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Variation studies in a wild groundnut species, *Arachis stenosperma* Krapov. & W.C. Gregory nov. sp.

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Abstract

Wild relatives of crop species are known to be sources of genetic diversity that can be used in crop improvement. However, they have not always been studied adequately for the variation that may exist within them, for traits which may have important implications from an evolutionary point of view and their use in breeding programmes. In the present study, a wild groundnut species, *Arachis stenosperma*, has been studied for variation between accessions collected from different sites in Brazil for morphological and certain nutritional traits, and for disease resistance. Multivariate analysis of 23 characters grouped 18 accessions into two clusters, while one accession, ICG 14927, was distinct from these. However, in protein profile they all appear identical. Hence, the variation appears to have arisen in response to the climatic conditions of their habitat, which has implications for use of these accessions in breeding programmes. The variation in these traits could not be associated with any phytogeographical regions. The dispersal of this species from its centre of origin and diversity to other parts of Brazil appears to be recent and without any identifiable selection pressures having operated.

Keywords: *Arachis stenosperma*; groundnut rust; principal coordinate analysis; protein; protein profile; variation

Introduction

Wild *Arachis* L. species have been found to be a reservoir of genetic diversity, particularly in relation to biotic and abiotic stresses which cause severe yield losses in groundnut production world-wide. This has generated great interest in collection, characterization and conservation of wild *Arachis* species. Evaluation of reaction to biotic stresses in some wild species has shown variation between accessions of the same species (Singh *et al.*, 1996), however, there are very few studies exploring variation between accessions, which could be important from an evolutionary point of view and have implications in their use in breeding programmes.

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In addition to *A. villosulicarpa* and *A. hypogaea*, the cultivated groundnut, *A. stenosperma* is a species reported to be cultivated for its kernels. Also, it has been reported to be resistant to the groundnut foliar diseases, groundnut rust (Subrahmanyam *et al.*, 1983) and both early and late leaf spots (Kolawale, 1976). It belongs to section *Arachis*, which contains a number of diploid wild *Arachis* species, besides the cultivated tetraploid species *A. hypogaea* and its wild form *A. monticola*. It is cross-compatible with the cultivated groundnut, despite differences in ploidy level, and therefore has been used by several groundnut workers for the introduction of resistance to leaf diseases (Smartt and Gregory, 1967; Moss, 1980; Gardner and Stalker, 1983; Singh, 1985, 1986a, b). Initially, *A. stenosperma* was collected from south coastal regions of Parana, São Paulo and the Rio de Janeiro province of Brazil. For a long

time it was believed that *A. stenoperma* had originated in the coastal region of Brazil in phytogeographical isolation. Plant explorers in the mid-1990s found it growing wild and in a semi-cultivated state in the central plateau region of Brazil in the Mato Grosso and Goias provinces (Fig. 1). Accessions with yellow or orange flowers were collected. It has been found cultivated in Mato Grosso do Sul. Southern Mato Grosso and Mato Grasso do Sul are considered to be the centre of the origin of the genus *Arachis*, from whence the genus spread to other parts of South America. Therefore, the presence of *A. stenosperma* in the Mato Grosso and Goias indicates a possible origin of this species in the central plateau of Brazil.

Materials and methods

The accessions used in the present study are listed in Table 1. Material was grown in a glasshouse at the ICRISAT farm, Patancheru, India. Plants were raised in 12-in. plastic pots in a 2:5:2 soil, sand and manure mixture. Pots with three plants of each accession were used and arranged in a randomized block design with three replications. Observations on both qualitative and quantitative traits were recorded in each replication using the preliminary descriptors produced for *Arachis* by the International Board for Plant Genetic Resources (IBPGR) and ICRISAT (IBPGR, 1990). To evaluate groundnut rust reaction of each accession, five leaves from each replicate were inoculated with a suspension of rust urediniospores and scored for reaction using the method described by Subrahmanyam *et al.* (1983).

