

The Comparison of Seed Protein Patterns within the Genus *Arachis* by Polyacrylamide Gel Electrophoresis

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Abstract. The seed protein patterns of 12 *Arachis* species were compared by polyacrylamide gel electrophoresis (PAGE), similarities between patterns were measured by the Jaccard index. Results obtained confirm the close relationships established between members of the genus on morphological grounds and support the more recent classification schemes. *A. villosa* and *A. correntina* could well be regarded as distinct species on grounds of protein differences while *A. macedoi* and *A. villosulicarpa* (although members of the same section, *Extranervosae*) show considerable differentiation of their protein patterns. Surprisingly, the form *A. × batizogaea* showed less similarity in protein pattern to those of its parental species than might have been expected. The principle value of seed protein pattern data appears to be in distinguishing species within sections.

The genus *Arachis* (*Leguminosae*, tribe *Hedysareae*, subtribe *Stylosanthineae*) has about 22 well described and 40 or more undescribed species (GREGORY *et al.* 1980), which are divided into 7 sections according to their morphological characters. Because the taxonomic classification of this genus is not definitive, it is helpful to bring further data to bear on elucidating relationships within the genus. Some attention has also been given to karyological characterization including the differentiation of two genomes within section *Arachis* and their distribution in the species of this section (KRAPOVICKAS and RIGONI 1952, SMARTT *et al.* 1978a, b, RESSLAR and GREGORY 1979).

Comparison of protein patterns can also help in elucidating species relationships in *Arachis* as was shown by CHERRY (1975, electrophoretic comparison of seed proteins and enzymes) and NEUCERE and CHERRY (1975), who compared seed proteins of 32 taxa by means of immunoelectrophoresis. BASHA (1979) observed differences in seed polypeptides of *Arachis hypogaea* cultivars and DAUSSANT *et al.* (1969) characterized main reserve proteins.

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Quite recently RESSLAR *et al.* (1981) compared 12 taxa by means of cyto-photometric determinations of DNA amount and their results support more or less the classification of GREGORY *et al.* (1980).

We have attempted to compare the seed proteins of 12 *Arachis* species by means of polyacrylamide gel electrophoresis (PAGE) and to investigate their relationships.

MATERIAL AND METHODS

We have analysed seed protein patterns of following species:

Species	Coll. No.
<i>Arachis batizocoi</i> KRAP. et GREG.	9484
<i>A. × batizogaea</i> KRAP. et FERN.	named hybrid
<i>A. cardenasii sensu</i> KRAP. et GREG.	10017
<i>A. correntina</i> (BURK.) <i>sensu</i> KRAP. et GREG.	7830
<i>A. correntina</i> (BURK.) <i>sensu</i> KRAP. et GREG.	7897
<i>A. correntina</i> (BURK.) <i>sensu</i> KRAP. et GREG.	9530
<i>A. correntina</i> (BURK.) <i>sensu</i> KRAP. et GREG.	9548
<i>A. hypogaea</i> L.	36
<i>A. hypogaea</i> L.	40
<i>A. hypogaea</i> L.	NC 2
<i>A. hypogaea</i> L.	NC 5
<i>A. hypogaea</i> L.	commercial sample ("Peanuts groundnuts", Produce of India)
<i>A. macedoi sensu</i> KRAP. et GREG.	10127
<i>A. monticola</i> KRAP. et RIG.	7264
<i>A. paraguariensis</i> CHOD. et HASSL.	9646
<i>A. rigonii</i> KRAP. et GREG.	10034
<i>A. villosa</i> BENTH	22585
<i>A. villosulicarpa</i> HOEHNE	14446
<i>Arachis</i> sp.	HLK 410

The seed material listed above was obtained from prof. W. C. Gregory of the Crop Science Department, North Carolina State University Raleigh (through Dr. J. Smartt). The authors wish to thank him for supplying the seed material.

