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Genetic diversity among *Arachis* species based on RAPDs

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Abstract

Thirty-two accessions of wild species of *Arachis* belonging to twenty-five species and grouped under six sections were used to study genetic relationship using RAPDs. Twenty-nine primers belonging to OPH 1-20 and and OPM 1-9 were used in the study. All the primers showed polymorphic bands and the number of bands varied from five to thirty-three. Similarity values (S_{ij}) for 464 pairwise comparisons among 32 accessions ranged from a minimum of 0 to a maximum of 49%, with an average of 15%. The data obtained from cluster analysis matched that obtained from multidimensional scaling (MDS). Cultivated species *A. hypogaea* grouped with *A. monticola*, a tetraploid wild species and a probable progenator of *A. hypogaea*. *A. stenosperma* accessions grouped together. Wild species from section *Arachis* with B genome formed two clusters, with one cluster having *A. batizocoi* showing distant relationship than the other cluster with *A. hoehnei* showing closer relationship. *A. glandulifera* having the D genome remained apart. It was possible to explain the grouping of wild species grouped as per their relationship with each other. The exception was the two accessions of *A. cardenasii* (ICG 11558 and 11559) from section *Arachis*. They did not group with any of the A, B or the D genome species from section *Arachis* nor with each other.

Key words: *Arachis*, cluster analysis, MDS, polymorphism, RAPD, wild species.

Introduction

The origins of modern *Arachis* can be traced to the valleys of South America, in the Brazil-Paraguay region [1] where it is distributed even today. What is grown today as cultivated groundnut in different parts of the world show great morphological variability but limited molecular polymorphism, but the wild species show both morphological and molecular diversity [2]. Based on morphological characters and cross compatibility relationships Krapovickas and Gregory [3] classified the genus *Arachis* into nine sections.

The study of genetic and taxonomic relationships require a flexible and reliable marker system to detect high levels of polymorphism. Traditionally a combination of morphological and agronomic traits has been used to measure genetic diversity, but most of the vegetative characteristics are influenced by environmental factors, and show continuous variation and have high degree of plasticity. In an attempt to overcome these problems, biochemical and molecular techniques have been used to monitor genetic and to solve taxonomic and phylogenetic problems. Data based on RFLP studies showed that sections *Arachis* and *Heteranthae* form two clearly defined groups and sections *Heteranthae*. *Caulorrhizae* and *Triseminatae* form a more closely related group [2]. Use of AFLP to study genetic relationship among *Arachis* species, grouped section *Arachis* species together, with *A. glandulifera* showing distant relationship to *A. hypogaea* and the A and B genome species [4]. Species from section *Erectoides* grouped with *A. glabrata* of section *Rhizomatosae*, and *A. rigoni* (section *Procumbentes*) showed close relationship with *A. dardani* (section *Heteranthae*).

RAPD markers have been used in evolutionary studies of wild species from section *Arachis* [5] and in the creation of genetic linkage map [6]. Fennell [7] used RFLPs to distinguish 17 wild species from five sections of *Arachis* and cultivated groundnut *A. hypogaea* and RAPDs to track introgression of alien genes in wide crosses. In the present study RAPDs were used to assess the genetic relationships among 32 accessions of wild species belonging to six sections.

Materials and methods

Genomic DNA was extracted from immature leaves of 32 accessions of *Arachis* species (Table 1) grown in the glasshouse and were numbered from 1–32. All the plants used were wild species plus a hybrid between two wild species, *A. paraguariensis* (ICG 8130) and *A. appressipila* (ICG 8128), which was given the number 22. The cultivated species *A. hypogaea* was also included in the study. Two of the wild species lost their identity hence their names (no. 17 and 24) have not been mentioned. Fresh immature leaves were harvested, lyophilized in liquid nitrogen and stored at –70°C, and DNA was extracted whenever necessary by the CTAB method [8]. RAPD-PCR was performed according to the protocols of Williams *et al.* [9]. Twenty-nine random 10-mer primers (OPH 1-20 and OPM 1-9; Operon Technologies) were used to amplify

