research has predominantly concentrated, a wealth of other, often undescribed species is still awaiting discovery of their potential, as comprehensively described by Valls and Simpson (Chapter 1, this volume). Research should include direct use of these species of mostly unknown agronomic value, and their use in interspecific breeding programs.

"New" species with the highest potential for agronomic use will probably be found in the section Procumbensae because of their high biomass production, general vigor, seed production potential, and their crossability with species of the sections Caulorhizae and Rhizomatosae (Gregory and Gregory, 1979). The germplasm base available of section Procumbensae, however, is still small (Table 2). Another promising section is Triseminalae because it contains perennial germplasm adapted to dry climates and hard, structured soils. As stated by Valls and Simpson (Chapter 1, this volume), the germplasm of this section needs to be increased. Species of little promise as forage plants may provide important genes for improving established forage species, especially when pests and diseases may become a problem in the future.

Genetic Diversity

Within the genus, most studies on genetic diversity were carried out on *A. hypogaea* and related wild species in the section Arachis. This research, including studies on morphology, phenology, agronomy, cytogenetics, reproductive biology, seed proteins, isozymes, and DNA, indicated that improved peanut cultivars and landraces represent an extremely narrow germplasm base with little genetic variation. Wild species, however, exhibit both genetic variation detectable by molecular analysis and a large amount of morphological and physiological variation (Halward et al., 1991; Kochert et al., 1991; Lu and Pickersgill, 1993; Paik-Ro et al., 1992; Stalker, 1990). The few studies on genetic diversity of wild species with forage potential, such as *A. glabrata* (Maass and Ocampo, n.d.), *A. pintoi* (Maass et al., 1993), and *A. sylvestris* (Veiga and Lopes, unpublished data), also showed a high degree of intraspecific variation.

Although *Arachis* species are expected to be mostly autogamous, little is known on the reproductive biology of wild *Arachis* species (Simpson et al., Chapter 4, this volume). Thus, no prediction can be made about the genetic identity and stability of germplasm accessions. In the case of *A. pintoi*, one accession is now widespread. It is thus important to investigate whether genetic erosion, shift, or drift has occurred since the original material was made available.

Most researchers have addressed genetic diversity in *Arachis* more at the interspecific rather than the intraspecific level. Such studies of morphological characters, seed protein, and isozyme patterns were helpful in classifying germplasm accessions, and assisted in gross taxonomic grouping of unidentified material (e.g., Lacks et al., 1991). For example, the enzymatic systems LAP and IDH made it possible to discriminate three species of section Extranervosae (*A. villosulicarpa*, VW 5913, and VKRSv 6556). In the same section, the species VPmSv 12939 (Terezina de Goiás) shows the exclusive MDH band 3 of zone three, the species VW 5913 (Araguari) is distinguished by ADH band 3, and *A. macedoi* by LAP band 9 (Table 3).

Table 3. Isoenzymatic variability in different accessions of section Extranervosae of the genus *Arachis*.

Isozyme zone, band		Species and accession[a]										
		A. villosulicarpa	*A. lutescens*	*A. prostrata*	*A. macedoi*	*Arachis* sp. Nobres	*Arachis* sp. Terezina de Goiás		*Arachis* sp. Carolina	*Arachis* sp. Araguari	*Arachis* sp. Araguaina	
		1	2	3	4	5	6	7	8	9	10	11
ADH (alcohol dehydrogenase)												
	2[b]	+	+	+	+	+	+	+	+	+	+	+
	3	-	-	-	-	-	-	-	-	+	-	-
IDH (iso citrate dehydrogenase)												
1	2	-	+	+	+	+	+	-	-	-	-	-
	3	-	-	-	-	-	-	+	+	+	+	+
2	1	-	-	-	-	-	-	-	-	-	-	+
	2	-	-	-	-	-	+	-	-	+	-	+
	3	+	-	+	+	+	-	+	-	-	-	-
	4	-	+	-	-	-	-	-	-	-	-	+
3	1	-	-	-	-	-	-	-	-	+	-	-
	2	+	-	-	-	-	-	-	-	-	-	+
	3	+	-	-	-	-	-	-	-	-	-	-
	4	+	-	-	-	-	-	-	-	-	-	-
LAP (leucine amino peptidase)												
	4[b]	+	+	+	-	+	+	+	+	+	+	+
	7	+	+	+	+	+	+	+	+	+	+	+
	9	-	-	-	+	-	-	-	-	-	-	-
MDH (malate dehydrogenase)												
1[b]	1	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+
3	1	+	+	+	+	+	+	+	+	+	+	+
	2	-	-	-	-	-	-	+	-	-	-	-
	3	-	-	-	-	-	-	+	-	-	-	-

a. Accessions: 1 = IAC; 2 = VW 5925; 3 = VKRSv 6638; 4 = VR 7533; 5 = VSStGdW 7861; 6 = VSW 9950; 7 = VPmSv 12939; 8 = VSStGdW 7821; 9 = VW 5913; 10 = VSStGdW 7784; 11 = VKRSv 6556.

b. Denomination of zones and bands exclude unresolved bands which do resolve in other accessions and/or species not presented here (e.g., see Table 5).