Defatted seed meal was extracted by buffered saline (KLOZ and KLOZOVÁ 1974). Polyacrylamide gel electrophoresis was carried out using the technique of DAVIS (1964) as modified by NOVACKÝ *et al.* (1966) at pH 8.3. Gel columns were stained with Coomassie brilliant blue R-250 and were densitometrically evaluated. From densitograms Rp values of single bands were obtained and protein patterns were drawn schematically (Fig. 1). Protein patterns were then compared by means of the Jaccard index (HADAČOVÁ *et al.* 1980); this evaluation was semi-quantitative in that intensity of single bands was taken into account. The result of this evaluation is an index of similarity (I) which expresses the relationship between protein patterns of the taxa compared.

RESULTS

The results obtained are presented in Fig. 1 and Tables 1 and 2. Fig. 1 shows seed protein patterns of the *Arachis* taxa compared (some of them in more than single accessions). We have densitometrically identified 27 bands in total, the highest number is 21 bands in 1 sample (of *A. hypogaea*) and the lowest is in *A. macedoi* with only 11 bands (Table 1). From Table 1 we can see that there is no band in common for all 19 samples compared. The most

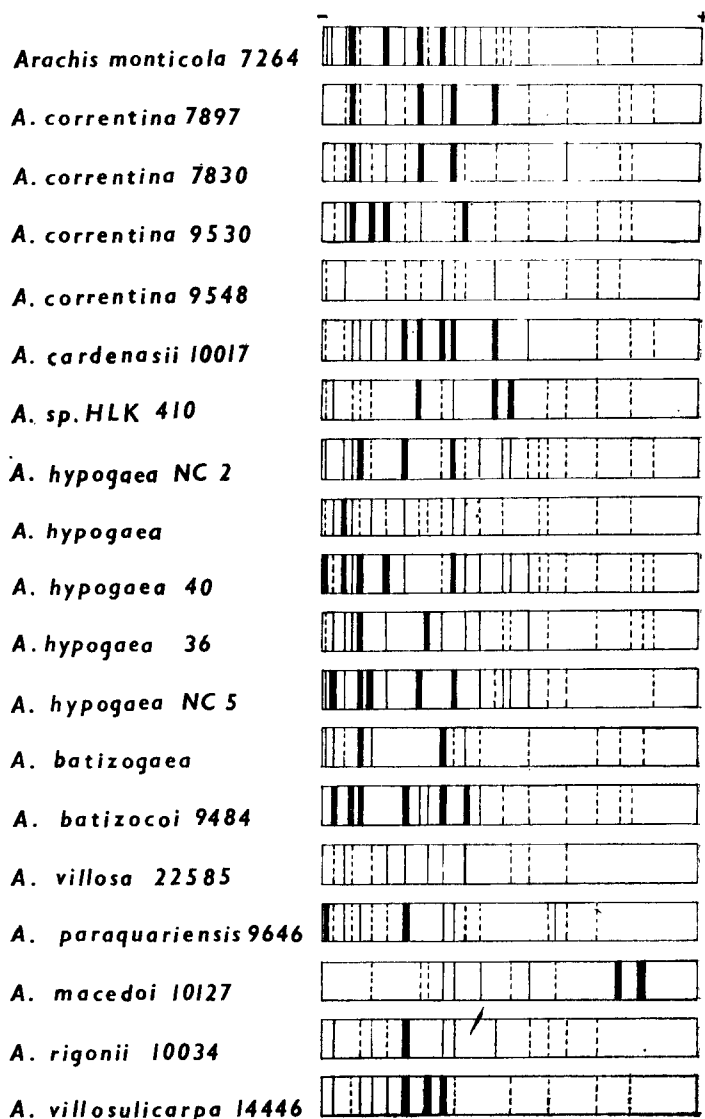


Fig. 1. Seed protein patterns of different *Arachis* species revealed by polyacrylamide gel electrophoresis.