Biochemical analysis

For analysis of total protein and oil content, and development of electrophoretic protein profiles, equal weights of seeds were harvested from each replicate. For measurement of oil and protein contents, equal weights of seed were placed in 50-ml kaimax glass culture tubes. Oil was extracted three times successively using 10 ml of hexane: diethyl ether (60:40) mixture as solvent. The contents were homogenized for 45 min. The supernatant was collected in a pre-weighed beaker after centrifugation for 15 min at 3000 × *g*. The contents were dried and weighed and oil content calculated. The remaining defatted meal was oven-dried at 55°C for 3 h and ground into a fine powder, which was used to determine nitrogen content in an auto-analyser. A factor of 5.46 was used for converting nitrogen into crude protein (Singh and Jambunathan, 1980). The protein profile of all accessions was obtained using the sodium dodecyl sulphate–polyacrylamide gel electrophoretic technique (SDS-PAGE) reported by Singh *et al.* (1991).

Statistical analysis

All quantitative traits, including protein and oil content, and rust reaction were analysed statistically using a randomized block design. By calculating the Eigen vector, principal coordinate analysis was carried out on 22 morphological features plus total protein. All characters showed significant variation, including protein and oil contents and rust reaction, but only morphological and

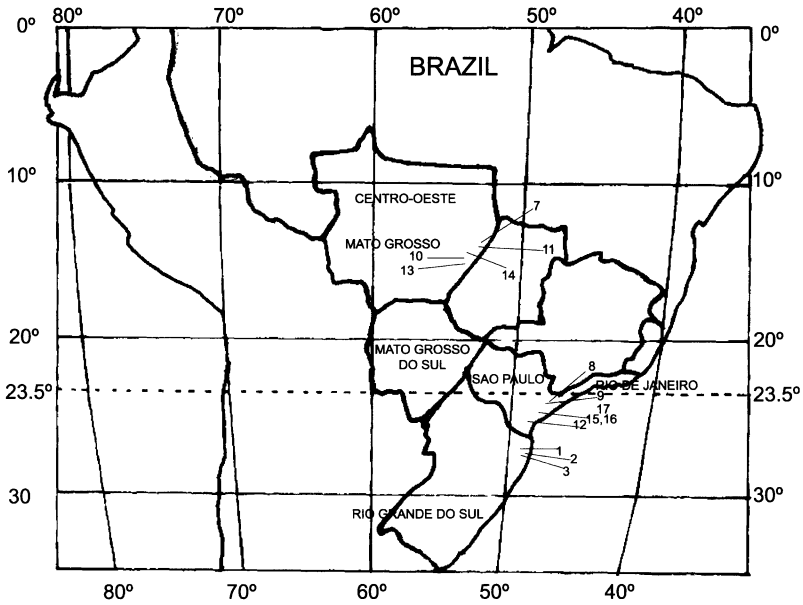

Fig. 1. Distribution of *Arachis stenosperma*.

Table 1. List of accessions of *Arachis stenosperma* used in the present study

Serial No.	Accession identity	Collector No.	Province	Latitude	Longitude	Altitude (m)
1	ICG 8125	HLK 408	Parana	25°24′S	48°44′W	3
2	ICG 8126	HLK 410	Parana	25°31′S	48°31′W	3
3	ICG 13171	V 7377	Parana	25°26′S	48°33′W	3
4	ICG 13233	CIAT 9960	–	–	–	–
5	ICG 13252	V 7762	Mato Grosso	–	–	–
6	ICG 13210	A 2796	Mato Grosso	–	–	–
7	ICG 14891	Jt 2	Mato Grosso	15°32′S	52°10′W	340
8	ICG 13188	V 7382-7	São Paulo	23°46′S	45°24′W	15
9	ICG 13173	7348	São Paulo	23°45′S	43°24′W	15
10	ICG 15157	V 9017	Mato Grosso	15°43′S	55°42′W	170
11	ICG 14927	V 12646	Mato Grosso	15°32′S	52°13′W	250
12	ICG 15160	V 10229	São Paulo	25°01′S	47°55′W	10
13	ICG 14868	V 10309	Mato Grosso	16°28′S	54°39′W	215
14	ICG 14874	12575	Mato Grosso	15°41′S	52°46′W	360
15	ICG 14884	V 13260	São Paulo	24°19′S	46°59′W	5
16	ICG 14885	V 13262	São Paulo	24°16′S	46°56′W	3
17	ICG 14882	V 13256	São Paulo	24°36′S	45°24′W	10
18		Ve 66	São Paulo	–	–	–
19	ICG 14873	V 12491	–	–	–	–