SOURCE: Galgaro and Lopes, unpublished data.

Other features, such as biotic and abiotic adaptation, are dealt with by other authors in this volume. But the amount of intraspecific genetic variation is practically unknown. In the following section, intraspecific, or at least intrasectional, variation of wild species will be emphasized.

Morphology

Morphological descriptions and classifications were carried out by Stalker (1990) for several species in the section Arachis and by Maass et al. (1993) for *A. pintoi* section Caulorhizae. According to Veiga and Lopes (unpublished data), the 18 accessions of *A. sylvestris* section Ambinervosae studied were discriminated by using characteristics such as plant size, stem branching pattern, branch size and pigmentation, presence or absence of trichomes on the leaflets, glossy leaflets, spots on the flower standard, and peg pigmentation. But it was not possible to correlate morphological characteristics and geographical distance among the different accessions.

Preliminary morphological descriptors compiled by IBPGR (1990) for wild *Arachis* species were utilized with adjustments. The usefulness of individual characters to describe intraspecific variation, however, depends on the species or section. As generalized descriptor lists tend to differentiate among species but not among accessions of each species, adjustments become necessary whenever several accessions of a single species are compared. Important characters, at this point, are those that allow for the best discrimination between accessions of a single species. Such characters are often good genetic markers, useful for studies of reproductive biology and population structure. Determination of morphological descriptors and characterization of new germplasm of *A. pintoi* are presently being carried out at CENARGEN and CIAT.

Biochemical markers

Several authors showed seed proteins to be a useful tool for classification (e.g., Klozová et al., 1983; Singh et al., 1991) in interspecific studies. The variation found among seed protein patterns made it possible to discriminate the three subspecies of *A. hypogaea* (Lopes et al., unpublished data). Data on intraspecific variation of seed proteins in wild *Arachis* species are not yet available.

Isozyme electrophoresis was used in some studies of genetic variation (Table 4). Several isozyme systems showed intraspecific polymorphism and were useful for describing existing variation and for fingerprinting accessions. Germplasm accessions of *A. sylvestris* and *A. villosulicarpa* showed strong polymorphism. Some accessions of *A. sylvestris* can be easily distinguished by specific bands, mainly with ACP and α, ß-EST (Table 4). Genetic variation was also detected within and among accessions of *A. villosulicarpa* studied with these same enzymatic systems, ACP and α, ß-EST (Galgaro and Lopes, n.d.).

Isozymes also may help to identify possible duplicate accessions in germplasm collections. Isozyme characterization has been initiated in seven available accessions of *A. villosulicarpa*, also involving the possible wild progenitor of this cultigen (Galgaro, 1991). Particularly useful isozymes researched across various species were esterases (Figure 2, Table 5). Several studies are presently being carried out that involve wild species of various sections of the genus *Arachis* to assess intra- and interspecific genetic diversity, such as studies on the isoenzymatic variability of different populations of *A. pintoi* from geographically isolated regions.

Molecular markers

Molecular markers have not yet been used in intraspecific studies of genetic diversity, except for the cultivated peanut and its wild relatives of section Arachis (Halward et al., 1991; Kochert et al., 1991; Paik-Ro et al., 1992). New and ongoing projects, however, intend to include RFLP and RAPDs in the characterization of intraspecific genetic diversity of wild *Arachis* species of other sections.

Table 4. Isoenzymatic variability in different accessions of *Arachis sylvestris*.

Isozyme bands	Accession[a]																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
ACP (acid phosphatase)																	
1	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
3	-	-	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
6	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
7	-	-	-	+	+	-	-	-	+	+	+	+	-	-	-	-	-
8	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
14	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+
15	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
16	-	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-
17	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-
18	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
19	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
21	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
22	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-
23	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
ADH (alcohol dehydrogenase)																	
1	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
G-6-Phosphate																	
1	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
2	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-
3	-	-	-	-	-	+	+	-	-	-	+	+	-	-	+	-	+
4	+	+	-	+	-	-	-	+	-	+	-	-	-	+	-	-	-
5	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
LAP (leucine amino peptidase)																	
1	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
2	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
3	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
4	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
5	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PRX (peroxidase)																	
1	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
3	+	+	+	-	+	-	-	-	-	+	+	-	+	+	+	+	+
4	+	+	+	-	-	-	+	+	-	+	+	-	+	+	+	+	+
5	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
EST (esterase)																	
1	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+
3	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-
4	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
5	-	-	+	+	+	-	-	-	+	+	-	-	-	-	-	-	-
6	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
9	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-
10	+	-	-	-	-	-	-	+	-	+	+	+	-	-	-	+	+
11	-	+	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
14	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
15	-	+	-	-	+	+	-	+	-	-	-	-	-	+	+	-	-
16	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
17	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-
18	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-	-	-
19	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+
20	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
22	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-
23	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-

