RESEARCH ARTICLE

Genetic relationships among *Arachis hypogaea* L. (AABB) and diploid *Arachis* species with AA and BB genomes

Fanley Bertoti da Cunha · Paula Macedo Nobile ·
Andrea Akemi Hoshino · Márcio de Carvalho Moretzsohn ·
Catalina Romero Lopes · Marcos Aparecido Gimenes

Abstract The cultivated peanut (*Arachis hypogaea* L.) is an allotetraploid, with two types of genomes, classified as AA and BB, according to cytogenetic characters. Similar genomes to those of *A. hypogaea* are found in the wild diploid species of section *Arachis*, which is one of the nine *Arachis* sections. The wild species have resistances to pests and diseases that affect the cultivated peanut and are a potential source of genes to increase the resistance levels in peanut. The aim of this study was to analyze the genetic variability within AA and BB genome species and to evaluate how they are related to each other and to *A. hypogaea*, using RAPD markers. Eighty-seven polymorphic bands amplified by ten 10-mer primers were analyzed. The species were divided into two major groups, and the AA and the BB genome species were, in general, separated from each other. The results showed that high variation is available within species that have genomes similar to the AA and the BB genomes of *A. hypogaea*.

Keywords *Arachis hypogaea* · Genetic variability · Peanut · RAPD · Wild species

F. B. da Cunha · A. A. Hoshino · C. R. Lopes ·
M. A. Gimenes
Departamento de Genética, Instituto de Biociências,
Universidade Estadual Paulista (UNESP), Rubião Junior,
Botucatu, SP, Brazil

P. M. Nobile
Instituto Agronômico Campinas – RGV, Caixa Postal
28, Campinas, SP 13001-970, Brazil

M. d. C. Moretzsohn · M. A. Gimenes (✉)
Embrapa Recursos Genéticos e Biotecnologia –
CENARGEN, Brasília, DF, Brazil
e-mail: gimenes@cenargen.embrapa.br

Introduction

Arachis hypogaea L., the cultivated peanut, is an important crop for oil production in tropic and subtropic areas. The cultivated peanut is an allotetraploid (AABB), native to South America, where the wild diploid *Arachis* species are also found. The wild diploid species are a very important germplasm for the peanut breeding programs, because they have some useful traits, such as resistances to diseases and pests (Pande and Rao 2001) that are not found in *A. hypogaea*.

Species of the genus *Arachis* were assembled into nine sections, according to their morphology, geographic distribution and crossability (Krapovickas and Gregory 1994). Section *Arachis* is the most important, since it includes species that have genomes closely related to *A. hypogaea*, which is also in this section. Thus, introgression of genes from these species should be easier than from more distantly related species (Holbrook and Stalker 2003). Several studies have shown that hybrids between *A. hypogaea* and some species of section *Arachis* have a high degree of chromosome pairing, allowing the introgression of genes by genetic

recombination (Singh and Moss 1984; Krapovickas and Gregory 1994; Mallikarjuna et al. 2004).

Three genomes have been identified in section *Arachis*, including the AA genome, found in most species, the BB genome, described for species such as *A. batizocoi* Krapov. et W. C. Gregory, *A. ipaënsis* Krapov. et W. C. Gregory, and *A. magna* Krapov., W. C. Gregory et C. E. Simpson, and the DD genome represented exclusively by *A. glandulifera* Stalker (Stalker 1991). *Arachis hypogaea* is believed to be an allotetraploid species, in large part because of the karyotypic differences in their chromosomes, where only one distinctively small chromosome pair (called A) is observed, and the diploid-like chromosome pairing during meiosis (Smartt and Gregory 1967). Molecular and cytogenetic evidences have indicated *A. duranensis* Krap. et W.C. Gregory (AA genome) and *A. ipaënsis* (BB genome), both from Section *Arachis*, as the most probable progenitors of *A. hypogaea* (Kochert et al. 1996; Seijo et al. 2004).

Improvement of the cultivated peanut using wild diploid species of *Arachis* has been focused on the AA genome, because the diploid AA genome species are well characterized and many accessions are available. The focus on the BB genome has been lower. *Arachis ipaënsis* has a unique known accession and the other BB genome species (lacking the small pair of chromosomes) have not been adequately studied. Thus, the analysis of genetic relationships among the BB genome species and *A. hypogaea* would be very useful for the selection of accessions and species to be used in the breeding programs.

