Seed storage protein variation in *Arachis* species

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Seventy-two accessions, representing 22 species from sections *Arachis*, *Erectoides*, *Extranervosae*, and *Triseminalae* of the genus *Arachis*, were screened for seed storage protein polymorphism. Variation was detected between sections, between genome types, between species, and in some cases between different accessions of the same species or different seeds of the same accession. *Arachis duranensis* and one accession of *A. cardenasii* were found to have identical protein patterns. The greatest dissimilarity was found between species of the section *Extranervosae* and species of the section *Triseminalae*. Those of section *Erectoides* showed much similarity with some species of section *Arachis*. Protein polymorphism was shown to distinguish the two subspecies of *A. hypogaea* (*fastigiata* and *hypogaea*) in 27 of 28 cases. The seed protein profile of *A. monticola* was a combination of seed protein profiles from the two *A. hypogaea* subspecies. The relatedness between the various species was calculated and those that had the greatest similarity with *A. hypogaea* were *A. spegazzinii* and *A. batizocoi*.

Key words: *Arachis*, groundnut, storage proteins, variation.

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Soixante-douze accessions, représentant 22 espèces des sections *Arachis*, *Erectoides*, *Extranervosae* et *Triseminalae* du genre *Arachis*, ont fait l'objet d'une étude portant sur le polymorphisme des protéines de réserve des graines. De la variation a été détectée entre les sections, les types de génomes, les espèces et, dans certains cas, entre différentes accessions d'une même espèce ou différentes graines d'une même accession. L'*Arachis* et une accession d'*A. cardenasii* se sont avéré posséder des profils protéiques identiques. La plus grande dissimilarité a été trouvée entre les espèces de la section *Extranervosae* et celles de la section *Triseminalae*. Les espèces de la section *Erectoides* ont présenté beaucoup de similarité avec certaines espèces de la section *Arachis*. Le polymorphisme des protéines a permis de distinguer les deux sous-espèces d'*A. hypogaea* (*fastigiata* et *hypogaea*) dans 27 cas sur 28. Le profil des protéines des graines d'*A. monticola* s'est révélé être une combinaison des profils protéiques des graines des deux sous-espèces d'*A. hypogaea*. Le niveau des liens entre les diverses espèces a été calculé et les espèces dont le degré de similarité a été le plus élevé avec l'*A. hypogaea* ont été l'*A. spegazzinii* et l'*A. batizocoi*.

Mots clés : *Arachis*, arachide, protéines de réserve, variation.

[Traduit par la rédaction]

Introduction

The genus *Arachis* consists of seven sections (Gregory et al. 1980; Ressler 1980; Stalker and Moss 1987), one of which, section *Arachis*, includes the cultivated groundnut *A. hypogaea* L. and its wild progenitor *A. monticola* Krap. et Greg., both of which are tetraploid ($2n = 4x = 40$). The remainder of the section consists of wild diploid species. These wild accessions constitute a valuable source of novel germplasm for the introgression of pest and disease resistance genes into *A. hypogaea* (Moss et al. 1988). The utility of species from other sections of the genus is limited by difficulties in producing fertile intersectional hybrids with *A. hypogaea* (Stalker and Moss 1987). Nine different genome types have been recognized in the genus: A, B, and D (section *Arachis*); E (section Erectoides); Ex (section *Extranervosae*); T (section *Triseminalae*); Am (section *Ambinervosae*); C (section *Caulorhizae*); and R (section *Rhizomatosae*; Smartt and Stalker 1982; Stalker 1985).

The study of seed protein profiles is a useful method for species identification, clarifying taxonomic and evolutionary problems, and studying genetic diversity (Ladizinsky and Hymowitz 1979) and has been successfully used with a wide range of plant species, for example, *Ricinus communis* (Sathaiah and Reddy 1985), *Lolium* sp., *Festuca* sp., *Vulpia* sp. (Bulinska-Radomska and Lester 1986, 1988), *Citrullus* sp. (Navot and Zamir 1987), *Capsicum* sp. (Panda et al. 1986), *Zizania* sp. (Duvall and Biesboer 1989), and *Brachypodium* sp. (Khan 1992). Klozova et al. (1983), Singh et al. (1991), and Bianchi-Hall et al. (1993) used seed protein profiles to examine relationships within the genus *Arachis*. Klozova et al. (1983) screened 10 wild species, five species of the cultivated groundnut, and one "synthetic" hybrid for seed protein polymorphisms and found this approach most useful for distinguishing species within sections. Singh et al. (1991) determined the seed protein profiles of nine section *Arachis* wild species, eight cultivars of *A. hypogaea*, two autotetraploids, and two synthetic hybrids and concluded that there was significant variation within the section, limited variation

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TABLE 1. *Arachis* accessions screened for polymorphic seed proteins

Species (genome type)	Accession No.	Collector*
Section *Arachis*		
A. batizocoi Krap. et Greg. (B)	9484	K
	30080	GKBSPSc
	30081	GKBSPSc
A. cardenasii (A)†	36019	KSSc
	36020	KSSc
	36033	KSSc
	36034-Y	KSSc
	36034-YF	KSSc
	36034-YO	KSSc
A. chacoense (A)	10602	GKP
A. duranensis (A)	7988	K
	30061	GKBSPSc
	30065	GKBSPSc
	30067	GKBSPSc
	30069	GKBSPSc
	30070	GKBSPSc
	30074	GKBSPSc
A. glandulifera Stalker (D)	30091	GKSSc
	30099	GKSSc
	30100	GKSSc
A. helodes Martius ex Krap. et Greg. (A)	30036	GK
A. kempffmercadoi (A)	30085	GKBSPScZ
	35001	GKSPScGB
A. khulaminii (A)	30035	GK
A. magna (A)	30097	GKSSc
A. monticola Krap. et Rig. (AB)	30062	GKBSPSc
	30063	GKBSPSc
A. otavoi (A)	30008	GK
A. spegazzinii (A)	10038	GKP
A. stenosperma (A)	408	HLK
	410	HLK
A. valida (A)	30011	GK
Section *Erectoides*		
A. appresipilla (E)	9990	GKP
	9993	GKP
	10002	GKP
	30003	GK
A. chiquitana (E)	36025	KSSc
A. paraguariensis Chod. et Hassl. (E)	565-66	HLKHe
	30109	GKPSc
A. rigonii Krap. et Greg. (E)	114	Unknown
A. stenophylla (E)	31026	GKPSc
Section *Extranervosae*		
A. villosilicarpa Hoehne (EX)	8142	Unknown
Section *Triseminalae*		
A. pusilla (Benth.) (T)	12922	GK

