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Key words: Arachis duranensis, groundnut rust, principal component analysis, protein profile, variability

Abstract

Forty-two accessions of *Arachis duranensis*, a wild groundnut species that has been reported as a source of resistance to several groundnut diseases, were studied for 30 quantitative traits including total protein content, oil content, and reaction to groundnut rust. Protein profiles were also investigated for variation at the molecular level. Principal component analysis was applied to 28 traits that showed significant variation. Of these, only five characters, namely, height of the main stem, length of apical leaflet on the main stem, length of isthmus between pods, width of seed, and reaction to groundnut rust, accounted for more than 61.4% of the total variation. Protein profiles of these accessions were broadly similar, except some accessions which differed in few bands. The importance of these variations in strategies for germplasm collection and breeding is discussed.

Introduction

Arachis duranensis Krapov. & W.C. Gregory (1994), a wild relative of the cultivated groundnut, is native to South America and is mainly found in a narrow strip in the western part of the "Chaco region", extending from Villamontes in southern Bolivia to El Tunal (near Joaguain Gonzalez) in northern Argentina (Salta prov.). This zone has a more or less uniform environment with little variation in latitutde, longitude, and altitude. Accessions of this species have shown resistance to groundnut rust caused by *Puccinia arachidis* (Subrahmanyam et al., 1983), and can be used in resistance breeding programs. However, there have been conflicting reports of their reaction to *Aspergillus flavus* (Ghewande et al., 1989, Mehan et al., 1992). Stalker (1990) used numerical taxonomic techniques to evaluate the structure and variation in species in the section *Arachis*, that contains the tetraploid cultivated groundnut, *A. hypogaea* L., and several diploid species including *A. duranensis*. He did not study intraspecific variation, however he found that most variation between species was confined to leaflet size, followed by branching habit and flower size and that most accessions belonging to a known species clustered together.

In the present study, an attempt has been made to investigate possible variation among *A. duranensis* accessions for morphological and biochemical traits, and for reaction to groundnut rust. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) gene bank has 42 accessions of *A. duranensis* (Table 1). Many of them have the same collection number but they have been maintained as separate entries because of variation observed during collection or maintenance and are considered as separate accessions (Simpson & Higgins, 1984). Possible intraspecific variation within these accessions was studied for 30 traits. Principal component and cluster analyses were performed on traits that showed significant variation. Variation at molecular level was also assessed through protein profiles. Though the habitat of these accessions is not environmentally very distinct, but an attempt has been made to assess the possible relationship between variation for the selected traits and the specific location

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of their origin. This could be useful to plant collectors in maximizing genetic diversity with minimum sample number and to plant breeders in identifying appropriate accessions for breeding programs.

Materials and methods

Five seeds of each of the 42 accessions of *A. duranensis* (Table 1) were germinated in 9 cm Petri dishes kept in a germination chamber maintained at 20 °C and 80% relative humidity. One-week-old seedlings were transplanted into three pots filled with a mixture of sterilized sand, soil, and manure (2:2:1). The pots were arranged in a randomized block design in three replications. Morphological observations were recorded on 60–70-day old plants in each replication using the preliminary descriptors for *Arachis* produced by the International Board for Plant Genetic Resources (IBPGR) and ICRISAT (IBPGR, 1990). To evaluate the reaction of each accession against groundnut rust, five leaves from each replicate were inoculated with a suspension of rust unredeniospores and scored for reaction as described by Subrahmanyam et al. (1983).

Biochemical analysis

For total oil content, protein content, and electrophoretic protein profile, equal weight of seeds harvested from each of the replicates were used. The protein profiles were resolved using the SDS-polyacrylamide gel electrophoresis technique described by Singh et al. (1991). For measuring the oil and protein contents, equal weight of seed was taken into a 50 ml Kimax 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 in a kinematic homogenizer for 45 min at a setting of five. The supernatant was collected in a pre-weighed beaker after centrifugation for 15 min at 3000 × g. The contents were dried and weighed to calculate the oil percentage. The remaining defatted meal was oven-dried at 55 °C for 3 h and ground ‘ɔ a fine powder using a mortar and pestle. This defatted meal was used to determine nitrogen in an autoanalyzer. A factor of 5.46 was used for converting nitrogen into crude protein (Singh & Jambunathan, 1980).

