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Phylogenetic relations in section Arachis based on seed protein profile

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Summary. Seed protein profiles of nine diploid species (2n=20), ten tetraploid accessions, two synthetic amphidiploids and two autotetraploids (2n=40) were studied using SDS-polyacrylamide gel electrophoresis. While the general profiles suggested considerable homology among these taxa in spite of speciation and ploidy differences, appreciable genetic differences were present to support the existing genomic divisions and sub-divisions in the section Arachis. A high degree of relationship was indicated between the two diploid species (*A. duranensis* containing the A genome and *A. batizocoi* (ICG 8210) containing the B genome) and tetraploids *A. monticola*/*A. hypogaea* (2n=40) containing AABB genome. Similar relationships were recorded between the AABB synthetic amphidiploid and the profile obtained from the mixture of protein of *A. duranensis* and *A. batizocoi,* suggesting that these two diploid species were the donors of the A and B genome, respectively, to tetraploid *A. monticola*/*A. hypogaea.*

Key words: *Arachis* – SDS-PAGE electrophoresis – Protein-profile – Homology – Statistical-distance

Introduction

Seed proten profiles have been a powerful technique for ascertaining genetic homology at the molecular level and for resolving taxonomic and phylogenetic problems (Ladizinsky and Hymowitz 1979). In the section Arachis of the genus *Arachis*, phylogenetic relationships among different species have been traced on the basis morphological, phytogeographical and cytogenetical data (Singh 1988). These studies revealed that most diploid wild species (2n=20) have a common A genome, whereas *A. batizocoi* has different genome, designated B. The A and B genomes are homoeologous and together constitute the cultivated tetraploid *A. hypogaea* (2n=40) (Smartt et al. 1978; Stalker and Dalmacio 1981; Singh and Moss 1982). Genome analysis (Singh and Moss 1984) and the hybridization of cultivars of *A. hypogaea* with synthetic amphidiploids of the A and B genome species (Singh 1988) revealed that *A. hypogaea* is a segmental allotetraploid evolved through the amphidiploidization of an AB hybrid. *A. batizocoi* is believed to be the more probable donor of the B genome and *A. duranensis* and/or *A. villosa* of the A genome.

Attempts have been made to establish species relationships among both broad groups and among species of the section Arachis using seed protein and isozyme profiles and immunochemical characterization. However, the inferences that can be drawn from these studies are fragmentary and have not always corresponded with those from previous investigations (Cherry 1975; Klozova et al. 1983a, b; Krishna and Mitra 1988). The present study is an extension of the genome analyses carried out by Singh and Moss (1982, 1984) and Singh (1988) using the same set of *Arachis* species and cultigens from *A. hypogaea* representing a spectrum of genomic variability. Protein profiles of the amphidiploids, autotetraploids and a mixture of proteins from two of the most probable ancestral wild *Arachis* species with A and B genomes were also studied to verify earlier conclusions. Only protein profiles were used because they are relatively stable and alter slowly during evolution (Margoliash and Fitch 1968; McDaniel 1970).

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Table 1. Distribution of denatured polypeptides in nine diploid species of section Arachis and ten tetraploid accessions of *A. monticola* and *A. hypogaea*

Group	Rf values	MW[a] (kDa)	Section Arachis diploid species, *A. monticola* and cultivars of *A. hypogaea*																						
			1	2	3	4	5	6	8+6	7	8+9	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
I	0.293	64	+	+	−	−	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	0.312	63	−	−	+	+	−	−	+	−	+	+	+	−	−	−	−	−	−	−	−	−	−	+	−	−	+
II	0.429	48	−	−	−	−	−	−	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	0.435	46	+	+	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
	0.447	45	+	+	+	+	+	+	+	+	−	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	−
	0.465	44	−	−	−	−	−	−	+	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+
	0.488	42	+	+	+	+	+	+	+	+	−	−	−	+	+	−	−	−	−	+	+	+	+	+	+	+	−
III	0.512	40	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+
	0.518	39	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	0.541	37	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
IV	0.606	31	+	+	−	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	0.635	29	−	−	+	+	−	−	+	−	+	+	+	+	+	−	−	+	−	+	+	+	+	−	−	−	+
	0.682	27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	0.753	23	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	0.759	22	−	−	−	−	+	−	+	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+
V	0.765	21.5	+	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+
	0.782	21.0	−	−	+	−	−	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	0.847	19.0	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	0.894	16.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+, Donets present; −, Donets absent