Table 1. *Arachis* species from different sections used in the study

Section	Species	Genome	S. No. on the dendrogram
Arachis	A. cardenasii ICG 11558	A	1
	A. cardenasii ICG 11559	A	10
	A. stenosperma ICG 8125	A	8
	A. stenosperma ICG 8137	A	16
	A. stenosperma ICG 8126	A	3
	A. stenosperma ICG 14868	A	26
	A. stenosperma ICG 14872	A	12
	A. villosa ICG 8144	A	4
	A. kempf-mercadoi ICG 8959	A	6
	A. kempf-mercadoi ICG 8164	A	14
	A. valida ICG 13230	B	19
	A. hoehnei ICG 8190	B	11
	A. benensis ICG 11551	B	5
	A. batizocoi ICG 8210	B	7
	A. magna ICG 8960	B	13
	A. ipaensis ICG 8206	B	15
	A. glandulifera ICG 15172	D	18
	A. monticola ICG 13177	AB	9
	A. hypogaea ICGS 44	AB	23
Procumbentes	A. chiquitana ICG 11560	P	2
	A. kretschmeri ICG 8191	P	27
	A. matiensis ICG 11557	P	20
	A. rigoni ICG 8904	P	25
Erectoides	A. major ICG 13262	E	28
Heteranthae	A. sylvestris ICG 14858	H	29
	A. dardani ICG 14923	H	31
	A. pusilla ICG 14898	H	32
Caulorhizae	A. pintoi ICG 14855	C	30
Rhizomatosae	A. glabrata ICG 8176	RR	21
--	*A. sps* (unknown)	--	24,17
--	A. *appressipila* ICG 8128 × *A. paraguariensis* ICG 8130	P × E	22

DNA in Perkin GeneAmp 9600 thermal cycler and PCR products were electrophoresed on 1.4% agarose gels, stained in ethidium bromide and photographed under UV illumination.

Randomly amplified polymorphic DNA (RAPDs) are dominant markers, and we assumed that each band corresponded to a single character with two alleles, presence or absence of a band, respectively. The resultant band patterns obtained were manually scored for the presence or absence of bands.

Pair-wise similarities (S_{ij}) between accessions (i,j) were estimated using Jaccard similarity coefficient $S_{ij} = N_{ij} / (N_i + N_j - N_{ij})$, where N_{ij} is the number of bands common in accessions i and j, N_i and N_j are the total number of bands in accessions i and j respectively. S_{ij} represents the proportion of bands in common between any two accessions and may range from 0 (no common bands) to 1 (identical band profile for two accessions). Based on genetic distance estrimates, $D_{ij} = 1 - S_{ij}$, multidimensional scaling (MDS) [10] was performed to see whether the observed molecular variation indicated any evidence of clustering among accessions. A dendrogram was constructed based on the Sij values using the clustering technique of unweighted pair group method of arithmetic means (UPGMA) [11] to confirm the clustering suggested by MDS. All computations were performed using GenStat statistical computing package.

Results and discussion

All the OPH and OPM primers used in the study produced polymorphic bands. A maximum of 33 polymorphic bands were observed in primer OPH-08 (Table 2). A total of 378 polymorphic bands were observed. Similarity values (S_{ij}) for 464 pairwise comparisons among 32 accessions ranged from a minimum of 0 to a maximum of 54%, with an average of 15%. The MDS plot and UPGMA-based dendrogram (Fig. 1) both provided quite similar grouping of the 32 accessions of wild species. The cultivated species *A. hypogaea* was grouped with its tetraploid wild relative *A. monticola* (ICG 13177). All the *Arachis stenosperma* accessions ICG 8126, ICG 8125, ICG 14872, ICG 8137, ICG 14868 collected from Mato Grosso and Parana regions of Brazil clustered together.