Multivariate analysis

Initially, 30 quantitative traits including reaction to groundnut rust were analyzed for significance of variation. Twenty-eight of these showed significant variation and were analyzed using principal component analysis (Table 2). Based on this analysis the accessions were clustered by using principal component analysis and Ward's method (Wishart, 1978).

Results

Morphology

Mean values, coefficients of variation, and variances between and within the accessions for each character are presented in Table 2. Principal component analysis showed significant variation for 24 characters at $p = 0.01$. Of these, 5 characters [height of the main stem (21.5%), apical leaflet length on main stem (7.1%), length of isthmus between pods (13.2%), seed width (4.6%), and reaction to rust (15.0%)] contributed to more than 61.4% of the variation (Table 3).

Cluster analysis following Ward's method grouped the accessions into six main groups. These groups are represented in a scatter diagram (Fig. 1) obtained from the principal component analysis. They were similar to the grouping based on principal component analysis. Although cluster analyses grouped accessions with greater morphological similarity together, the clusters did not necessarily include all the accessions from the same site or nearby sites. For example, cluster nos. III and V, which have only three accessions each, had two accessions each from either the same location or from nearby location and the third from a little distant location. Whereas such accessions as 13242, 13189, 13190, 13191, 13192, 13193, and 13194, though originating from the same location, fell into different clusters. Similarly, the variation in reaction to groundnut rust, which contributed significantly to variation between accessions, was randomly distributed among accessions originating from both Bolivia and Argentina. Moreover, the degree of variation in reaction to rust was qualitatively low because most accessions expressed either an immune or a hypersensitive reaction. No accession was susceptible.

Table 1. Details of *Arachis duranensis* accessions studied

Sl No	ICRISAT[1] identity	Collector no.[2]		Country[3] Origin	Province S	Latitude W	Longitude (m)	Altitude
1	8123	K 7988		ARG	SAN MARTIN	22° 19	63° 43	500
2	8195	GKBSPSc	30060	ARG	JUJUY	24° 22	65° 27	940
3	8196	GKBSPSc	30061	ARG	JUJUY	24° 16	65° 12	1050
4	8199	GKBSPSc	30064	ARG	JUJUY	24° 23	65° 07	940
5	8200	GKBSPSc	30067	ARG	SALTA	23° 03	63° 56	380
6	8201	GKBSPSc	30069	BOL	TARIJA	21° 48	63° 33	575
7	8202	GKBSPSc	30070	BOL	TARIJA	21° 53	63° 38	600
8	8204	GKBSPSc	30073	BOL	TARIJA	21° 44	63° 33	625
9	8205	GKBSPSc	30075	BOL	TARIJA	21° 18	63° 27	450
10	8957	GKBSPSc	30074	BOL	TARIJA	21° 26	63° 27	425
11	11550	GKBSPSc	30068	ARG	SALTA	22° 51	63° 56	350
12	11552	KSBScC	36002-1	ARG	SALTA	UN	UN	600
13	11553	KSBScC	36002-2	ARG	SALTA	UN	UN	600
14	11554	KSBScC	36003-1	ARG	SALTA	UN	UN	600
15	13236	GKBSPSc	30061 A	ARG	JUJUY	24° 16	65° 12	1050
16	12162	GKBSPSc	30067 D	ARG	SALTA	23° 03	63° 56	380
17	13248	KSBScC	36002	ARG	SALTA	UN	UN	600
18	13249	KSBScC	36003	ARG	SALTA	UN	UN	600
19	13250	KSBScC	36004 OR	ARG	JUJUY	24° 16	65° 12	1100
20	13216	KSBScC	36004 Y	ARG	JUJUY	24° 16	65° 12	1100
21	13218	KSBScC	36006	ARG	SALTA	UN	UN	UN
22	13161	KSBScC	36006-2	ARG	SALTA	UN	UN	UN
23	13242	KSSc	38900	ARG	SALTA	24° 41	64° 16	450
24	13189	KSSc	38900-1	ARG	SALTA	24° 41	64° 16	450
25	13190	KSSc	38900-2	ARG	SALTA	24° 41	64° 16	450
26	13191	KSSc	38900-3	ARG	SALTA	24° 41	64° 16	450
27	13192	KSSc	38900-4	ARG	SALTA	24° 41	64° 16	450
28	13193	KSSc	38900-5	ARG	SALTA	24° 41	64° 16	450
29	13194	KSSc	38900-6	ARG	SALTA	24° 41	64° 16	450
30	13243	KSSc	38902	BOL	TARIJA	21° 19	63° 27	450
31	13196	KSSc	38902-1	BOL	TARIJA	21° 19	63° 27	450
32	13197	KSSc	38902-3	BOL	TARIJA	21° 19	63° 27	450
33	13198	KSSc	38902-4	BOL	TARIJA	21° 19	63° 27	450
34	13173	KSSc	38903	ARG	SALTA	22° 12	63° 41	500
35	13199	KSSc	38903-1	ARG	SALTA	22° 12	63° 41	500
36	13200	KSSc	38903-2	ARG	SALTA	22° 12	63° 41	500
37	13201	KSSc	38903-3	ARG	SALTA	22° 12	63° 41	500
38	13202	KSSc	38903-4	ARG	SALTA	22° 12	63° 41	500
39	13184	KSSc	38904	ARG	SALTA	23° 15	63° 23	250
40	13203	KSSc	38904-2	ARG	SALTA	23° 15	63° 23	250
41	13204	KSSc	38904-3	ARG	SALTA	23° 15	63° 23	250
42	13205	KSSc	38904-4	ARG	SALTA	23° 15	63° 23	250