Molecular markers have been used to the analysis of genetic variability and establishment of relationships in many different plant species, including *Arachis* (Gimenes et al. 2002; Moretzsohn et al. 2004; Tallury et al. 2005).

The aim of this study was to establish the genetic relationships among *A. hypogaea* and diploid species with AA and BB genomes and to analyze the genetic variability of this germplasm, using Random Amplified Polymorphic DNA (RAPD).

Material and methods

Plant material

Fifty individual plants of 28 accessions of 12 section *Arachis* species were analyzed (Table 1). All plants

Table 1 Accessions of sections *Arachis* and *Trierectoides*, genus *Arachis*, the number of samples per accession and life cycle of each species analyzed using RAPD markers

Species	Collection No.	Number of samples	Life cycle
AB genome			
A. hypogaea	Mf 1640	2	Annual
	V 12577	2	Annual
	W 725	1	Annual
	As 433	1	Annual
	FT 85062	1	Annual
	FT 85183	1	Annual
	Mf 1560	1	Annual
	Pd 3147	2	Annual
	FT 85273	1	Annual
	V 12548	1	Annual
	V 10522	1	Annual
	FT 85209	1	Annual
A. monticola	V 14165	1	Annual
B genome			
A. batizocoi	K 9484	4	Annual
A. gregoryi[a]	V 6389	1	Annual
A. hoehnei	V 9146	5	Annual
	KG 30006	1	Annual
	V 13985	2	Annual
A. magna	V 13760	1	Annual
	V 13748	1	Annual
A genome			
A. aff. kuhlmannii	V 13721	3	Unknown
A. cardenasii	GKP 10017	3	Perennial
A. duranensis	V 14167	1	Annual
A. microsperma	V 14041	2	Perennial
A. schininii[a]	V 9923	2	Unknown
A. simpsonii	V 13732	2	Perennial
	V 13728	5	Perennial
	V 13746	1	Perennial
Section *Trierectoides*			
A. guaranitica	V 13600	1	Perennial
A. tuberosa	V 13501	1	Perennial

[a] New species, recently described by Valls and Simpson (2005)

were obtained from *Arachis* Germplasm Bank, located at Embrapa Genetic Resources and Biotechnology–Cenargen (Brasília, Brazil). *Arachis tuberosa* Benth. and *A. guaranitica* Chodat et Hassl. of section

Trierectoides were included as outgroups. Section *Trierectoides* is believed to be the most ancient section in the genus *Arachis* (Krapovickas and Gregory 1994).

DNA extraction

Total DNA was extracted from leaves as described by Ferreira and Grattapaglia (1998). The DNA concentration was estimated at 260 nm wavelength in a GENESYS 5 Spectrophotometer (Spectronic Instruments).

RAPD analysis

Each reaction was performed in a volume of 13 μl consisting of 3 μl of DNA (5 ng/μl), 1.04 μl of BSA (10 μg/μl), 1.04 μl of dNTPs (2.5 mM each), 1.3 μl of 10x *Taq* DNA reaction buffer, 0.4 μl of $MgCl_2$ (50 mM), 0.2 μl of *Taq* DNA Polymerase (1 U), 3.0 μl of primer (1 μM) and 3.0 μl of water. Ten 10-mer primers were included in the analysis (Table 2). The amplification cycle was as following: 40 cycles of 1 min at 92°C, 1 min at 35°C, 2 min at 72°C and a final extension of 72°C for 5 min A PTC 100 thermal cycler (MJ Research) was used for the DNA amplification. The PCR products were electrophoresed in 1.5% agarose gels, stained with ethidium bromide and visualized under UV light.

Table 2 Number of polymorphic fragments scored per each RAPD primer included in the analysis

Primers	No. of bands scored
OPA18	9
OPN1	12
OPN2	5
OPN4	9
OPW2	5
OPW3	11
OPW6	10
OPW9	8
OPW13	9
OPW16	9
Total	87

Data analysis

The presence or absence of each polymorphic fragment was scored for all samples. A genetic distance matrix was obtained using Nei and Li's coefficient (Nei and Li 1979) and submitted to cluster analysis using Neighbor Joining. The analyses were performed using the computer program Treecon (version 1.3b – Van der Peer and De Watcher 1994).