TABLE 1
List of present bands and their intensity

Arachis	Coll. No.	Rp × 100																								Σ values of band intensity												Σ bands present
		1	3	6	8	10	13	17	22	26	28	32	35	38	42	46	48	50	55	58	60	62	65	73	79	82	85	88										
<i>monticola</i>	7264	2	2	2	3	1	3	2	3	1	3	2	3	2	2	2	1	1	1				1	1	1	1	19	35										
<i>correntina</i>	7897			1	3	1	3	2	1	3	2	3	3	1	3		3	1	1	1		1	1	1	1	1	14	24										
<i>correntina</i>	7830	1	1	3	2	1	3	2	1	3		3	1	1	1	1	1	1	1		2	1	1	1	1	1	15	24										
<i>correntina</i>	9530	1	2	3		3	3	1	2			1	3	1	3	1	1	1	1		1	1	1	1	1	1	15	25										
<i>correntina</i>	9548	2		2			1	1	1	1	2	1	1	1	2	1	2	1	1		1	1	1	1	1	1	13	17										
<i>cardenasii</i>	10017	1	1	2	2	2	2	2	3	3	3	3	3		3	3		2								1	15	30										
sp.	HLK410	1	2	1	1	1	1	1	3	3	1	2			3		3		3	1	1	1	1	1	1	1	14	22										
<i>hypogaea</i>	NC 2	2	2	2	3	1	3	2	1	3	1	3	1	2	3	2	2	1	1	1	1	1	1	1	1	1	19	31										
<i>hypogaea</i>		1	2	3	2	1	2	1	2	1	1	2	2	1	1	1	1	1	1		1	1	1	1	1	1	19	27										
<i>hypogaea</i>	40	3	1	3	2	3	3	2				1	3	2	2	2	2	2	2	1	1	1	1	1	1	1	21	38										
<i>hypogaea</i>	36	1	2	2	2	3	2			3	2	2	2	2	2	2	1	1	2	1	1	1	1	1	1	1	18	30										
<i>hypogaea</i>	NC 5	2	3	2	3	3	2			3		3	3	2	2	2	1	2	2	2	1	1	1			1	16	33										
× <i>batizogaea</i>		2	2	1	3	3	2			3	3	1	2	1	1	1	2	2	1	1	1	1	1	1	1	1	13	21										
<i>batizocoi</i>	9484	3	3	3		3	3		3	2	2	3	3	2	1	1	1	1	1		1	1	1	1	1	1	15	30										
<i>villosa</i>	22585	1	2	1	1	1	2	2	2	2	2	2	2	2	2	1	1	1	1		1	1	1	1	1	1	12	18										
<i>paraguariensis</i>	9646	3	1	2	1	2	1	1	3		1	2	2	1	1			1	2	1	1	2	1	1	3	3	16	25										
<i>macedoi</i>	10127									1	2	1	3	2	2	2	2	1	2	1	1	1					11	19										
<i>rigonii</i>	10034	2		1	2	1	2	1	3		2	2	2		2		1	1	1	1	1	1	1	1	1	1	12	19										
<i>villosulicarpa</i>	14446	2	1	2	2	2	3			3	3	1			2		1	1	1	1	1	1	1	1	1	1	13	23										
Σ 19		11	14	14	14	15	13	14	14	11	7	16	16	12	10	9	7	9	15	3	9	2	12	14	7	12	4	6										

= most intensive band; 2 = intensive band; 1 = less intensive band.

3 = most intensive band; 2 = intensive band; 1 = less intensive band.