[1]ICRISAT identity = ICG = ICRISAT Groundnut Accession Number
[2]Source: Catalog of Germplasm collection in South America, 1976–83 (Simpson & Higgins, 1984)
[3]Country of origin: ARG = Argentina, BOL = Bolivia
[4]UN = Unknown

Table 2. Statistics and variance of 28 characters in 42 accessions of *Arachis duranensis* (n = 20), characters measured in mm

Variable No.	Character	Mean (mm) S.E.	CV%	Source of Variance	
				Between accession (42)[1]	Within accession (126)[1]
1	Days to flowering	32.71 ± 3.84	11.8	109.63**	14.80
2	Height of main stem	386.10 ± 100.81	26.1	392607.00**	10163.00
3	Thickness of stem	5.24 ± 0.94	17.4	1.55	0.83
4	No. of laterals	6.87 ± 2.63	38.4	20.05**	6.39
5	Hairiness	6.38 ± 0.87	13.7	4.96**	0.76
6	Apical leaflet length (main axis)	33.52 ± 3.22	9.6	146.25**	10.40
7	Apical leaflet width (Main axis)	18.19 ± 2.06	11.3	27.64**	4.26
8	Basal leaflet length (Main axis)	28.92 ± 3.07	10.6	99.88**	9.42
9	Basal leaflet width (Main axis)	15.08 ± 1.75	11.6	16.17**	3.07
10	Apical leaflet length (Lateral)	19.41 ± 1.90	9.8	38.53**	3.62
11	Apical leaflet width (Lateral)	13.40 ± 1.66	12.5	18.44**	2.78
12	Basal leaflet length (Lateral)	16.44 ± 1.81	11.1	28.71**	3.31
13	Basal leaflet width (Lateral)	11.25 ± 1.44	12.8	10.11**	2.07
14	Leaflet color	3.06 ± 0.23	7.7	0.16**	0.05
15	Hypanthium length	42.33 ± 4.75	11.2	66.33**	22.63
16	Standard petal length	11.07 ± 0.62	5.6	0.73	0.39
17	Standard petal width	12.73 ± 0.86	6.8	2.60**	0.75
18	No. of pod/plant	137.30 ± 43.83	31.9	6887.00**	1921.00
19	Pod length	11.76 ± 1.17	1.0	55.93**	13.80
20	Pod width	5.69 ± 0.26	0.4	2.38**	0.69
21	Pod Isthmus length	41.11 ± 5.10	12.4	505.79**	26.07
22	Seed length	10.00 ± 0.40	0.4	23.99**	1.67
23	Seed width	4.92 ± 0.23	0.4	1.37**	0.54
24	25-seed weight	2.99 ± 0.33	11.1	0.40**	0.11
25	Reaction to rust	1.31 ± 0.15	11.8	0.60**	0.02
26	Percent protein	25.11 ± 2.86	11.4	18.95**	8.17
27	Percent oil	54.83 ± 2.99	5.5	14.21	8.94
28	Seed moisture	4.04 ± 1.04	25.9	0.40	1.09