Results

The 10 primers amplified 87 polymorphic fragments. The average number of polymorphic fragments per primer was 8.7, ranging from five using primers OPN2 and OPW2 to 12 fragments using primer OPN1 (Table 2). Eight bands (9.2%) were shared between the AA and BB genome species analyzed, four bands (4.6%) were found only in the BB genome species, and ten bands (11.5%) were exclusive to the AA genome species. Thirteen bands (15%) were shared among AA, BB and AABB (*A. hypogaea* and *A. monticola* Krapov. et Rigoni) genome species, seven (8%) between AA and AABB (two of them were shared between *A. duranensis* and AABB) and three (3.4%) among species with BB and AABB genomes. In the BB genome group, the largest number of fragments was shared between *A. batizocoi* and *A. magna*, and the lowest between *A. batizocoi* and *A. hoehnei* Krapov. et W. C. Gregory. No bands were found to be unique to *A. hypogaea*.

The genetic relationships among the accessions analyzed are shown in Fig. 1. The accessions were grouped into two major clusters: the first one included accessions of *A. hoehnei*, *A. schininii* (Valls et Simpson), *A. aff. kuhlmannii* Krapov. et W. C. Gregory, *A. cardenasii* Krapov. et W. C. Gregory, *A. microsperma* Krapov., W. C. Gregory et Valls and *A. simpsonii* Krapov. et W. C. Gregory and the second included two sub-groups, being one composed by accessions of *A. hypogaea, A. monticola* and *A. duranensis* and the other subgroup composed by the accessions of three BB genome species: *A. batizocoi*, *A. magna* and *A. gregoryi* (Valls et Simpson). *Arachis tuberosa* and *A. guaranitica* (section *Trierectoides*) were used as outgroups.

Very low polymorphism was detected among the 12 accessions of *A. hypogaea* analyzed. On the other

Fig. 1 Genetic
relationships among 12
Arachis species with A and/
or B genome
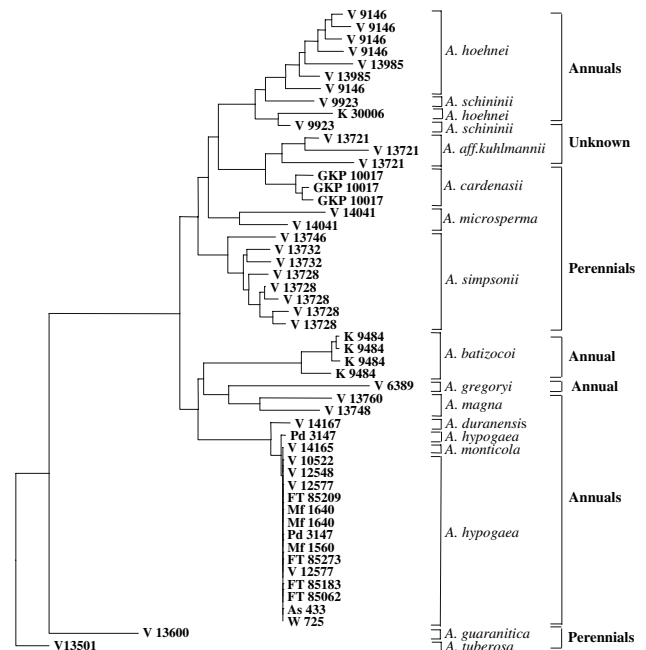
hand, high polymorphism was found within and among accessions of the wild diploid species analyzed (Fig. 1).

Discussion

In general, all species grouped according to their genomes. Accessions of five out of the six AA genome species included in the present study (*A. cardenasii*, *A. microsperma*, *A. simpsonii, A. schininii*, and *A. aff. kuhlmannii*) grouped together (Fig. 1). Close relationships among AA genome species were also detected in previous studies using molecular markers (Hilu and Stalker 1995; Moretzsohn et al. 2004) and crossability data (Singh and Moss 1984). Hybrids between AA genome species are usually highly fertile, with 10 bivalents (Stalker et al. 1991), also indicating they are closely related. A cross between *A. cardenasii* and *A. hypogaea* subsp. *fastigiata* var. *fastigiata* (Smartt and Gregory 1967) resulted in a tetraploid interspecific hybrid population that comprised introgression lines with many important characteristics, such as high levels of resistance to *Cercospora arachidicola*, moderate resistance to *Cercosporidium personatum* (Stalker and Beute 1993), and resistances to several insects (Stalker

and Campbell 1983). These lines showed introgression of *A. cardenasii* chromosome segments into both genomes of *A. hypogaea* (Garcia et al. 1995) and were used to the identification of molecular markers linked to nematode resistance genes (Garcia et al. 1996). *Arachis microsperma*, *A. simpsonii*, *A. schininii*, and *A. aff. kuhlmannii* have not been used in crosses with *A. hypogaea*, but, based on our results, introgression of chromosome segments from these species into the cultivated peanut should also be possible.