Table 2. Primers with more than 15 bands

OPH primer	No. of bands produced
OPH 05	17
OPH 07	26
OPH 08	33
OPH 09	25
OPH 11	19
OPH 12	19
OPH 13	26
OPH 14	18
OPH 15	31
OPH 16	20
OPH 17	22
OPH 18	22
OPH 19	25
OPH 20	25

There were two clusters of B genome species. *Arachis hoehnei* (ICG 8190) and *A. benensis* (B genome species of section *Arachis*; ICG 11551) grouped together. Some of the other B genome species *A. batizocoi* (ICG 8210), *A. magna* (ICG 8960), and *A. ipaensis* (ICG 8206) grouped together at the other end of the dendrogram. *A. glandulifera* (ICG 15172), the only known D genome species from section *Arachis*,

remained aloof from all the *Arachis* species. Two accessions of *A. cardenasii* (ICG 11558 and ICG 11559) did not show any relationship. They placed themselves at the two ends of the dendrogram (Fig. 1).

A. magna formed a unique group showing distant relationship with *A. hypogaea*. This is in concurrence with the study of Halward *et al.* [5] wherein *A. batizocoi* formed distant group, but the exception being grouping

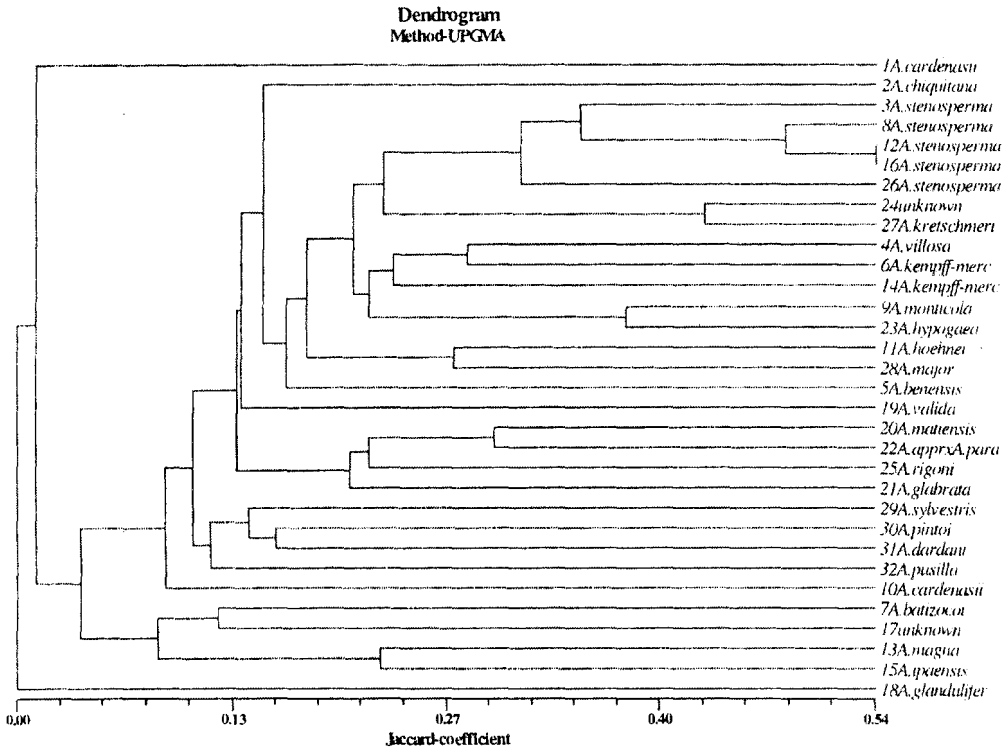

Fig. 1. UPGMA-based dendrogram of Arachis species based on RAPD data

Based on karyo-morphological similarities [12] and molecular studies [13] *A. duranensis* is the A genome donor of cultivated groundnut. According to Singh and Moss [12] *A. batizocoi*, which crosses freely with *A. hypogaea* but produces sterile hybrids, is the B genome donor species. Kochert *et al.* [13] proposed *A. ipaensis* as the B genome species based on their results from RFLP studies. According to the present study neither *A. ipaensis* nor *A. batizicoi* can be the B genome donor of *A. hypogaea*. In the cluster analysis, although both of them together with *A. magna* formed a group, they showed distant relationship to *A. hypogaea*. Recently more species with B genome have been identified [18], amongst which *A. hoehnei* is one of them. In the cluster analysis as well, it was observed that *A. hoehnei* showed very close relationship to *A. hypogaea*. According to the present study *A. hoehnei* is the probable B genome donor species of *A. hypogaea* because *A. hoehnei* crosses with *A. hypogaeae* to produce fertile hybrids (Mallikarjuna personal observation). It is the only B genome species in close association with *A. hypogaea* and *A. monticola* as well as with the other A genome species. In the present study *A. batizocoi* and *A. ipaensis* together with and of *A. ipaensis* with *A. batizocoi* and *A. magna*. This supports the observation of Krapovickas and Gregory [3] and Kochert *et al.* [14] that *A. ipaensis* and *A. magna* are closely related.