[1] Degree of freedom
* = significant at 1%

Protein profile

Protein profiles of 42 accessions are presented in Fig. 2. Eleven major bands were resolved with little variation between the accessions. The bands were divided into three groups, representing three units of groundnut protein, when compared to the reference protein profile of *A. hypogaea* (Krishna et al., 1986). The first group is of conarchin, consisting of a single major band of a high molecular weight protein, 66.2 kilodaltons (kDa);

Table 3. Characters contributing to most of the variability

Characters	Percent contribution
Height of main stem	21.5
Reaction to rust	15.0
Pod Isthmus length	13.2
Apical leaflet length (main axis)	7.1
Seed width	4.6
Total	61.4

No. of accessions = 42
No. of quantitative traits causing significant difference = 24

the second group of acidic arachins, containing five major bands of protein with molecular weight between 30 to 45 kDa; and the third group of basic arachins containing five major bands of proteins with molecular weight ranging from 17 to 27 kDa. several minor bands of low molecular weight proteins were also observed. The accession ICG 8957 expressed quantitative differences through differential intensity of the same band and a qualitative difference with an additional band of 22 kDa (Fig. 2). ICG 8201, 8123, and 11552 have two minor additional bands at 22 and 50 kDa (Fig. 2). Accessions ICG 13184, 13203, 13204, and 13205, which shared an identical profile and are probably the forms of a single accession, differed from others in the bands at 43, 25, and 15 kDa. There again the accession 8205 had bands at 25 and 15 kDa similar to the four accessions, and the band at 42 kDa similar to other accessions (Fig. 2). Accessions 8205 and 13194 both had bands at 29 and 30 kDa, while the remaining accessions had only either of the two. Accessions, 13242, 13189, 13190, 13191, 13192, 13193, and 13194, collected from the same location show some variation in their profiles (Fig. 2). The differential intensity of the same band in the above accessions probably reflects quantitative differences, while the presence of additional bands reflects qualitative differences between accessions. A similarity between profiles of accessions (particularly of those originating from the same location) implies that they are merely duplicates of the same original collection.

Discussion

Arachis species have been primarily classified on the basis of morphological similarities and dissimilarities (Gregory et al., 1973; Gregory & Gregory, 1979). Through principal component analysis, Stalker (1990) has shown that the accessions of a known species cluster together, manifesting more definitive relationships between accessions of a species. In the present study, 23 of the 28 characters (mostly morphological quantitative traits) contributed very little to the variation observed in *A. duranensis*. The greater part of variation (61.4%) was accounted for by only five characters, namely, height of the main stem, length of apical leaflet on the main stem, length of isthmus between pods, seed width, and reaction to rust. This supports the grouping of all the accessions of *A. duranensis* into one cluster on the basis of morphological features (Stalker, 1990). It also indicates that *A. duranensis*, which is distributed over a narrow strip of land with nearly uniform phytogeographic conditions has not developed significant variation for most morphological traits. Even those accessions that originated from distant places exhibit similarity for most of the morphological features, probably because of identical selection pressure under broadly similar environment. In the present study, this situation has been further compounded by duplicates maintained as separate accessions reducing the actual variation. Cluster analysis, however, produced six clusters but the clustering, probably because of the above reasons, does not show any pattern of association between a specific morphological character or a set of variation and a specific site of collection.