[a] MW, Molecular weight

1 = *A.* sp. HLK-410
2 = *A. chacoense*
3 = *A. cardenasii*
4 = *A. correntina*
5 = *A. villosa*
6 = *A. duranensis*
7 = *A.* sp. 10038
8 = *A. batizocoi*
9 = *A.* sp. 30081
10 = *A. monticola*
11 = *A.* Sp. 30063

A. hypogaea ssp *fastigiata* var '*fastigiata*'
12 = ICG 7368
13 = ICG 11596

A. hypogaea ssp *fastigiata* var '*vulgaris*'
14 = ICG 11175
15 = ICG 11595

A. hypogaea ssp *hypogaea* var '*hypogaea*'
16 = ICG 10825
17 = ICG 12064

A. hypogaea ssp *hypogaea* var '*hypogaea*'
18 = ICG 11609
19 = ICG 12065

20 = *A. batizocoi* × *A. chacoense* (4×)
21 = *A.* sp. 10038 × *A.* sp. HLK-410 (4×)
22 = *A. duranensis* (4×)
23 = *A. batizocoi* (4×)

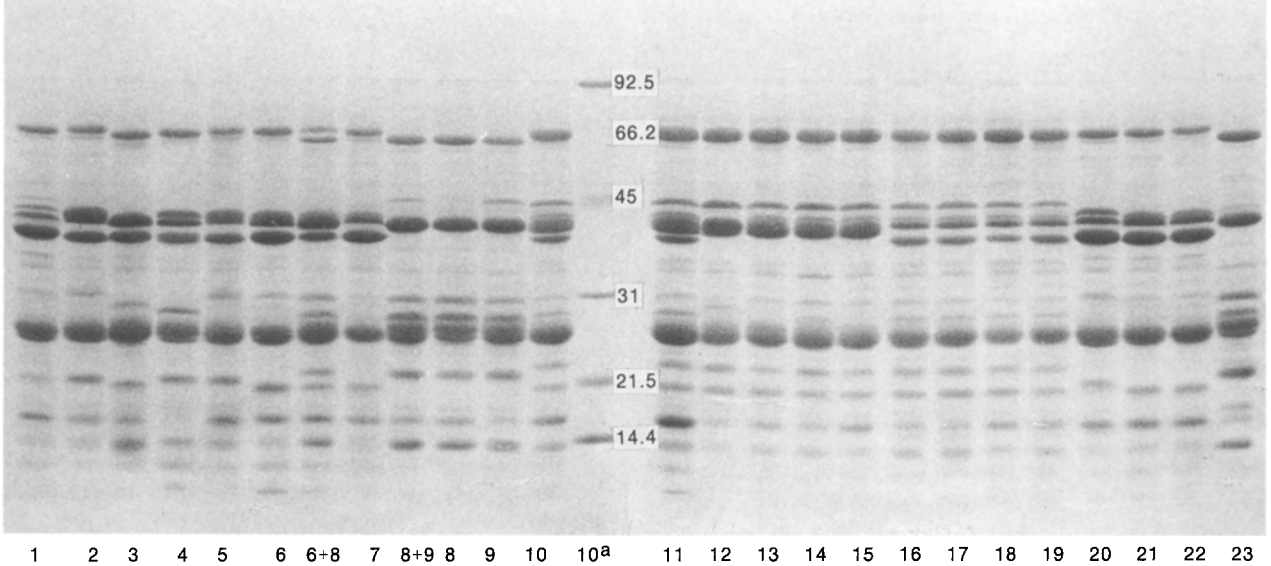

Fig. 1. Profiles of seed protein of *Arachis* species. For genotype number see Table 2. *Track 10 a* contains the molecular weight markers