*B, Banks; G, Gregory; Gb, Gibbons; H, Hammons; He, Hemsy; K, Krapovickas; L, Langord; P, Pietrarelli; S, Simpson; Sc, Schinini; Z, Surita.

†Species without an authority have not been described and names given are *nomina nuda* and may change.

among cultivars, and that genomic divisions within section *Arachis* were justified. Bianchi-Hall et al. (1993) studied seed protein polymorphism among 55 accessions of diploid section *Arachis* species and detected variation within and between species. The present study extends the work of these authors to include a total of 72 accessions, representing 22 species and six genome types from four sections of the genus, *Arachis*, *Erectoides*, *Extranervosae*, and *Triseminalae*; the cultivated groundnut was represented by 28 accessions, which include both subspecies. Attention was focused on variation between sections, genome types, species, and where possible, between accessions within species. The relatedness of diploid section *Arachis* wild species to the cultivated groundnut is also discussed.

Materials and methods

The groundnut accessions used in this study are listed in Tables 1 and 2. All seeds were supplied by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, except those of the three accessions of *A. glandulifera*, which were a gift from Prof H.T. Stalker, North Carolina State University, N.C., U.S.A. Wild

TABLE 2. *Arachis hypogaea* accessions screened for polymorphic seed proteins

	Identity	Synonyms	Origin
	***A. hypogaea* ssp. *hypogaea* var. *hypogaea* (AB)**		
Virginia type			
Bunch subtype			
ICG 8265	P 2416	59-127 N.C.Acc 17194	Senegal
ICG12158	Chunseong	300357	Korea
ICG 8859	Tamale	PI 268947 NCAC 16608	Ghana
ICG 11148	Makulu Red	PI 371965	Uganda
ICG 2985	AH 6712	A 6/36	Myanmar
ICG 2384	DHT 192 OV. Chiqitano	PI 313949 N.C.Acc 17139	Bolivia
ICG 2252	Feng Lee Tai Kung Don Gujarat Narrow Leaf	PI 269710 N.C.Acc 38	Japan
ICG 2741			India
Runner subtype			
ICG 2837	Spanish Peanut		Argentina
ICG 7028	VRR 171		India
ICG 2621	KANO 38	EC 1541 AH 6637	Nigeria
ICG 2645	Che Tse Hua Seng	EC 16668	China
ICG 2706	Asiriya Mwitunde	EC 25188	Tanzania
ICG 4416	Venezuela		Venezuela
	***A. hypogaea* ssp. *fastigiata* var. *fastigiata* (AB)**		
Valencia type			
ICG 10522	Uganda Erect	PI 268551	Uganda
ICG 10566	5101	PI 313200	Congo
ICG 8360	Lonyun 6104	NCAC 18121	Thailand
ICG 6230	RCM 597-1	PI 262092 NCAC 16141	Bolivia
ICG 1267	AH 7229		Malaysia
ICG 1924	RCM 593	PI 262087	Brazil
	***A. hypogaea* ssp. *fastigiata* var. *vulgaris* (AB)**		
ICG 2192	AH 7173	EC 4580 CPI 12156	Brazil
ICG 365	Mani Blanca 61	SH 61 PI 161867	Argentina
ICG 2233	Philippine Pink		Philippines
ICG 10351	Makanga Spanish 1		Malawi
ICG 3200	Kou Pi Hua Seng	EC 16669	China
ICG 10194	55-19		Senegal
ICG 3196	Kalamadi	EC 4082	Kenya

NOTE: "PI" numbers are cited by the source of germplasm. N.C.Acc., North Carolina State University accession number; ICG, ICRISAT groundnut germplasm number.

species were selected to give emphasis to section *Arachis*, as most of these can be crossed with *A. hypogaea*, and the cultivars were selected to represent diverse origins, and to include members of the subspecies and botanical varieties available. Individual seeds were manually ground between two layers of Watman No. 1 filter paper with a carpenter's hammer. Material from several seeds were pooled and aliquots of ground seed (8 mg) were extracted for 1 h at room temperature in 200 μL of the following extraction buffer: Tris–HCl (pH 6.8), 65 mM; sodium dodecyl sulphate, 2% w/v; Pyronin G (BDH), 0.01% w/v; glycerol, 11% w/v; mercaptoethanol, 5% w/v. Samples were then boiled for 3 min after which they were centrifuged briefly to remove particulate matter. Aliquots 20–40 μL were electrophoresed through 10% SDS–polyacrylamide gels at 20 mA for 6–8 h (Laemmli 1970). Both neat and diluted samples of each accession were used, depending on whether faint or strong bands were being scored. Protein bands were visualized by staining the gels in 95% ethanol, 0.4% w/v Coomassie Blue R (Sigma). All accessions were scored plus (+) or minus (−) for each polymorphic protein band. The results were analysed statistically by cluster analysis and by Nei's coefficient of similarity (Nei and Lei 1979).