Reaction to groundnut rust accounted for significant variation, but it ranged only from immunity to hypersensitivity, and no accession was found susceptible. Acessions from different regions of Bolivia as well as Argentina were involved in this variation. This indicates that variation for reaction to such a disease may exist between accessions but only for relatively low magnitude for resistance as well as susceptibility. This can possibly be explained as a consequence of minor quantitative differences in the synthesis of certain biochemicals (as is reflected by the differential intensity of bands in protein profiles of several accessions), which influence some components of resistance. Therefore, in such species as *A. duranensis* which have a restricted distribution, only a limited degree of variation for reaction to the disease between accessions is expected. The extremes that have been reported in the literature are probably due to variations in the techniques of evaluating the reaction to the pathogen or due to variation in the pathogen. Further investigations can illustrate the precise nature of variation in different accessions and their evolution.

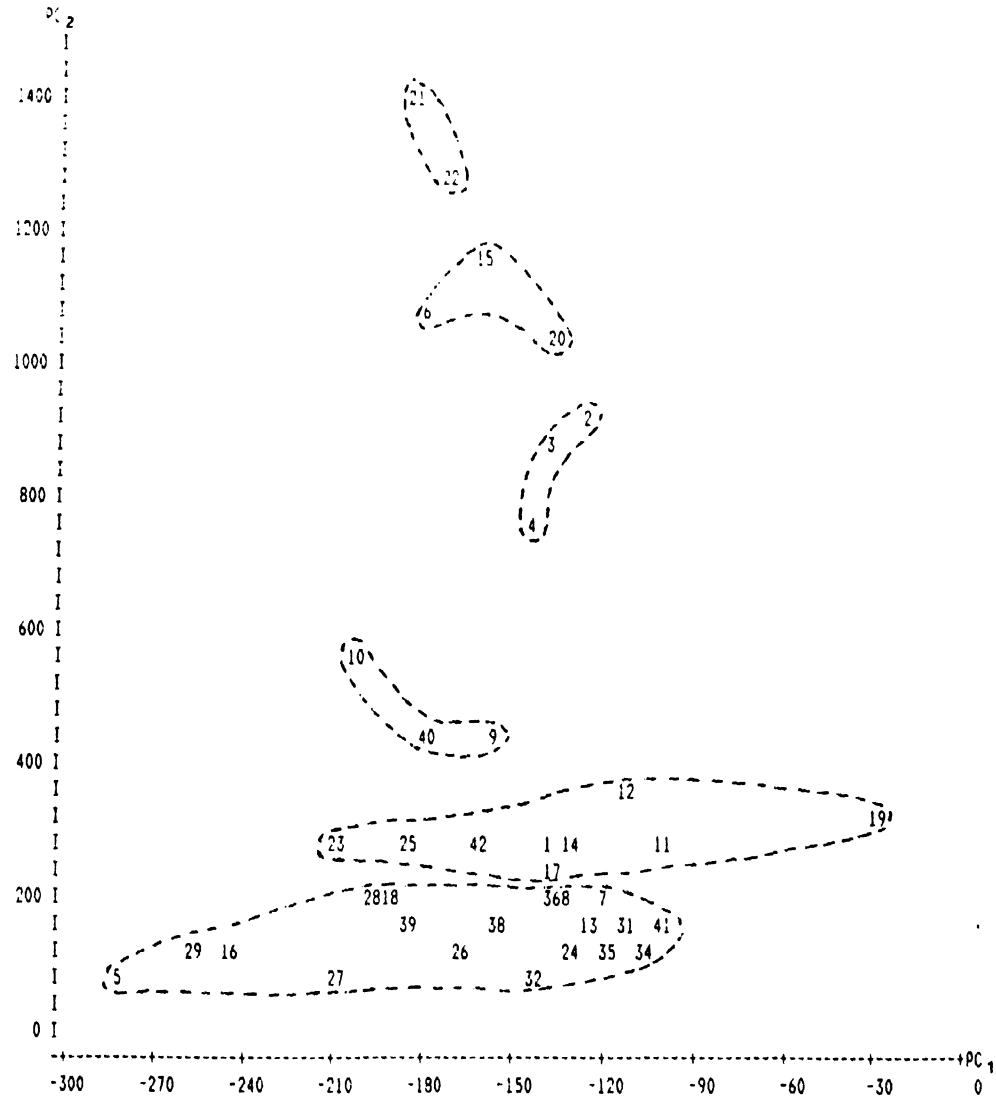

Fig. 1. Scatter diagram obtained from principal component analysis. Points coinciding are 7 and 30, 26 and 33, 17 and 37; the numbers in scatter diagram correspond to serial no. of accession no. in Table 1; lines circles represent clusters obtained, using principal component analysis and Ward's method.

The above pattern of variation is similar to the variation observed by Stalker (1990) in other wild *Arachis* species of section *Arachis* in general and of *A. duranensis* and *A. cardenasii* Krapov. et W.C. Gregory in particular. Where, principal component analysis was able to discern more definitive relationships between the accessions of the same species than the cluster analysis. He further observed that in *Arachis*, although the cluster analysis grouped accessions with morphological similarities, but taxa which look alike do not always equate with biological species, and that in species as *A. stenosperma* Krapov. et W.C. Gregory, the accessions collected from far and distinct places (the eastern seacoast and south central Brazil) were morphologically alike, whereas in species as *A. batizocoi* Krapov. et W.C. Gregory which has a restricted distribution like *A. duranensis*, of the four accessions, one accession 30080 differed significantly in its morphology. The variation observed among accessions of *A. duranensis* is very limited, when compared to cultivated groundnut, *A. hypogaea*, which is cultivated over 80 countries distributed over the tropics, subtropics and warm temperate zones extending between 40° N and 40° S. It has a significant variation for both botanic and agronomic traits evolved in various parts of the world because of differential selection pressure.