TABLE 2
Indexes of similarity according to Jaccard

<i>Arachis</i>	Coll.No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
<i>monticola</i>	7264	1		43	46	40	51	39	43	55	52	54	41	36	51	39	39	22	35	38
<i>correntina</i>	7897	2	47	66	44	41	64	39	31	28	32	26	33	18	32	27	26	23	34	24
<i>correntina</i>	7830	3	43	66	58	32	50	35	34	34	38	26	39	25	35	31	32	19	30	27
<i>correntina</i>	9530	4	46	44	40	40	38	27	27	40	31	28	32	28	38	39	28	13	29	26
<i>correntina</i>	9548	5	40	41	40		34	26	30	29	28	21	25	36	24	35	40	20	38	18
<i>cardenasii</i>	10017	6	51	32	40	34		41	42	39	39	33	40	31	33	26	38	20	44	43
sp.	HLK410	7	39	50	38	27	41		33	36	28	24	38	23	24	18	31	21	41	32
<i>hypogaea</i>	NC 2	8	43	34	27	30	42	33		49	73	45	49	33	39	29	56	22	32	32
<i>hypogaea</i>		9	55	34	40	29	39	36	49		51	55	33	37	39	36	44	21	39	43
<i>hypogaea</i>	40	10	52	38	31	28	39	28	73	51	58	48	48	44	36	33	47	21	27	30
<i>hypogaea</i>	36	11	54	26	28	21	33	24	45	55	58	40	40	42	46	41	34	26	23	43
<i>hypogaea</i>	NC 5	12	41	33	32	25	40	38	49	33	48	40		32	26	21	32	24	30	24
<i>× batizogaea</i>		13	36	25	28	36	31	23	33	37	44	42	32		38	26	39	25	33	33
<i>batizocoi</i>	9484	14	51	32	38	24	33	24	39	39	36	46	26	38		33	31	23	29	39
<i>villosa</i>	22585	15	39	31	39	35	26	18	29	36	33	41	21	26	33	39	39	19	32	41
<i>paraguariensis</i>	9646	16	39	26	32	28	40	38	31	44	47	34	32	39	31	39	19	42	41	
<i>macedoi</i>	10127	17	22	19	13	20	20	21	22	21	21	26	24	25	23	19	19	23	20	
<i>rigonii</i>	10034	18	35	30	29	38	44	41	32	39	27	23	30	33	29	32	42	23	23	50
<i>villosulcarpa</i>	14446	19	38	24	27	18	43	32	32	43	30	43	24	33	39	41	41	20	50	

frequent bands are Nos. 32, 35 (present in 16 samples) and 10, 55 (present in 15 samples).

A. hypogaea is characterized by the presence of bands 1, 6, 10, 42, 48, 60 and by the absence of bands 46, 62 and 79.

In *A. correntina* bands 28, 42, 48, 50, 58, 60, 62, 85 are typically absent.

Table 2 shows the similarity in seed protein patterns of different taxa which is expressed by the s.c. index of similarity — I (when I is higher than 50, then the compared protein patterns are similar and more related; when I is lower than 50, the patterns are less similar and also less related).

Thus we can see high similarity between different cultivars of *A. hypogaea* with the exception of No. 5. A similar situation exists for *A. correntina* where No. 9548 differs appreciably from other accessions of this taxon.

The protein pattern of *A. monticola* is according to this evaluation very similar to those of *A. hypogaea*, *A. batizocoi* and *A. cardenasii*. The latter is also very similar to those of two samples of *A. correntina*, *A. paraguariensis* closely resembles one sample of *A. hypogaea* while *A. rigonii* and *A. villosulicarpa* are very similar to each other.

The lowest index of similarity has been found for *A. macedoi*, where the highest value is 26. *A. villosa*, *A. × batizogaea* and an undescribed taxon *A. sp.* Col. No. HLK 410 which is nearest to *A. rigonii* and *A. cardenasii* (I = 41), are less similar to the other taxa.

According to those results *A. hypogaea* is most similar to *A. monticola* (I = 52, 55, 54) and then to *A. paraguariensis* (I = 51). Further removed stand *A. batizocoi* (I = 46), *A. × batizogaea* (I = 44), *A. villosulicarpa* (I = 43), *A. villosa* (I = 41), *A. correntina* (I = 40), *A. cardenasii* (I = 40).

The lowest similarity of *A. hypogaea* is to *A. macedoi* (I = 26), *A. sp.* Col. No. HLK 410 (I = 38) and to *A. rigonii* (I = 39).

DISCUSSION

Recently considerable attention has been given to the classification of the genus *Arachis* and considerable data obtained by different methods and techniques are at our disposal now for elucidating natural relationships within this taxon. There exist several views on classification of the genus *Arachis* differing slightly in the number of the sections and distribution of species into sections and series (GREGORY *et al.* 1973, KRAPOVICKAS 1973, GREGORY *et al.* 1980). RESSLAR (1980) published a review of the nomenclature of the genus *Arachis* according to the International Code of Botanical Nomenclature (STAFLEU 1978). Ressler follows Gregory, who proposes 7 sections with several series. We shall compare our conclusions on protein pattern relationships with these taxonomic schemes.

It can be said that *Arachis* species can be distinguished by their seed protein pattern although the extent of differentiation is not extreme; — this last statement is supported by the fact that crossability exists between the species within the section, although rarely some sterile hybrids can be obtained between the species of different sections (*e.g.* SMARTT 1979).