protein data were analysed using NTSYS software and distance coefficients were calculated (Rohlf, 1992). A range of hierarchical clustering techniques, namely single linkage (SLINK), complete linkage (CLINK), the unweighted pair group method of arithmetical means (UPGMA) and Ward's minimum variance were used to classify accessions. Clustering of 19 accessions revealed by UPGMA was the most effective and produced three distinct groups. The dendrogram constructed was based on the distance coefficient produced by UPGMA. Principal coordinate analysis was performed to substantiate the grouping pattern based on UPGMA using the distance coefficient. Furthermore, principal coordinate analysis was used to assess the relative importance of various traits contributing to the variance. To assess the extent of variability within accessions, variance was calculated for individual accessions and then pooled over all accessions for a trait.

Results

Table 2 presents results from simple statistical analysis of variability data from 19 accessions relating to mean values, coefficient of variation, and variance between and within accessions for 25 traits. Analysis of variance showed significant variation for all 25 characters, indicating statistically significant differences between accessions at $P < 0.01$.

The dendrogram generated by UPGMA was based on a distance coefficient of 22 quantitative characters including protein content but excluding oil content and rust reaction of 19 accessions. This grouped them into two main clusters with accession ICG 14927 from Mato Grosso being completely distinct from the rest of the accessions (Fig. 2). The remaining accessions expressed rather less dissimilarity and clustered into two major groups. Dissimilarity within the group was narrow. Group one contains ICG 8125, 8126, 13171, 13210, 14891, 15157, 14882, 13188 and 13252, and group two contains ICG 13233, 14884, 14873, 15160, 14885, 14868, 14874 and Ve 66. Both groups have representation from nearly all regions of distribution of *A. stenosperma*. Only the three collections from Parana came together in the same group, with ICG 8126 and 13171 closer to each other than to ICG 8125. Among the remaining accessions of group one the majority (four) were from the Mato Grosso region of central Brazil, while the second group consists of accessions mostly from the east coast of Brazil. The above groupings are supported by principal coordinate analysis, which assessed the relative contribution of various traits to total variation and also served to validate the groupings (Fig. 3). The first three roots of the principal coordinate analysis accounted for 60% of variation. In the first root are leaf length and the width of leaves on laterals and standard petal length; in the second root are stipule length, pod length and seed length, while in the third root the number of secondary branches and pod widths contributed most substantially to the variation recorded.

ICG 14873 from the São Paulo region and ICG 14868 from Mato Grosso scored 1 against groundnut rust on a nine-point scale, indicating the highest degree of resistance. ICG 8125, 8126, 13210, 13188, 13173 from coastal areas scored 2–4, indicating some resistance to rust, while ICG 13233, 13252, 14891 and 14927 mostly from

Table 2. Variability parameters for various characteristics in 19 accessions of *Arachis stenosperma*