Arachis sylvestris (ICG 14858) grouped with *A. pusilla* (ICG 14898) and *A. dardani* (ICG 14923), wild species belonging to section *Heteranthae* and collected from different regions of Brazil clustered with *A. pintoi* (ICG 14855), which belongs to section *Caulorrhizae*. *A. glabrata* (ICG 8176) which belongs to section *Rhizomatosae* came in close proximity with *A. rigoni* (ICG 8904) and a hybrid between *A. appressipila* (ICG 8128) and *A. paraguariensis* (ICG 8130) and *A. matiensis* (ICG 11557) from section *Procumbentes*. *A. chiquitana* (ICG 11560) a wild species from section *Procumbentes* did not group with other members of the section *Procumbentes*, instead showed relationship to *A. valida* (ICG 13230), a section *Arachis* member. *A. kretschmeri* (ICG 8191), belonging to section *Procumbentes*, did not group with either *A. chiquitana* or other members of the section *Procumbentes*, instead showed closer relationship to the *A. stenosperma* accessions (ICG 8125, 8137, 8126, 14868, 14872) on one end and to

A. villosa (ICG 8144) and *A. kempff-mercadoi* (ICG 8164) on the other (Fig. 1).

Based on the above observation it can be concluded that section *Caulorrhizae* evolved from section *Heteranthae*. Galgaro *et al.* [2] have also shown close relationship between sections *Heteranthae* and *Caulorhizae*. Three species from section *Procumbentes viz. A. matiensis*, a hybrid between *A. paraguariensis* (section *Erectoides*) and *A. appressipila* (section *Procumbentes*) and *A. rigoni* grouped together. *A. glabrata* from section *Rhizomatosae* placed itself close to the group. *A. glabrata*, the tetraploid member from section *Rhizomatosae*, has evolved with one genome donor from section *Arachis* [15] and with a probable donor from section *Erectoides*. The members of sections *Erectoides* and *Procumbentes* share close relationship.

A. hypogaea grouped closely with its tetraploid relative from section *Arachis, A. monticola*. According to one school of thought, *A. monticola* is a progenator species of *A. hypogaea* [16]. These two species show close relationship with *A. kempff-mercadoi* and *A. villosa*. According to *in situ* hybridization studies *A. villosa* is the A genome donor of *A. hypogaea* [17]. All the *A. stenosperma* grouped together at one end of the dendrogram and close to *A. hypogaea* complex. An interesting observation from the present study was the placement of *A. kretschmeri* (section *Procumbentes*) close to *A. hypogaea* and *A. stenosperma* complex. *A. chiquitana* a member of section *Procumbentes*, also placed close to the *A. stenosperma* complex. *A. kretschmeri* and *A. chiquitana* when crossed with *A. hypogaea* set large number of bold but immature seeds. Unlike other members of section *Procumbentes* such as *A. matiensis, A. rigoni* and *A. appressipila* when crossed with *A. hypogaea*, hybrid embryos aborted at an early stage when the seeds are less than 2.5–3.0 mm, with a tiny globular to heart shaped embryo less than 0.2–0.3 mm in size. According to Valls (personal communication) close to the border of Bolivia and Brazil where *A. chiquitana* thrive, tetraploid *A. hypogaea* is grown by the native people which resemble *A. chiquitana* pods but are big in size. Hence, it is possible that *A. chiquitana* or its ancestors might have indirectly contributed a genome for the evolution of *A. hypogaea*.

Although RAPDs have not been able to clearly distinguish *Arachis* species into their respective sections, it was possible to draw some important conclusions based on this study. With the development of SSR markers in *Arachis* it needs to be seen if groupings emerge section wise.

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