Results

Screening of *Arachis* germplasm for seed protein polymorphism revealed 24 polymorphic bands ranging in size from 17 600 to 49 500 Da, which were numbered 1–24 in order of decreasing size (Table 3). Figure 1 gives the scores of all accessions represented graphically in band map form (Powell et al. 1991). All *Arachis* species showed unique profiles with the exception of *A. cardenasii* and *A. duranensis*. No intraspecific variation was detected among the seven *A. duranensis* accessions tested. Four accessions of *A. cardenasii* were screened, one of which, 36034, had been separated into three lines (36034-Y, 36034-YF, and 36034-YO), where Y, YF, and YO refer to flower colour. Profiles of 36034-YO and 36034-Y were identical to each other but also to *A. duranensis*, while 36034-YF showed a

TABLE 3. The molecular sizes of the 24 polymorphic seed proteins that were detected in *Arachis* germplasm

Protein band	Approximate molecular mass (Da)
1	49 500
2	48 400
3	38 000
4	37 200
5	32 400
6	31 600
7	30 200
8	29 500
9	29 300
10	28 800
11	28 200
12	27 500
13	26 900
14	26 300
15	26 000
16	22 900
17	21 600
18	21 100
19	20 800
20	20 200
21	19 600
22	19 300
23	18 600
24	17 600

unique profile, as did the other three accessions of *A. cardenasii* (36019, 36020, and 36033) (Fig. 1). Figure 2*a* shows some of the variation found in *A. cardenasii* germplasm; in total, six polymorphic proteins were detected. Collection sites of 36019 and 36020 were within about 10 km of each other, as were 36033 and 36034, but these two areas were about 120 km apart. There is therefore variation over a fairly short distance at each location.

Three accessions of *A. batizocoi* were screened. Although 30080 and 30081 were collected close together (7 km), these two collections differ by an additional protein band (No. 8) in 30081 (Fig. 2*b*). Accession 30080 was identical to 9484 collected 56 km away. Stalker et al. (1991) in a morphological and karyotypic study of five accessions of *A. batizocoi*, including 9484 and 30081, found differences between them and presented evidence that chromosomal translocations may have been responsible for these differences. They could distinguish between all accessions, including 30080 (cited as 30097 in their papers; H.T. Stalker, personal communication) and 30082, collected within 2 km of each other.

Only one accession of *A. chacoense*, 10602, was available for study. Variation between seeds was detected, whereby some seeds possessed an additional protein band (band No. 7; Fig. 2*c*). A total of 16 individual seeds were tested of which half scored positive for band No. 7 and half scored negative. We therefore refer to the absence of this extra band as 10602/A and its presence as 10602/B.

Twenty-eight accessions of the cultivated groundnut *A. hypogaea* L. were screened for polymorphism. These accessions represented ssp. *hypogaea* var. *hypogaea*, ssp. *fastigiata* var. *fastigiata*, and ssp. *fastigiata* var. *vulgaris*. Two polymorphic proteins were detected, band No. 7, which was present in ssp. *hypogaea* only and band No. 6, which was present in ssp. *fastigiata* only (both var. *fastigiata* and var. *vulgaris*). One exception to this was observed: accession 10351, which is *A. hypogaea* ssp. *fastigiata*, had a profile identical to *A. hypogaea* ssp. *hypogaea* (Fig. 3). *Arachis monticola*, the wild progenitor of *A. hypogaea*, which was represented by two accessions, was shown to possess both protein band Nos. 6 and 7, in addition to all other markers found in *A. hypogaea* germplasm (Fig. 3). Seed proteins did not distinguish two market types, Runner and Bunch, of *A. hypogaea* ssp. *hypogaea* var. *hypogaea* (Fig. 3). *Arachis hypogaea* ssp. *hypogaea* var. *hirsuta* was not available for study.

The band map generated from protein data orders genotypes such that those with similar profiles are more likely to be placed close to each other than those with dissimilar profiles (Powell et al. 1991). The band map highlights the similarities and differences among accessions for protein variation (Fig. 1). It is immediately obvious that the five accessions of *A. appresipilla*, collected very close to each other, are identical to each other, whereas the six examples of *A. cardenasii* are nonidentical and are dispersed in the band map. The number of protein bands present in each accession ranged from 5 in *A. pusilla* to 15 in the two accessions of *A. monticola*. Protein bands placed to the right of the band map occur at lower frequencies than those placed to the left; this facilitates the identification of those that are unique or rare. Protein band Nos. 11 and 21 were uniquely present in *A. khulaminii* and *A. pusilla*, respectively, whereas band Nos. 10 and 23 were uniquely absent from *A. pusilla*. Marker 8, while present in four accessions, was detected in only two species, *A. batizocoi* Krap. et Greg. and *A. villosulicarpa* Hoehne.

A similarity matrix and a minimum spanning tree derived from seed protein data are shown in Table 4 and Fig. 4, respectively. Interspecific similarities ranged from 0% (*A. pusilla/A. paraguariensis* and *A. pusilla/A. stenophylla*) to 92% (*A. chacoense/A. stenosperma*). A similarity of 92% represents a difference of one protein band only, in this case band No. 17. Although this protein band distinguishes *A. chacoense* from *A. stenosperma*, it was present in 38 accessions in total (Fig. 1). Among the accessions of *A. cardenasii*, the similarity varied from 91% (36019/36020) to 64% (36033/36034-YS), whereas similarity among the A genome species of section *Arachis* as a whole varied from 92% (*A. chacoense/A. stenosperma*) to 49% (*A. chacoense/A. khulaminii*; Table 4). The *A. cardenasii* accessions form a cohesive group on the minimum spanning tree (Fig. 4) with the exception of 36034-YF, and it should be noted that 36034-Y and 36034-YO (which have an identical profile to and occupy the same position on the minimum spanning tree as *A. duranensis*) are grouped with the other *A. cardenasii* accessions. It should also be noted that *A. cardenasii* 36033 is as similar to *A. duranensis* as it is to *A. cardenasii* 36030 (83.3%; Fig. 4). The extent of variation found in *A. cardenasii* germplasm, both between collection sites and at one site (36034) may indicate that this species is an outbreeder.