Variable No.	Character	Mean (± SE)	CV (%)	Source of variance: Between accessions (18)[a]	Source of variance: Within accessions (51)[a]
1	No. of primary branches	4.03 ± 0.09	10.09	0.774**	0.166
2	No. of secondary branches	8.21 ± 0.43	9.14	31.23**	0.563
3	Height of main stem (cm)	14.12 ± 0.21	6.78	172.64**	0.918
4	Thickness of main stem (mm)	4.92 ± 0.19	8.58	6.04**	0.179
5	Stipule length (mm)	17.11 ± 0.84	6.75	124.50**	1.335
6	Length of adnation of stipule (mm)	6.50 ± 0.21	8.53	7.54**	0.308
7	Petiole length (cm)	5.64 ± 0.22	5.94	8.46**	0.113
8	Leaflet colour (1–3 scale)	1.64 ± 0.08	8.03	1.35**	0.018
9	Leaflet length main stem (mm)	30.19 ± 0.46	3.43	36.036**	1.072
10	Leaflet width on main thorn (mm)	12.02 ± 0.26	4.73	11.136**	0.324
11	Leaflet length on laterals (mm)	26.77 ± 0.79	2.72	111.22**	0.532
12	Leaflet width on laterals (mm)	12.55 ± 0.38	5.09	24.87**	0.408
13	Hypanthium length (mm)	36.87 ± 0.46	3.65	33.60**	1.808
14	Standard petal length (mm)	12.31 ± 0.12	4.69	1.842**	0.334
15	Standard petal width (mm)	17.21 ± 0.23	3.15	9.32**	0.294
16	Peg length (cm)	17.02 ± 0.50	7.72	41.12**	0.72
17	Pod length (mm)	16.57 ± 0.20	2.17	7.41**	0.12
18	Pod width (mm)	5.95 ± 0.05	3.50	0.35**	0.044
19	Seed length (mm)	13.14 ± 0.14	2.05	3.79**	0.072
20	Seed width (mm)	4.80 ± 0.06	4.32	0.67**	0.043
21	25 seed mats (g)	4.53 ± 0.07	2.03	0.96**	0.008
22	Pod yield (number per plant)	34.59 ± 2.36	0.77	994.94**	0.070
23	Protein (%) dry seed mass	58.58 ± 0.78	1.92	104.98**	1.261
24	Oil (%)	45–54.1[b]			
25	Reaction to rust (1–9 scale)	1–7[c]			

[a] In parentheses are degrees of freedom for between and within accession variances.
[b] Range of nuclear magnetic resonance oil percentage at 5% moisture level among accessions investigated.
[c] Range of reaction to rust on 1–9-point scale after Subrahmanyam *et al.* (1983): 1 highly resistant and 9 highly susceptible.
** Significant at probability $P < 0.01$.

Mato Grosso region scored 5–6, indicating a moderate level of resistance. No accession was found to be fully susceptible. This indicates variability between accessions in their reaction to groundnut rust without any geographical association of accessions with levels of resistance.

The protein content of accessions on a defatted dry seed weight basis ranged from 43% in the case of ICG 8125 to 65.2% in ICG 13233. Based on the distance coefficient of protein data, the 19 accessions fell into four major groups. The accessions ICG 8125 and 13173 collected from the coastal region contained the lowest quantity of protein (42.7 and 46.9%) followed by accessions ICG 8126, Ve 66 and ICG 14885 (53.7–54.8%) and accessions ICG 13171, 14927, 14882, 13210 and 15157 (58.7–59.8%). The accessions ICG 13233, 13252, 14873, 13188, 14891, 15160, 14868, 14874 and 14884 expressed the highest protein contents ranging from 60.86 to 65.2%. The last group consists of accessions with high seed protein content from both central and coastal regions of Brazil. Based on oil content, which could not be estimated for all accessions due to lack of sufficient seed, the accessions could be grouped into three broad groups. The first group with oil contents from 40 to 45% contained accessions ICG 13188 and 13233, the second group with oil contents between 45 and 50% contained accessions ICG 14868, 13173, 14891, 13171, 8126 and 8125, while the third group with oil contents of more than 50% contained ICG 14874, 15160, 14927, 15157 and 13210 (Table 3). Plotting of total defatted seed protein contents against oil contents of the various accessions showed that ICG 14873, 15157 and 13210 are quite distinct. They have higher defatted protein contents of 29.2, 28.3 and 27.8% combined with high oil contents of 54.1, 53.9 and 52.9%, respectively (Fig. 4).

The protein profile of 19 accessions resolved 10 major bands without any variation between accessions. The bands can be divided into three groups representing three units of groundnut protein, compared to the reference protein profile of *A. hypogaea* (Krishna *et al.*, 1986).