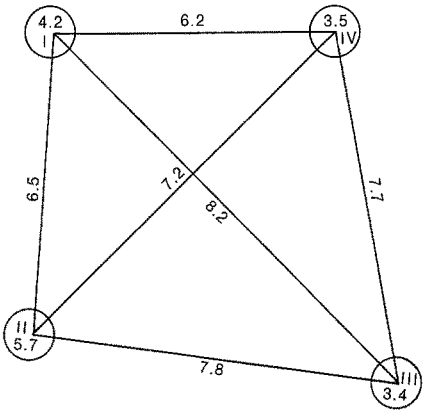

Fig. 2. Clusters (I-IV) on the basis of percentage dissimilarities taken as the statistical distance between accessions

Materials and methods

Details of some taxa used in present investigation have been listed in a previous communication (Singh and Moss 1982). The details of an additional accession of *A. batizocoi,* (*A.* sp. 30081) and of *A. monticola* (*A.* sp. 30063) representing other branching habits, and of the cultigens of *A. hypogaea* from four cultivar groups, representing three botanical varieties and two subspecies, are given as footnote in Table 1.

Analysis of protein profile by SDS-polyacrylamide gel electrophoresis

One seed of each taxa was decorticated and ground in a mortar with pestle. About 100 mg of powdered sample was defatted with 1 ml *n*-hexane. The tubes containing the powdered sample were mixed for 30 min and the hexane was decanted. The defatted cake was dried under vacuum, and about 10 mg of the powdered cake was taken for protein extraction by mixing it with 1 ml buffer (TRIS-HCl pH 7.5, 50 m*M*) for 60 min. The samples were centrifuged for 5 min at 13,000 rpm in an Eppendorf centrifuge, the supernatant was removed and the protein was estimated by the Lowry et al. method (1951).

Samples were treated with TRIS buffer containing sodium dodecyl sulfate (SDS), glycerol and bromophenol blue according to the method of Laemmli (1970). Electrophoresis was conducted on 12.5%, polyacrylamide slab gels containing SDS by loading samples containing equal amounts of protein; the gels were later stained with Coomassie Brilliant Blue G-250. The molecular weight markers used were: phosphorylase b, 92.5 kDa; bovine serum albumin, 66.2 kDa; ovalbumen, 45 kDa; carbonic anhydrase, 31 kDa; soybean trypsin inhibitor 21.5 kDa, and lysozyme 14.4 kDa.

Variations in the position of the bands in any lane were expressed in Resolution factor (Rf) values. The bromophenol blue dye front at the bottom of the gel was arbitrarily given the value 1, while the top of the gel was given a value of zero. The Rf value of a particular variant band was proportional to the distance between the two reference standards (i.e., 0 and 1). The Rf value for each band was computed from the mean of observations obtained from three independent electrophoretic runs of separate extractions.

The percentage similarity between different pairs of species and cultigens was calculated by adapting the method of Ladizinsky and Hymowitz (1979).

Treating percentage dissimilarity as the generalized statistical distance, we grouped the taxa into clusters using the Tocher method (Rao 1952).

Results

The protein profiles of 23 species and cultigens are presented in electrophoregrams (Table 1, Fig. 1). A total of 19 bands were resolved in these taxa. The maximum number of bands in any taxon (i.e., 14) was recorded in the tetraploid *A. monticola.* Rf values of these bands ranged from 0.293 to 0.894. On the basis of Rf values the bands were classified into five groups: (1) very slow moving (0.293–0.312), (2) slow moving (0.429–0.488), (3) medium moving (0.512–0.541), (4) fast moving (0.606–0.759) and (5) very fast moving (0.765–0.894) (Table 1).

Diploid species (2n=20)