Arachis batizocoi and *A. glandulifera* Stalker, while classified in section *Arachis*, possess genome types B and D, respectively, which are different from all other diploid section *Arachis* species (Smartt et al. 1978*a*; Stalker 1991; Stalker et al. 1991). This is reflected in their seed protein profiles (Fig. 4 and Table 4). The most similar section *Arachis* species to *A. batizocoi* is *A. octavoi*, which has only 44%

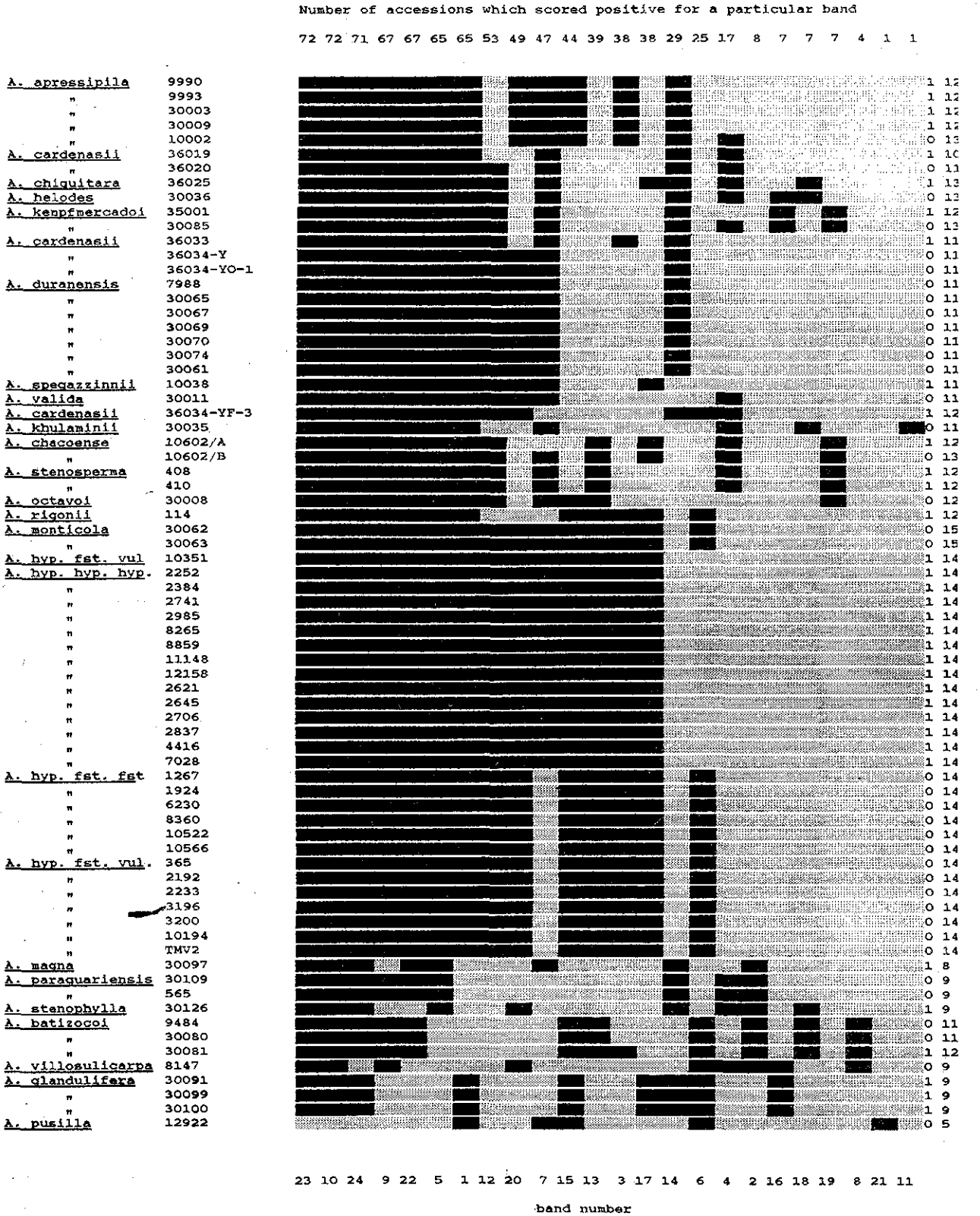

FIG. 1. Band map of polymorphic seed proteins detected in *Arachis* germplasm. ■, protein band present; □, protein band absent. The row of numbers along the bottom of the band map identifies each band by its number. The row of numbers along the top and bottom of the band map gives the number of accessions that scored positively for each band, e.g., band No. 23 (bottom) was present in 72 (top) of the accessions tested. The column of zeros and ones that occur at the right-hand side of the band map are indicators of change; if two adjacent accessions have the same value, whether one or zero, they have identical seed protein profiles. The column of markers on the extreme right of the band map indicates the number of markers present in each accession, e.g., *A. appressipilla* 9990 scored positive for 12 markers. Note that both types of *A. chacoense* 10602 are represented (see text for further details).