Three of the four BB genome species included in this analysis (*Arachis magna*, *A. gregoryi* and *A. batizocoi*) also grouped together. *Arachis magna* is morphologically very similar to *A. ipaënsis* (Krapovickas and Gregory 1994), which is the most probable donor of the BB genome to *A. hypogaea* (Kochert et al. 1996; Seijo et al. 2004). The identification of species with high homology to the BB genome of *A. hypogaea* is important, because the success of breeding program that aims at the introgression of genes from diploid species depends on the choosing of species that have genomes similar to the species being improved.

Only two species did not cluster according to their genomes, *A. hoehnei* (BB genome) and *A. duranensis* (AA genome). *Arachis hoehnei* accessions grouped to

the AA genome species and showed the smallest number of shared fragments with *A. batizocoi* accessions, that has a BB genome. *Arachis hoehnei* has been classified as a BB genome species (Fernández and Krapovickas 1994; Holbrook and Stalker 2003). Genetic relationship studies based on microsatellite (Moretzsohn et al. 2004) and AFLP markers (Tallury et al. 2005) corroborated this classification. In contrast, phylogeny analysis of genus *Arachis* based on the sequence variation of internal transcribed spacers (ITS) of rDNA genes (Bechara 2001) and on sequence data of the *trn*T-F region (Tallury et al. 2005) placed *A. hoehnei* in the AA genome clade. Cytogenetic studies are currently underway and should elucidate the genomic classification of *A. hoehnei* (Andrea Peñaloza, personal communication).

Arachis duranensis was placed very closely to *A. hypogaea* and distantly from the other AA genome species analyzed. *Arachis duranensis* is considered to be the most probable donor of the AA genome to *A. hypogaea* (Kochert et al. 1996; Seijo et al. 2004). Thus, this clustering result could be explained by the strict relation among the genomes of *A. duranensis* and *A. hypogaea*.

The tetraploid species (*A. monticola* and *A. hypogaea*) were very close related, as expected. Hybrids between *A. monticola* and *A. hypogaea* showed high fertility levels (Kaprovickas and Gregory 1994). The close relationship between these two species has been reported by many authors, who proposed different hypothesis to explain this tight relation. Krapovickas et al. (1974) suggested that *A. monticola* could have originated through a crossing between *A. hypogaea* and a wild diploid species. Pickersgill (1986) and Lu and Pickersgill (1993) suggested that *A. monticola* and *A. hypogaea* are probably not distinct species. Lanham et al. (1994) pointed out that *A. monticola* may have arisen by the hybridization between two subspecies of *A. hypogaea*. However, the most accepted hypothesis is that *A. monticola* is the allotetraploid ancestral progenitor of *A. hypogaea* (Kochert et al. 1996; Moretzsohn et al. 2004).

The low polymorphism observed in *A. hypogaea* accessions in the present study is in agreement with previous data (Kochert et al. 1996; Gimenes et al. 2002). The general low level of variability in the cultivated peanut has been attributed to its origin, through a single and recent polyplodization event

followed by successive selection during breeding efforts—thus resulting in a highly conserved genome (Young et al. 1996). The morphological variation observed in this species is most probable due to the variation in few genes, as proposed by Kochert et al. (1991).

RAPD markers characterized all accessions and individuals of the wild species. This is very important for the maintenance of this material, since mislabeling and duplicates can be identified. Molecular markers have shown to be useful to the identification of mislabeling in *Arachis* species (Kochert et al. 1996). The diploid species analyzed showed high polymorphism within and among accessions. High polymorphism was also detected among accessions of wild diploid species using different molecular markers (Kochert et al. 1991; Moretzsohn et al. 2004; Tallury et al. 2005).

The present work has been useful to show the genetic relationships among the species of section *Arachis*. Furthermore, it has showed that RAPD markers can provide an easy, low cost and quick way for the identification of accessions maintained in germplasm collections.

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