Seven species from genome A and two accessions of *A. batizocoi* containing genome B showed a similar number of total bands. The A genome species had a greater number of similar bands and thereby a higher percent similarity (50–100%). They displayed a greater genetic homology among themselves than they did with the two accessions of *A. batizocoi* and, therefore, statistically formed a separate clusters (Figs. 1, 2; Table 2). Within the genome A species, *A. stenocarpa* and *A. chacoense* (Fig. 1, lanes 1 and 2) had an identical number of bands with similar mobility and thereby 100% genetic homology, but certain bands in *A. chacoense* did not resolve to a similar intensity, particularly in group II at Rf values 0.429, 0.435 and 0.447. *A. cardenasii* and *A. correntina* had the least number of similar bands with other A genome species with percentage similarity ranging from 47% to 67%. On the basis of dissimilarity, these two species statistically formed another group (Fig. 2). The other A genome species, *A. stenocarpa, A. chacoense, A. villosa, A. duranensis* and *Arachis* sp. 10038 and the synthetic autotetraploids and amphidiploids were genetically similar with percentage similarity ranging from 54% to 100%. The two *A. batizocoi* accessions formed one group with its tetraploid and differed from each other by a single band at Rf value 0.429. *Arachis* sp. 10038 (A genome) had the highest (53%) number of similar bands to that of *A. batizocoi.*

Tetraploid species (2n=40)

The protein profile of tetraploid *A. monticola* revealed the largest number of bands. The majority of these were also expressed in cultivars of *A. hypogaea,* except for bands at Rf values of 0.465, 0.488 and 0.635, which are present in the cultivars of one subspecies and absent in cultivars of another subspecies. Statistically, the cultivars belonging to subspecies *hypogaea* with 93% similarity were closer to *A. monticola* than the cultivars belonging to subspecies *fastigiata.* Protein profiles of two accessions of *A. monticola* were identical with 100% genetic homology, although morphologically they differed in

Table 2. Percentage similarities between diploid species of section Arachis and tetraploid accessions of *A. monticola and A. hypogaea*

Taxa[a]	1	2	3	4	5	6	8+6	7	8+9	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1		100	50	62	92	83	63	75	29	31	29	56	56	47	53	50	53	60	60	60	60	85	75	75	38
2			50	62	92	83	63	75	29	31	29	56	56	47	53	50	53	60	60	60	60	85	75	75	38
3				67	47	62	67	67	40	43	40	60	60	40	47	53	47	64	64	64	64	64	67	67	40
4					57	50	56	54	31	33	31	41	41	24	29	35	29	44	44	44	44	64	43	43	40
5						77	69	69	35	38	35	53	53	44	50	47	50	56	56	56	56	79	69	69	44
6							73	91	29	31	29	67	67	57	64	60	64	71	71	71	71	85	91	91	29
8+6								67	53	56	53	71	71	53	59	65	59	65	65	65	65	75	67	67	53
7									24	25	24	60	60	50	57	53	57	64	64	64	64	77	82	82	24
8+9										91	100	47	47	47	44	50	44	41	41	41	41	33	31	31	83
8											91	41	41	40	38	44	38	35	35	35	35	35	33	33	91
9												47	47	47	44	50	44	41	41	41	41	33	31	31	83
10													100	79	86	93	86	93	93	93	93	59	71	71	39
11														79	86	93	86	93	93	93	93	59	71	71	39
12															92	85	92	71	71	71	71	50	62	62	38
13																92	100	79	79	79	79	56	69	69	35
14																	92	86	86	86	86	53	64	64	41
15																		79	79	79	79	56	69	69	35
16																			100	100	100	63	77	77	33
17																				100	100	63	77	77	33
18																					100	63	77	77	33
19																						63	77	77	33
20																							77	77	41
21																								100	31
22																									31
23																									

[a] see Table 1

their branching pattern. Protein profiles of cultivars belonging to the two cultivar groups of subspecies *fastigiata* resolved minor differences, but not those of cultivars belonging to the subspecies *hypogaea*.

Diploid species versus tetraploid species

Most of the bands that were resolved in diploid species with the A or B genome were also present in tetraploid *A. monticola* and cultivars of *A. hypogaea*. However, a band resolved at Rf value 0.435 in almost all A genome species and a band of Rf value 0.512 resolved in B genome of *A. batizocoi* were absent from the profiles of tetraploid taxa; an additional band at Rf value 0.753 was present.

Experimental polyploids

In the profiles of the amphidiploid and autotetraploids, the *A. batizocoi* × *A. chacoense* (AABB) amphidiploid resolved the highest number of 13 bands with 47–59% bands being similar to those observed in tetraploid *A. monticola* and cultivars of *A. hypogaea*. The profile from the mixture of proteins of *A. batizocoi* and *A. duranensis* (the two most probable ancestors of *A. hypogaea*) resolved 15 bands with 71% similarity to that of *A. monticola*.