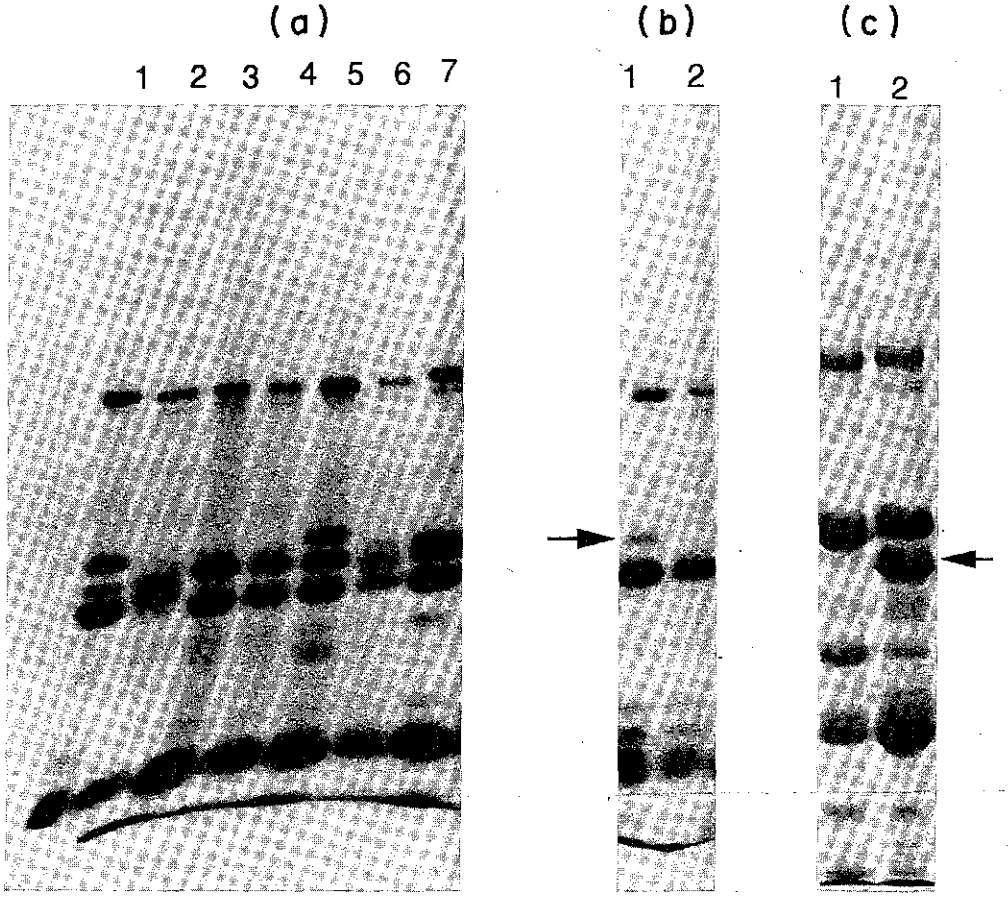

FIG. 2. Intraspecific polymorphism detected in seed proteins of wild *Arachis* germplasm. (*a*) Lane 1, *A. appresipilla* 9990; lane 2, *A. cardenasii* 36034-YF; lane 3, *A. cardenasii* 36034-YO; lane 4, *A. cardenasii* 36034-Y; lane 5, *A. cardenasii* 36033-Y; lane 6, *A. cardenasii* 36020; lane 7, *A. cardenasii* 36019. (*b*) Lane 1, *A. batizocoi* 30081; lane 2, *A. batizocoi* 9484; arrow, band No. 3. (*c*) Lane 1, *A. chacoense* 10602 type A; lane 2, *A. chacoense* 10602 type B; arrow, band No. 7.

similarity to *A. batizocoi* 30081; *A. batizocoi* has more in common with the section *Erectoides* species *A. rigonii* Krap. et Greg., which has 60% similarity with *A. batizocoi* 9484 and 53% with 30080/81. Interspecific crosses between *A. batizocoi* and A genome species of section *Arachis* have very low fertility levels (Smartt et al. 1978*b*). *Arachis glandulifera* has its greatest similarity in section *Arachis* with *A. kempfmercadoi*, or *A. cardenasii* 36034-YF (both 40%), while it has 50% similarity with *A. rigonii* and 40% with *A. appresipilla*, both in section *Erectoides*. *Arachis glandulifera* is distinguished from other section *Arachis* species on morphological and karyotypic grounds. In addition, *A. glandulifera* does not cross-hybridize with *A. hypogaea*, and hybrids between *A. glandulifera* and either *A. duranensis* (A genome) or *A. batizocoi* (B genome) are sterile (Stalker 1991). *Arachis batizocoi* (30080) and *Arachis glandulifera* were distinct from each other, having only 33% similarity. *Arachis glandulifera* has 41% similarity with *A. hypogaea*/*A. monticola*, as close to those as to any other species in section *Arachis*.

Arachis monticola possesses the A and B genomes similar to *A. hypogaea* and is generally considered to be a wild type of the cultivated groundnut. In this study, the seed protein profile of *A. monticola* was identical to a combination of markers found in the two subspecies of *A. hypogaea* and from these results it would not be unreasonable to propose that *A. monticola* gave rise to both *A. hypogaea* ssp. *hypogaea* and *A. hypogaea* ssp. *fastigiata* (Fig. 3). Conversely, *A. monticola* may have arisen by hybridization of the two subspecies. *Arachis hypogaea* is represented in Fig. 4 and Table 4 by *A. monticola* (i.e., it occupies the same position).