Discussion

The genus *Arachis* has been divided into nine sections with 69 species on the basis of morphological similarities (Krapovickas and Gregory, 1994). Stalker (1990)

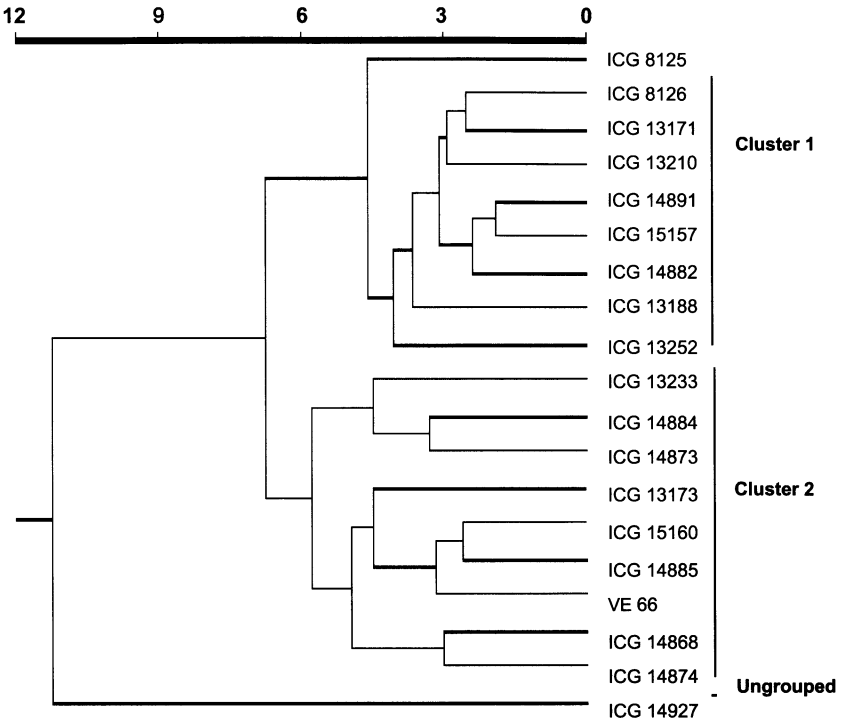

Fig. 2. Dendrogram generated by UPGMA based on distance coefficients of morphological and protein data for 19 accessions of groundnut.

performed principal component analysis on a large number of accessions of *Arachis* species with a selected set of quantitative traits which produced clustering of accessions within species, indicating at the same time both distinctness of individual accessions and their degree of relatedness. Singh *et al.* (1996) carried out principal component analysis on 42 accessions of *Arachis duranensis*, which grouped accessions originating from different regions of a narrow strip of land in the western part of the Chaco region extending from Villamontes, southern Bolivia to El Tunal in northern Argentina into six clusters but without any specific association with phytogeographical regions of the distribution. In the present study most of the characters studied contribute to variation, though the degree of dissimilarity between accessions is narrow, as reflected in the dendrogram generated by UPGMA, except for ICG 14927 which has a greater degree of dissimilarity (Fig. 2). This is one of the accessions from the Mato Grosso location (Araguaina) which has orange flowers, the highest pod yield, higher oil and protein contents, and moderate rust resistance (unlike other accessions), probably because of its semi-cultivated status. In the remaining accessions the distribution of variation is nearly uniform as reflected in significance levels of differences. Cluster analysis produced three groupings, but without any association of a cluster to any specific phytogeographical region, cf. *A. duranensis* (Singh *et al.*, 1996). A similar level of

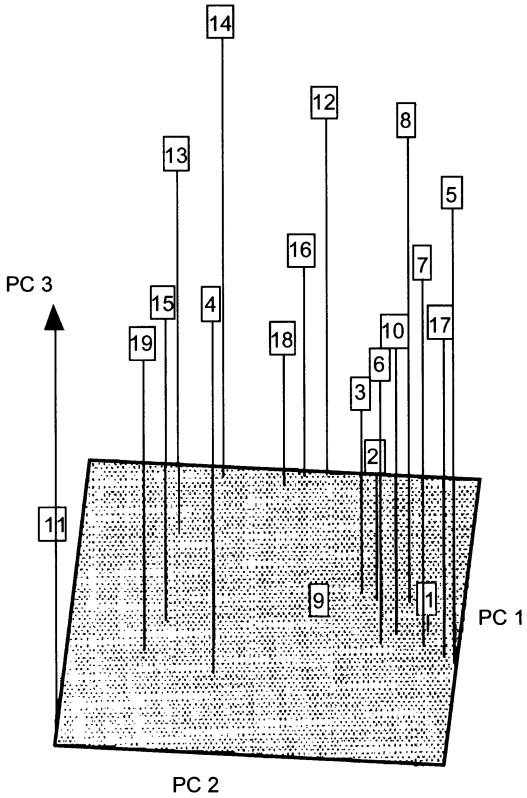

Fig. 3. Principal coordinate analysis for *Arachis stenosperma* based upon morphological characters and protein contents in the whole seed of all 19 accessions.