The clustering on the basis of percentage dissimilarity (taken as an indication of genetic non-homology and divergence) as per Tocher's method resulted in four groups (Fig. 2). Group I contains mostly A genome species along with autotetraploids and amphidiploids; group II, *A. cardenasii* and *A. correntina*; group III, two accessions of *A. batizocoi* and its autotetraploid; group IV, all tetraploid taxa.

Discussion

The protein profiles in the taxa studied have the potential to trace intra- and interspecific relationships among species of section Arachis. The consistency of the protein profile suggests that each species has a reproducibly stable profile as a consequence of its specific gene arrangement (Ladizinsky 1975).

The largest fraction of the protein profiles in the species of section Arachis were homogenous even after ploidy differences, corroborating the conclusions of Klozova et al. (1983b) based on immuno-chemical methods. This suggests that they belong to a common ancestral stock. Percentage similarity of bands (Table 2) and statistical distance calculated over dissimilarity (Fig. 2) reflect, however, appreciable genetic variability among them and also among the diploid species within a genome. *A. cardenasii* and *A. correntina* differed from the A genome species with a significantly lower number of similar bands (Fig. 2), suggesting a sub-group status. Singh and

Moss (1982) inferred the same for *A. cardenasii* based on karyomorphology. Differences resolved between *A. correntina* and *A. villosa* (earlier suggested to be varieties of the same species) and other A genome species represent minor genetic differences between these taxa. It justifies the separate specific entity of *A. correntina* (Klozova et al. 1983 b). *Arachis* sp. 30081 has a profile nearly identical to that of *A. batizocoi* except for an additional band at Rf value 0.429, indicating a higher homology between the two taxa. The presence of the 48-kDa polypeptide in all of the tetraploid taxa places *Arachis* sp. 30081 closer to them with a greater probability of being a donor of the B genome to *A. monticola* and *A. hypogaea* than the earlier accession of *A. batizocoi* (9484).

Among the tetraploid taxa, the accessions of *A. monticola* and the cultivars of *A. hypogaea* expressed higher levels of similarity (71–100%) in their bands. Statistically, they were included in one group (Fig. 2), suggesting that these are forms of the same basic species with minor genetic differences (Singh and Moss 1984). The nearly identical profiles among the cultivars of *A. hypogaea* subspecies *hypogaea* indicate that electrophoresis of denatured protein at 12.5% gel does not resolve the minor genetic differences that may have occurred at the microevolutionary level.

Most of the genetic factors resolved by electrophoresis of the A and B genome diploid species can be traced to the *A. monticola* and *A. hypogaea* accessions. For example, major polypeptides observed in different mobility groups of A genome species and in B genome accession *Arachis* sp. 30081 were also found in the *A. monticola,* and *A. hypogaea* accessions (Fig. 1, Table 1). This confirms earlier postulations that a hybrid of the A and B genome species has evolved *A. monticola.* Since *A. monticola* is a tetraploid, it must have evolved through the process of amphidiploidization. This was also visible by the tetrameric nature of certain darker bands.

A. duranensis with the A genome had a higher number of similar bands (67%) to *A. monticola* than any other diploid species, and thus it was the probable donor of the A genome to *A. monticola.* This supports our earlier conclusions based on cytogenetic evidence (Singh and Moss 1982, 1984; Singh 1988) and does not support the suggestions by Cherry (1975) for *A. villosa* and by Krishna and Mitra (1988) for *A. cardenasii.* Similarly, *Arachis* sp. 30081 seems to be the B genome donor. These inferences were corroborated by the mixed protein profile of *A. duranensis* and *A. batizocoi* with 71% genetic homology to *A. monticola,* and the profile of AABB synthetic amphidiploid (*A. batizocoi* × *A. chacoense*) with 59% homology. However, this synthetic amphidiploid statistically groups with the A genome species probably because of the dominance of the genetic factors contributed by the A genome. The protein profiles of section Arachis species were less differentiated and suggest *A. duranensis* and *A. batizocoi* (accession 30081) as the most probable ancestor of *A. hypogaea.*

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