Much speculation on the identity of the diploid precursors of *A. monticola*/*A. hypogaea* exists in the literature (Smartt et al. 1978*a*; Klozova et al. 1983; Stalker and Moss 1987; Krishna and Mitra 1988; Kochert et al. 1991; Singh et al. 1991; Halward et al. 1992; Paik-Ro et al. 1992; Bianchi-Hall et al. 1993). The results presented here indicate that *A. spegazzinii* (73% similarity with *A. monticola*) or *A. octavoi* (69% similarity with *A. monticola*) may be candidates for the A genome donor, while *A. batizocoi*, which is the only B genome diploid species identified to date, shares 50% similarity with *A. monticola*. Given that the seed protein profiles reported here distinguish the A and B genomes (see discussion above on *A. batizocoi*) these percentage similarities lend weight to the argument that *A. batizocoi* (B genome) and *A. spegazzinii* (A genome) or *A. octavoi* (A genome) may be considered as possible genome donors to the cultivated groundnut. On examining the profiles in more detail (Fig. 5), it becomes apparent that all bands present in *A. monticola* are to be found in either *A. batizocoi* (30081) or *A. spegazzinii*, whereas if *A. batizocoi* is combined

with *A. octavoi* one band (No. 20) is present in *A. monticola* but absent from the combination. Three bands (Nos. 2, 8, and 18) are present in *A. batizocoi* but absent from *A. monticola*.

The five section *Erectoides* species that were included in this study represented two series, *Tetrafoliolatae* and *Procumbense*. The most similar, 64%, were *A. paraguariensis* and *A. stenophylla*, both *Tetrafoliolatae* (Table 4). Similarities in *Procumbensae* species range from 47 to 60% and 24 to 57% for similarities between *Tetrafoliolatae* and *Procumbensae* species. Although this is in line with the taxonomic series affiliations, with the exception of *A. stenophylla*, each species exhibited greater similarity with non-*Erectoides* species, e.g., *A. chiquitana/A. helodes*, 86%. This is reflected in the dispersion of the *Erectoides* species throughout the minimum spanning tree with each of the three main groupings including at least one *Erectoides* species (Fig. 4).

Arachis villosulicarpa and *A. pusilla* were the sole representatives of sections *Extranervosae* and *Triseminalae*, respectively, available for this study. These species were distinguished from each other (8% similarity) and from all other species (Table 4). The **B** genome species *A. batizocoi* was the most similar to *A. villosulicarpa* (43%), while the D genome species *A. glandulifera* was the closest to *A. pusilla* (27%).

The **B**, D, Ex, and T genome species are grouped together in the minimum spanning tree, although the degree of similarity between the genome types is not particularly high (see Fig. 4).

Discussion

It has been estimated that, on morphological, karyotypic, and cross fertility criteria, the genus *Arachis* consists of 15–25 species with precise assignations of all accessions being incomplete (Stalker and Moss 1987; Stalker 1990), though up to 77 species have been suggested (IBPGR 1990). Estimates of the extent of variation within a genus or within a given species can be instrumental in clarifying such situations. In addition, a knowledge of the relatedness between species can assist in designing programs for gene mapping and for the introgression of novel characters from wild species into cultivars. For these reasons a range of *Arachis* germplasm was screened for polymorphic seed proteins. Twenty-four polymorphic proteins were identified by screening 72 accessions representing 22 species and six genome types.

All species had individual profiles with the exception of *A. cardenasii* 36034, which was identical with all the accessions of *A. duranensis*. Interspecific variation among accessions of *A. cardenasii*, *A. chacoense*, *A. batizocoi*, and *A. hypogaea* was also detected using this method, while it was observed that other species, e.g., *A. appresipilla* (five accessions), *A. duranensis* (seven accessions), and *A. glandulifera* (three accessions), showed no such variation. Klozova et al. (1983), Singh et al. (1991), and Bianchi-Hall et al. (1993) also examined seed protein variation in *Arachis* germplasm and identified 27, 19, and 25 polymorphisms, respectively. The disparity in the number of markers detected may reflect differences in extraction procedures or electrophoresis conditions. Singh et al. (1991) found that among six section *Arachis* species, *A. chacoense* and *A. stenocarpa* (accession HLK 410, now known and identified in this paper as *A. stenosperma*) were identical to each other, though differences were detected in the present study. A similar outcome was found with *A. cardenasii* 36034-Y and 36034-YO, which were identical to *A. duranensis*. In the present study one protein band, No. 3, was found to be polymorphic among accessions of *A. batizocoi*. Singh et al. (1991) also identified a single polymorphism between the *A. batizocoi* accessions 9484 and 30081, which corresponds in gel position to marker No. 3, although the estimation of size differs somewhat, 38 000 for band No. 3 compared with 48 000 Da for that of Singh et al. (1991). These authors also reported that *A. batizocoi* clustered separately from the other species tested. In contrast with the present study, they observed three protein bands that distinguished the two subspecies of *A. hypogaea*. However, it is noteworthy that their protein bands of 44 000 and 42 000 Da probably correspond to band Nos. 6 and 7 in the present study and that these two proteins distinguish the two subspecies of *A. hypogaea*. The third *A. hypogaea* polymorphism detected by Singh et al. was a protein of 45 000 Da, which was present in all but one of the accessions studied. This accession was ICG 7368, which was not available for the present study. The present study found *A. monticola* to be equally similar to both subspecies of *A. hypogaea*, while the third polymorphic band detected by Singh et al. (1991) resulted in *A. monticola* having greater similarity with subspecies *hypogaea*. Bianchi-Hall et al. (1993) investigated seed protein polymorphism in