Table 3. Information on value traits studied on each accession of *Arachis stenosperma*

Serial No.	Accession identity	Protein (%)		Oil (%)[c]	Reaction to rust[d]
		In total seed[a]	In defatted meal[b]		
1	ICG 8125	42.76	21.4	49.8	2.1 ± 0.1
2	ICG 8126	53.73	28.3	47.4	2.1 ± 0.1
3	ICG 13171	58.70	29.9	49.0	–
4	ICG 13233	65.23	35.9	45.0	7.3 ± 0.0
5	ICG 13252	63.66	–	–	6.1 ± 0.1
6	ICG 13210	58.96	27.8	52.9	2.3 ± 0.0
7	ICG 14891	62.50	31.3	49.5	5.5 ± 0.2
8	ICG 13188	64.00	35.6	44.4	2.4 ± 0.1
9	ICG 13173	46.90	23.9	49.1	2.6 ± 0.1
10	ICG 15157	59.83	27.5	53.9	–
11	ICG 14927	58.73	29.2	50.2	5.9 ± 0.3
12	ICG 15160	62.86	31.2	50.3	–
13	ICG 14868	60.86	32.5	46.6	1.0 ± 0.0
14	ICG 14874	61.43	30.7	50.0	–
15	ICG 14884	61.80	–	–	–
16	ICG 14885	54.80	–	–	–
17	ICG 14882	58.70	28.5	51.4	–
18	Ve 66	54.00	–	–	–
19	ICG 14873	63.66	29.2	54.1	1.0 ± 0.0

[a] Calculated at N × 5.46 factor.
[b] Calculated from total.
[c] Range of nuclear magnetic resonance oil percentage at 5% moisture level among accessions investigated.
[d] Range of reaction to rust on 1–9-point scale after Subrahmanyam *et al.* (1983): 1 highly resistant and 9 highly susceptible.

variation between accessions collected from distant places, i.e. Parana and São Paulo in the eastern coastal region and Mato Grosso in central Brazil, without any association of their distinctive features with phytogeographical regions, suggests that the spread of various accessions of this species from the Mato Grosso region, the centre of origin and diversity of genus *Arachis* (Singh and Simpson, 1994) to the coastal regions is very recent. Such a short time-scale may have been insufficient to generate significant levels of genetic drift and shifts for specific traits in specific areas to establish apparent phytogeographical association, also introduced accessions probably were not selected for specific traits before they were introduced to new areas and underwent similar selection pressures in their new environments.

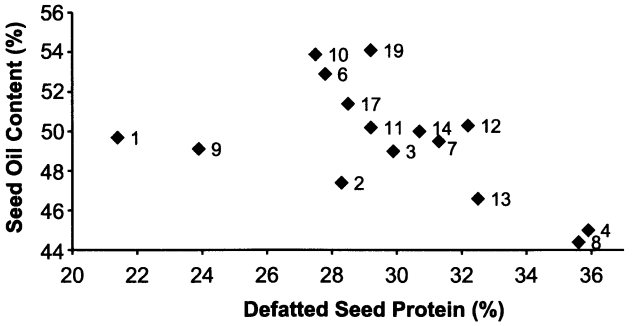

Fig. 4. Relationship between 15 accessions of *Arachis stenosperma* based on seed oil and defatted protein content of the whole seed. The number against plotted values refers to an accession's serial number as listed in Table 1.

Most accessions showed resistance to groundnut rust with a significant level of variation in their reaction. This ranged from a high degree of resistance with virtually no disease development to hypersensitivity in varying degrees, with production of only small necrotic lesions but no subsequent development of disease (Subrahmanyam *et al.*, 1983). Accessions showing this variation are from both the Mato Grosso region of central Brazil and the Parana and east coast regions of São Paulo. At the beginning of this study, following Krapivickas and Gregory (1994), it was presumed that accessions from the Mato Grosso, the primary centre of origin and diversity of the genus *Arachis*, would have a higher degree of resistance than accessions collected from the coastal region; because indigenous people would have introduced and selected more nutritious and high-yielding types in coastal regions without consideration of disease resistance. Accessions ICG 13252, 14891 and 19927, all from the Mato Grosso, showed different resistance levels indicating no geographical association. Nevertheless, overall significant variations would have implications in rust resistance breeding programmes and would require selection, as parents, of those accessions with higher and more stable resistance.