a. Accessions: 1 = VVeSv 6001; 2 = VVeSv 6180; 3 = VKRSv 6547; 4 = VKRSv 6575; 5 = VKVeSv 7294; 6 = VVeSv 8494; 7 = VVeSv 8516; 8 = VRSv 10980; 9 = VRSv 11020; 10 = Bi 664; 11 = VKVeSv 7037; 12 = VKVeSv 7071; 13 = VVeSv 8345; 14 = VVeSv 8373; 15 = VVeSv 8386; 16 = VVeSv 8435; 17 = VVeSv 8520.

SOURCE: Veiga and Lopes, unpublished data.

Table 5. Isozymes used in intraspecific or intrasectional studies of genetic diversity in wild *Arachis* germplasm, polymorphism encountered, and resolution of system.

Isozyme system	*A. pintoi* (section Caulorhizae)[a]	*A. glabrata* (section Rhizomatosae)[b]	Section Arachis[c]		*A. sylvestris* (section Ambinervosae)[d]		Section Extranervosae[e]
	peg tip	rhizome tip	leaves	pollen	leaves	seeds	seeds
ACP (acid phosphatase)	++[f]	++	-	-	+	++	-
ADH (alcohol dehydrogenase)	-	-	o/-	-	o/o	+	-
AP (amino peptidase)	-	-	++	-	-	-	-
CAT (catalase)	-	-	-	-	o/+	o/+	-
DIA (diaphorase)	+	+	-	-	-	-	-
EST (esterase)	++	++	-	-	+	++	++
GOT (glutamate oxalo-acetate transaminase)	+	+	-	-	-	-	-
IDH (iso citrate dehydrogenase)	-	-	o/-	-	o/o	+	++
LAP (leucine amino peptidase)	-	-	-	-	o/o	+	++
MDH (malate dehydrogenase)	o/-	o/-	++	++	o/+	o/+	++
ME (malic enzyme)	o/-	o/-	o/-	++	o/o	o/+	-
MNR (menadione reductase)	-	-	++	++	-	-	-
6-PGDH (6-phospho gluco dehydrogenase)	-	-	o/-	-	o/o	o/+	-
PGI (phospho gluco isomerase)	-	-	++	++	-	-	-
PGM (phospho glucomutase)	o/-	o/-	++	-	-	-	-
PRX (peroxidase)	o/-	o/-	++	-	+	+	-
SKDH (shikimic dehydrogenase)	-	-	++	-	-	-	-

a. Maass et al. (1993).
b. Maass and Ocampo (n.d.).
c. Lu and Pickersgill (1993).
d. Veiga and Lopes (unpublished data).
e. Galgaro (1991); Galgaro and Lopes (unpublished data).
f. ++ = polymorphism existent, good resolution; + = polymorphism existent, acceptable resolution; o/+ = no polymorphism, good resolution; o/o = no polymorphism, bad resolution; - = not reported.

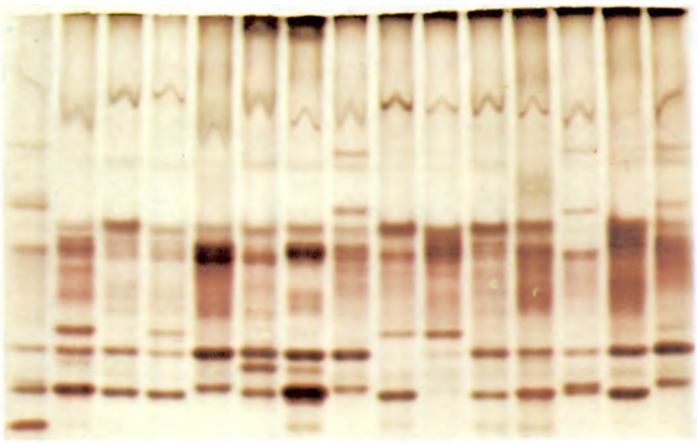

Figure 2.
Intraspecific variation in isozyme pattern of α, β–EST in rhizome-tip tissue of *Arachis glabrata* (Maass and Ocampo, unpublished data). (From left: CIAT 9086, 9095, 9097, 9079, 9093, 9098, 9085, 9083, 9081, 9073, 9092, 9072, 9078, 9100, 9075).