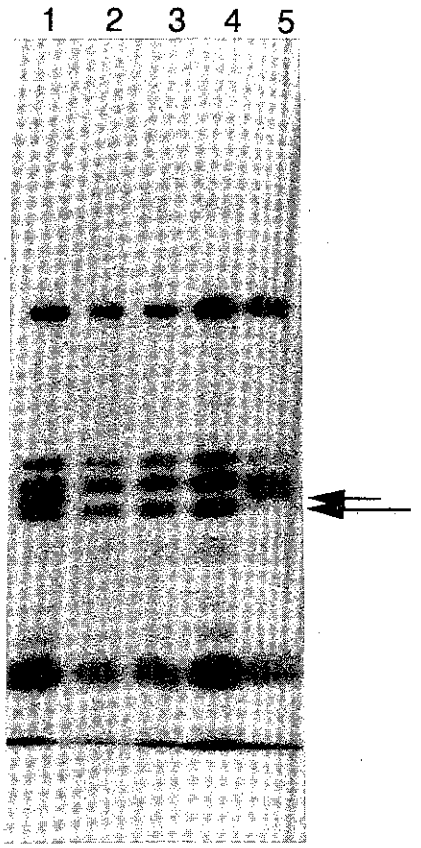

FIG. 3. Seed protein polymorphism detected in *Arachis hypogaea* and *A. monticola* germplasm; band Nos. 6 (upper) and 7 (lower) are indicated by arrows. Lane 1, *A. monticola* 30062; lane 2, *A. hypogaea* ssp. *fastigiata* var. *vulgaris* 10351; lane 3, *A. hypogaea* ssp. *hypogaea* var. *hypogaea* 2706; lane 4, *A. hypogaea* ssp. *hypogaea* var. *hypogaea* 2384; lane 5, *A. hypogaea* ssp. *fastigiata* var. *fastigiata* 1267.

TABLE 4. Similarity matrix (%) of *Arachis* species based on protein profiles analysed using Nei's coefficient of similarity

Section	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
ER	1. *appresipilla*	100																								
AR	2. *cardenasii* 36019	69	100																							
AR	3. *cardenasii* 36020	64	91	100																						
ER	4. *chiquitara*	56	77	85	100																					
AR	5. *helodes*	56	77	85	86	100																				
AR	6. *kempfmercadoi*	60	69	77	67	79	100																			
AR	7. *cardenasii* 36033	77	75	83	71	71	77	100																		
AR	8. *duranensis*	77	75	83	71	71	77	83	100																	
AR	9. *spegazzinii*	64	62	69	71	60	64	69	83	100																
AR	10. *valida*	64	75	83	71	71	64	69	83	83	100															
AR	11. *cardenasii* 36034-YF	60	69	77	67	67	60	64	77	64	77	100														
AR	12. *chacoense*	47	64	71	73	63	67	60	60	71	71	56	100													
AR	13. *stenosperma*	50	69	77	67	67	71	64	64	64	77	60	92	100												
AR	14. *otavoi*	60	57	64	56	56	71	64	64	64	64	50	79	85	100											
ER	15. *rigonii*	60	47	44	47	39	41	53	44	53	44	50	56	50	60	100										
AR	16. *monticola/hypogaea*	69	47	53	56	47	50	63	63	73	63	59	65	59	69	80	100									
AR	17. *khulaminii*	53	75	69	71	71	53	57	57	57	69	53	60	64	53	44	44	100								
AR	18. *magna*	54	64	58	50	50	54	58	58	46	46	43	40	43	43	33	35	46	100							
ER	19. *paraguariensis*	50	73	67	57	57	50	54	54	43	54	62	47	50	40	40	33	54	70	100						
ER	20. *stenophylla*	40	46	43	47	47	31	33	43	33	43	50	29	31	24	24	26	43	55	64	100					
AR	21. *batizocoi* 30081	35	31	29	33	33	28	29	29	29	29	35	33	35	44	53	44	38	36	43	33	100				
AR	22. *batizocoi* 9484 + 30080	41	29	28	32	32	26	35	28	28	28	33	32	33	41	60	50	35	33	40	31	92	100			
EX	23. *villosulicarpa*	24	27	25	22	29	29	18	25	25	33	40	22	24	17	24	26	25	21	38	38	43	40	100		
AR	24. *glandulifera*	40	36	33	38	38	40	33	33	33	25	40	29	24	31	50	41	25	31	29	29	33	31	29	100	
TR	25. *pusilla*	21	15	14	13	13	13	14	14	14	14	13	13	13	21	21	25	14	8	0	0	14	13	8	27	100

NOTE: Those species that demonstrated no intraspecific variation are represented only once; those in which such variation occurred are represented by more than one accession. *Arachis chacoense* 10602 is represented by type B (see text for details). *Arachis hypogaea* is represented by *A. monticola*. *Arachis cardenasii* 36034-Y and 36034-YO were identical to *A. duranensis* and are therefore represented by *A. duranensis*.