The difference in reaction may be due to quantitative differences in the accumulation of certain polyphenols in cells that restrict fungal growth after initial infection thereby producing varying levels of resistance.

Based on the above hypothesis regarding the spread of species through the actions of local people, materials collected from the coastal region might be expected to have higher protein contents. However, analysis showed that there was no such association and that different levels of protein and oil contents occur in accessions collected from different regions. In fact, most accessions from the coastal region are comparatively inferior in protein contents (Table 3), though high protein content (around 63%) was recorded in accessions from both the Mato Grosso and the coastal region, hence suggesting that the spread of this species from central Brazil to the coastal regions had taken place without application of any selection pressure for these characters. With regard to oil content, most accessions collected from the Mato Grosso have a comparatively higher oil content than those collected from coastal regions. Furthermore, the analysis showed that some accessions, ICG 14873, 15157 and 13210 (Fig. 4), have high oil and moderate protein contents, the common negative correlation between the two traits does not apply in this group of accessions. It is important from a nutritional breeding point of view to develop genotypes with well-balanced or both high oil and protein levels (Table 3).

Therefore, observations made in the present investigation with regard to variation between accessions of *A. stenosperma* are similar to those of Stalker (1990) in wild species of section *Arachis*, and among accessions of *Arachis duranensis* by Singh *et al.* (1996), where principal components analysis was able to establish more definite relationships between the accessions of different species and between those of the same species. They have observed that morphologically similar accessions need not necessarily originate from the same locality and that the accessions which originate in different localities or geographical regions may have very low levels of morphological and genetic differences. In the present study, variation observed between the accessions of *A. stenosperma* is limited even after a relatively wide dispersion, due to its recent introduction and insufficient time to result in significant and visible levels of genetic drift or shift, and probably no effective selection pressure was experienced by this species for any trait during its introduction. Although, the range of distribution compared to the cultivated groundnut (which is distributed widely in the tropics, sub-tropics and warm temperate regions extending between 40° North and 40° South) is negligible, *A. stenosperma* expresses appreciable variation in rust reaction between accessions occurring within a range of a few thousand kilometres; whereas in the cultivated groundnut variation for rust resistance is very limited and mostly confined to accessions originating from a very restricted region (Peru) compared to its world-wide distribution.

At the biochemical level, all accessions exhibited identical protein profiles, indicating that they belong to a somewhat conservative species with little significant genetic variation. This observation is consistent with recent dispersal of this species from central to coastal regions of Brazil. Levels of variation recorded for morphological characteristics between accessions may be due to differential environmental selection pressures acting on micromutation, which are also apparent in the variation for rust reaction, and total oil and protein contents. Environmental variation, particularly in soil conditions, might favour variation in production of some chemical components, which in turn may produce variation in morphological features, such as leaf size, colour and overall growth, and in reaction to diseases and the chemical composition of seeds. This is quite clear in the case of the cultivated groundnut, *A. hypogaea*, which dispersed from its centre of origin in southern Bolivia/north-west Argentina to different parts of South America. Two sub-species have evolved, *A. hypogaea hypogaea* and *A. hypogaea fastigiata*, and six botanical varieties which are distinct in their morphological, genetical and physiological characteristics. This presumably has occurred during the process of adaptation to different agro-climatic conditions. In the light of these observations, from the present study we can conclude that all accessions of *A. stenosperma* studied in the present investigation are genetically rather similar. The differences observed in morphological and biochemical features may be primarily due to their adaptation to environmental conditions. Variation between accessions of this species does exist for some of the important features, such as reaction to groundnut rust and total oil and protein contents. This is of significance and has implications in use of this species in breeding programmes by selection of appropriate parents for incorporation of desirable traits in the cultigen.

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