Molecular markers are better tools than biochemical markers, because they offer an almost infinite degree of polymorphism, are less affected by the environment, and can also be analyzed as Mendelian genes. Molecular markers are also used to address genetic diversity, classification, and phylogeny studies, relevant to germplasm management, and as a tool in breeding and selection by tagging genes and manipulating useful agronomic traits.

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Annex 1. National and institutional prefixes of collection numbers for germplasm.

Abbreviation	Institution
BRA	Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnologia (CENARGEN), Brazil
CIAT	Centro Internacional de Agricultura Tropical, Cali, Colombia
CPI	Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia
IAC	Instituto Agronômico de Campinas, Campinas, SP, Brazil
ICG	International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India
ILCA	International Livestock Centre for Africa, Addis Ababa, Ethiopia
IRFL	University of Florida, Gainesville and Fort Pierce, FL, USA
IRI	Rockefeller Research Institute, Matão, SP, Brazil
PI	Plant inventory number of USDA, Beltsville, MD, USA

Annex 2. Identification of principal collectors of *Arachis* germplasm.

Abbr.	Collector, Institution, Country
Bi	L. Bianchetti, CENARGEN/EMBRAPA, Brasília, DF, Brazil
Bm	B.L. Maass, CIAT, Cali, Colombia
Db	M. Dib Bechara, UNESP, Botucatu, SP, Brazil
Fa	L. Faraco de Freitas, CENARGEN/EMBRAPA, Brasília, DF, Brazil
Fd	M.S. França-Dantas, CPAC/EMBRAPA, Planaltina, DF, Brazil
G	W.C. Gregory, NCSU, Raleigh, NC, USA
Ga	M.L. Galgaro, UNESP, Botucatu, SP, Brazil
Gd	I.J. Godoy, Instituto Agronômico, Campinas, SP, Brazil
Ge	M.A.N. Gerin, Instituto Agronômico, Campinas, SP, Brazil
Gr	A. Gripp, CENARGEN/EMBRAPA, Brasília, DF, Brazil
H	R.O. Hammons, USDA-ARS, Tifton, GA, USA
J	L. Jank, CNPGC/EMBRAPA, Campo Grande, MS, Brazil
K	A. Krapovickas, Universidad Nacional del Nordeste, Corrientes, Argentina
L	W.R. Langford, USDA-ARS, Tifton, GA, USA
M	J.P. Moss, ICRISAT, Patancheru, India
Mt	J.C. Millot, Facultad de Agronomía, Univ. de la República, Montevideo, Uruguay
P	J. Pietrarelli, INTA, Manfredi, Argentina
Pm	R.N. Pittman, USDA, Griffin, GA, USA
Pn	P. Pinheiro, CPAC/EMBRAPA, Planaltina, DF, Brazil
Po	A. Pott, CPAP/EMBRAPA, Corumbá, MS, Brazil
Pz	E.A. Pizarro, CIAT, Brasília, DF, Brazil
Q	C.L. Quarin, Universidad Nacional del Nordeste, Corrientes, Argentina
R	V.R. Rao, ICRISAT, Patancheru, India
Ro	D.M.S. Rocha, CENARGEN/EMBRAPA, Brasília, DF, Brazil
Rs	R.C. Santos, CNPA/EMBRAPA, Campina Grande, PB, Brazil
S	C.E. Simpson, Texas A&M University, Stephenville, TX, USA
Sc	A. Schinini, Universidad Nacional del Nordeste, Corrientes, Argentina
St	H.T. Stalker, NCSU, Raleigh, NC, USA
Sv	G.P. Silva, CENARGEN/EMBRAPA, Brasília, DF, Brazil
V	J.F.M. Valls, CENARGEN/EMBRAPA, Brasília, DF, Brazil
Va	S.E.S. Valente, UNESP, Botucatu, SP, Brazil
Ve	R.F.A. Veiga, Instituto Agronômico, Campinas, SP, Brazil
Vi	J.G.A. Vieira, CENARGEN/EMBRAPA, DF, Brazil
Vn	R.O. Vanni, Universidad Nacional del Nordeste, Corrientes, Argentina
W	W.L. Werneck, CENARGEN/EMBRAPA, Brasília, DF, Brazil
Wi	D.E. Williams, USDA, Beltsville, MD, USA
Ws	W. Silva, CPAC/EMBRAPA, Planaltina, DF, Brazil