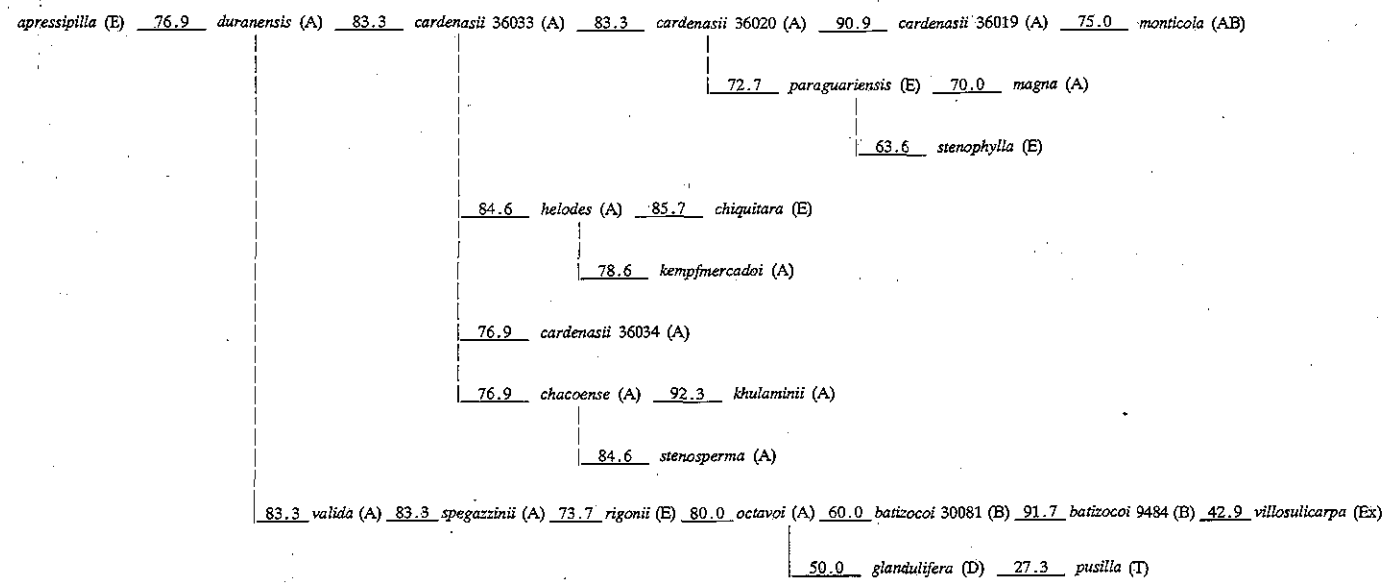

FIG. 4. Minimum spanning tree of *Arachis* species based on seed proteins. Species that showed no intraspecific variation are represented once only; those in which such variation occurred are represented by more than one accession. *Arachis chacoense* 10602 is represented by type B (see text for details). *Arachis hypogaea* is represented by *A. monticola*.

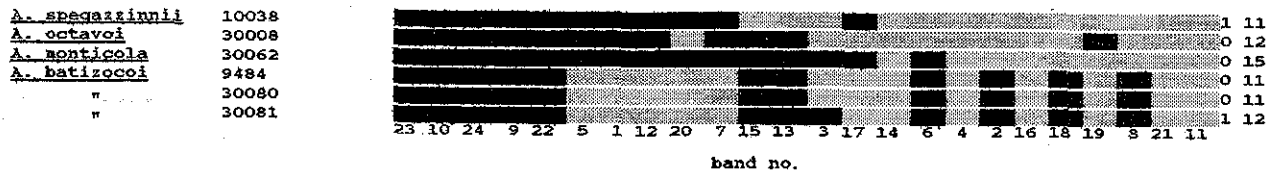

FIG. 5. Band map of selected *Arachis* accessions showing the relatedness of the possible donors of the A and B genomes, found in the cultivated peanut, to *A. monticola*.

section *Arachis* wild species. In agreement with the present study, they found variation in *A. cardenasii* germplasm, but in contrast, they reported differences between accessions of *A. duranensis*, which the present study did not detect. Variations that they reported for *A. helodes* and *A. correntina* were not investigated here because of unavailability of seed material.

The question of the possible diploid progenitor species of *A. hypogaea/A. monticola* has been addressed by various researchers using different methodologies. *Arachis batizocoi*, being the only B genome species as yet identified, is usually considered as being closely related to the B genome donor of the AB tetraploid species. This assumption is supported by cytogenetic data (Smartt et al. 1978*a*), by results obtained from seed protein studies (Klozova et al. 1983; Singh et al. 1991; the research reported here), and from a study of arachin immunoprecipitates (Krishna and Mitra 1988). However, results from restriction fragment length polymorphism (RFLP) analysis did not indicate a particularly close relatedness of *A. batizocoi* to the cultivated groundnut (Kochert et al. 1991; Paik-Ro et al. 1992). Bianchi-Hall et al. (1993) and the present study report three additional protein bands found in *A. batizocoi* but not in *A. hypogaea*. The former authors consider this as further evidence that *A. batizocoi* is not a progenitor of *A. hypogaea*. The present study and that of Singh et al. (1991) and Bianchi-Hall et al. (1993) show that there is variation between accessions, so it is possible that the ancestral accessions that would give a perfect match with *A. monticola/A. hypogaea* no longer exist or have not been collected to date. Several diploid species have been proposed as the possible donor of the A genome to the *A. hypogaea/A. monticola*, including *A. cardenasii* (Smartt et al. 1978a; Klozova et al. 1983; Krishna and Mitra 1988), *A. duranensis* (Krishna and Mitra 1988; Singh et al. 1991; Kochert et al. 1991; Paik-Ro et al. 1992), *A. ipaensis* (Kochert et al. 1991), and *A. spegazzinii* (Kochert et al. 1991; the present study). As yet, no diploid species consistently having sufficient similarity to the cultivated groundnut to be considered as the definitive donors of the A and B genomes to *A. hypogaea/A. monticola* have been identified. The variation identified in *A. cardenasii* must strengthen the case for this being an ancestral species.

Assessments of variation within the genus based on seed proteins, RFLPs or random amplified polymorphic DNAs (RAPDs) agree that while there is considerable variation within the genus as a whole, there is surprisingly little variation among cultivars of *A. hypogaea* (Singh et al. 1991; Kochert et al. 1991; Halward et al. 1992; the present study). Singh et al. (1991) detected 19 protein bands in total of which 3 were polymorphic among cultivars. In the research reported here 24 markers were identified of which only 2 were polymorphic among cultivars. It may be, as Kochert et al. (1991) suggested, that very obvious gross morphological polymorphisms are the result of a small number of subtle genotypic differences. In this report several groups among the A genome species were delineated on the minimum spanning tree (Fig. 4). However, the E genome species did not form a cohesive group. This agrees with results obtained using RAPDs whereby it was suggested that at least some section *Erectoides* species had greater similarity to section *Arachis* species than would be expected based on morphological or

cytogenetic data (Halward et al. 1992). Species with B, D, Ex, or T genomes were clearly distinguished by seed protein polymorphism.

In summary, polymorphic seed proteins were useful in estimating variation among different accessions of certain *Arachis* species and could distinguish between species and between genome types, although the results do not always reflect the differences between sections of the genus that have been determined based on crossability between species, morphology, and on cytogenetic data.

Acknowledgements

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