Protein pattern relationships agree quite well with the classification scheme of Krapovickas and Gregory (GREGORY *et al.* 1980). Some difficulties in the interpretation of the results do arise, nevertheless, The species with the most distinctive protein pattern is *A. macedoi*, a member of the section

Extranervosae; the pattern in this species differs as much from that of *A. villosulicarpa* (also in section *Extranervosae*) as it does from those of species in sections *Erectoides* and *Arachis*. It is quite apparent however that on morphological and hybridological grounds both *A. macedoi* and *A. villosulicarpa* are correctly placed in the same section. The problem we have to face in this connection is probably not dissimilar to that which confronted taxonomists on selecting morphological criteria for distinguishing species and in determining the most appropriate level of the taxonomic hierarchy at which to use them. To take as an example that of leaf form, lanceolate leaflets are found in species of three sections *Arachis*, *Erectoides* and *Rhizomatosae*. This character obviously has no diagnostic value in distinguishing sections but is of value in drawing distinctions between species within sections. Similarly one could argue that the value of protein character differences is the level of species within a section. It is possible that similar protein types and patterns have been conserved in different sections or perhaps even that convergent evolution has occurred. The example of the similarity between protein patterns in *A. hypogaea* and *A. paraguariensis* is a case in point.

The potential diagnostic value of protein patterns within a section can be illustrated by considering what some authorities consider as two botanical varieties of *A. villosa*, namely var. *villosa* and var. *correntina*. Protein patterns are sufficiently different to support the suggestion of KRAPOVICKAS (1973) and GREGORY (GREGORY *et al.* 1973) that they should be recognized as distinct species *A. villosa* and *A. correntina*, respectively.

A difficulty arises in considering *A. × batizogaea* in relation to its parental species, *A. hypogaea* and *A. batizocoi*. The protein pattern is more similar to some accessions of *A. hypogaea* than to anything else (*A. hypogaea* 38, I = 44, *A. hypogaea* 40, I = 42, *A. batizocoi*, I = 38). However it is not clear precisely how this form arose cytologically as it originated spontaneously as a tetraploid ($2n = 4x = 40$) *A. batizocoi* ($2n = 2x = 20$) which presumably was $2n = 3x = 30$.

It is also of interest to note that indexes of similarity between *A. monticola* and *A. hypogaea* accessions are very similar indeed to those between the various *A. hypogaea* accessions themselves. This supports the contention of GREGORY and GREGORY (1976) that *A. monticola* is close to the ancestry of *A. hypogaea* and they are in fact in the same biological species (SMARTT 1979). Following the recommendation of HARLAN and DE WET (1975) they should be assigned subspecific status in *A. hypogaea*. It is also of interest that both *A. cardenasii* and *A. batizocoi*, show an equally high index of similarity to *A. monticola* (I = 51) which supports the suggestion of SMARTT *et al.* (1978a) that these two species are the most likely sources at the genomes of *A. hypogaea* known at the present time.

The result of protein pattern analysis at an admittedly limited selection of *Arachis* species are not only consistent with the taxonomic scheme of Krapovickas and Gregory (GREGORY *et al.* 1980) but have shown remarkable parallels in the determination of the most appropriate and effective level of the taxonomic hierarchy for their use. In some ways morphology of *Arachis* is conservative in an evolutionary sense and it seems probable that some protein characters at least may also be so. It is clearly apparent that further study can be justified of both the range of protein variation within taxa and the number of taxa actually studied.

REFERENCES

- BASHA, S. M. M.: Identification of cultivar differences in seed polypeptide composition of peanuts (*Arachis hypogaea* L.) by two-dimensional polyacrylamide gel electrophoresis. — *Plant Physiol.* **63** : 301–306, 1979.
- CHERRY, J. P.: Comparative studies of seed proteins and enzymes of species and collections of *Arachis* by gel electrophoresis. — *Peanut Sci.* **2** : 57–65, 1975.
- DAUSSANT, J., NEUCERE, N. J., YATSU, L.: Immunochemical studies of *Arachis hypogaea* proteins with particular reference to the reserve proteins I. Characterization, distribution and properties of α -arachin and α -conarachin. — *Plant Physiol.* **44** : 471–479, 1969.
- DAVIS, D. J.: Disc-electrophoresis II. method and application to human serum proteins. — *Ann. N. Y. Acad. Sci.* **121** : 404–427, 1964.
- GREGORY, W. C., GREGORY, M. P.: Groundnuts: *Arachis hypogaea* — *Leguminosae* — *Papilionatae*. — In: SIMMONDS, N. W. (ed.): *Evolution of Crop Plants*. Pp. 151–154. Longman Group Ltd., London—New York 1976.
- GREGORY, W. C., GREGORY, M. P., KRAPOVICKAS, A., SMITH, B. A., YARBROUGH, J. A.: Structure and genetic resources of peanuts. — In: *Peanut Culture and Uses*. Pp. 47–133. Amer. Peanut Research and Education Association, Stillwater 1973.
- GREGORY, W. C., KRAPOVICKAS, A., GREGORY, M. P.: Structures, variation, evolution and classification in *Arachis*. — In: SUMMERFIELD, R. J., BUNTING, A. H. (ed.): *Advances in Legume Science*. Pp. 469–481. Royal Botanic Gardens, Kew 1980.
- HADAČOVÁ, V., TURKOVÁ, V., HADAČ, E., KLOZOVÁ, E.: Comparison of seed proteins of some representatives of the genus *Pisum* from the point of view of their relationship. Comparison by disc electrophoresis. — *Biol. Plant.* **22** : 7–16, 1980.
- HARLAN, J. R., DE WET, J. M. J.: On Ó Winge and a prayer: The origins of polyploidy. — *Bot. Rev.* **41** : 361–390, 1975.
- KLOZ, J., KLOZOVÁ, E.: The protein euphaseolin in *Phaseolinae* — a chemotaxonomical study. — *Biol. Plant.* **16** : 290–300, 1974.
- KRAPOVICKAS, A.: Evolution of the genus *Arachis*. — In: MOAV, R. (ed.): *Agricultural Genetics — Selected Topics*. Pp. 427–441. John Wiley & Sons, New York 1973.
- KRAPOVICKAS, A., RIGONI, A. A.: Estudios citológicos en el genero *Arachis*. — *Rev. Invest. Agr. (B. Aires)* **5** : 289–294, 1952.
- NEUCERE, N. J., CHERRY, J. P.: An immunochemical survey of proteins in species of *Arachis*. — *Peanut Sci.* **2** : 66–72, 1975.
- NOVACKÝ, K., MACKO, J., HÄSSLER, J.: [Remarks on disc electrophoresis of plant proteins on acrylamide gel.] In Slovak. — *Biológia (Bratislava)* **21** : 64–68, 1966.
- RESSLAR, P. M.: A review of the nomenclature of the genus *Arachis*. — *Euphytica* **29** : 813–817, 1980.
- RESSLAR, P. M., GREGORY, W. C.: A cytological study of three diploid species of the genus *Arachis* L. — *J. Hered.* **70** : 13–16, 1979.
- RESSLAR, P. M., STUCKY, J. M., MIKSCH, J. P.: Cytophotometric determination of the amount of DNA in *Arachis* L. Sect. *Arachis* (*Leguminosae*). — *Amer. J. Bot.* **68** : 149–153, 1981.
- SMARTT, J.: Interspecific hybridization in the grain legumes — A review. — *Econ. Bot.* **33** : 329 to 337, 1979.
- SMARTT, J., GREGORY, W. C., GREGORY, M. P.: The genomes of *Arachis hypogaea*. 1. Cytogenetic studies of putative genome donors. — *Euphytica* **27** : 665–675, 1978a.
- SMARTT, J., GREGORY, W. C., GREGORY, M. P.: The genomes of *Arachis hypogaea*. 2. The implications in interspecific breeding. — *Euphytica* **27** : 677–680, 1978b.
- STAPLEU, F. A. (ed.): *International Code of Botanical Nomenclature*. Adopted by the 12th International Botanical Congress, Leningrad, July 1975. — Bohn. Scheltema and Holkema, Utrecht 1978.