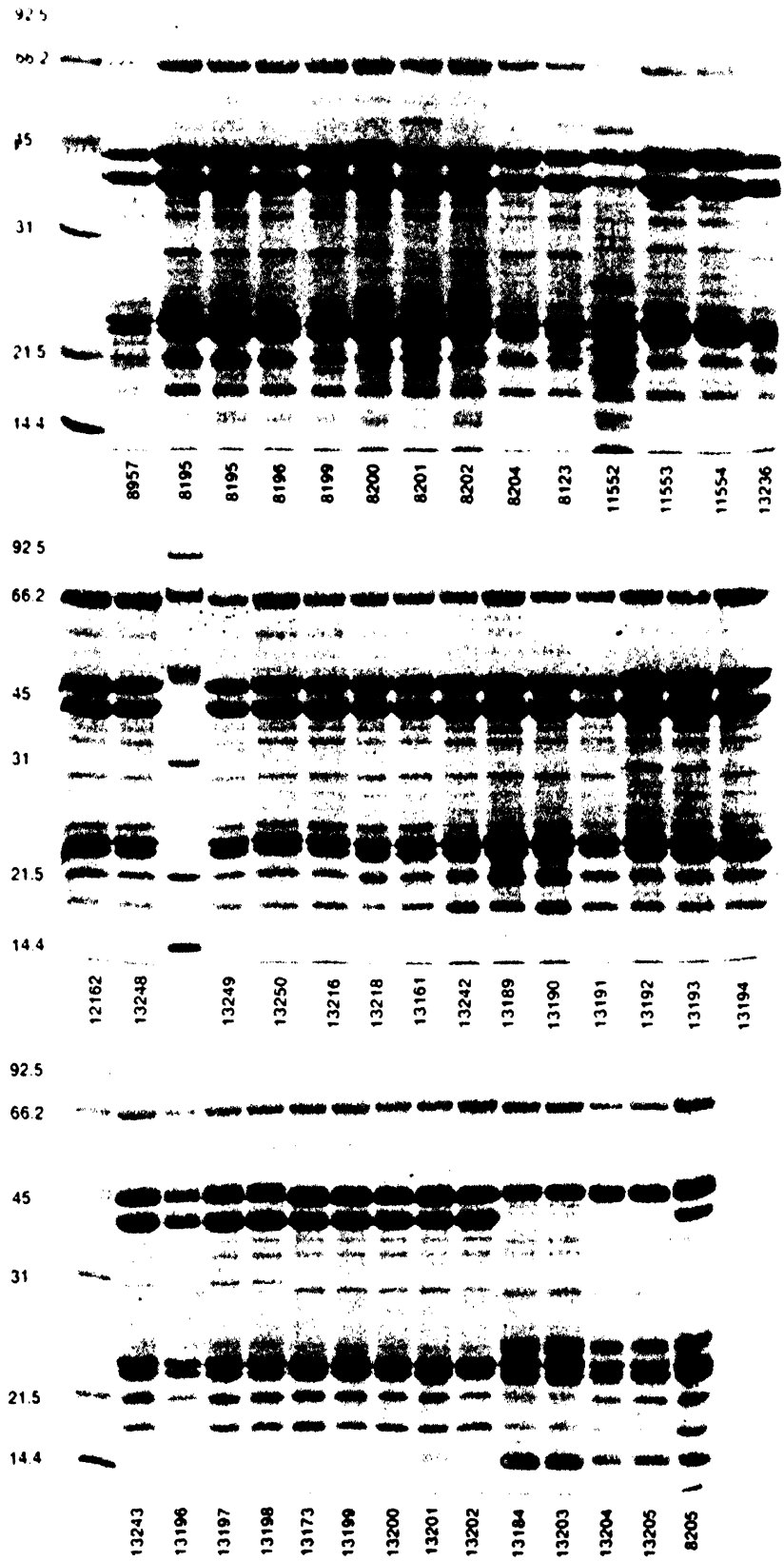

Fig. 2. Protein profiles of 42 accessions of *A. duranensis*. The number below each track represents the identity of genotype. * Sample protein for molecular weight.

However, in *A. hypogaea* also variation for certain traits such as resistant to rust and late leaf spot is confined to accessions of a specific subspecies, originated from a limited area. Eighty-seven percent of resistant accessions belong to *A. hypogaea subsp. fastigiata* Waldron, of which 75% have originated only in Peru (Subrahmanyam et al., 1989). Therefore, in general in species that has a large number of accessions originating from diverse eco-geographic conditions one can expect greater intraspecific variation than a species with restricted distribution.

At molecular level, most of the accessions broadly exhibited a similar pattern of protein profiles, indicating that most of these accessions are the members of the same conservative species. The quantitative and qualitative differences exhibited in protein profiles of certain accessions, such as 8957, 8123, 8201, 8205, 11552 originating from little distinct geographical conditions compared to those of the remaining accessions, and similarity between profiles of accessions such as 13192, 13193, 13194, and 13184, 13203, 13204, 13205 originating from the same location suggest that the protein profiles are able to resolve both the genetic variation and the similarity between accessions, and therefore can be utilized in identification of distinct accessions as well as duplicates. These observations are in line with the reports by Bianchi-Hall et al. (1993), where they observed significant variation between accessions of *A. duranensis* and *A. correntina* (Birk.) Krapov. et W.C. Gregory but not between the accessions of other species. However, they implied this to the analysis of protein profile in larger number of accessions in these two species than others. Further, the variation observed in protein profiles of accessions originating from the same location, such as 13242, 13189, 13190, 13191, 13192, 13193, and 13194 (Fig. 2) suggests that if genetic variation exists even between the accessions originating from the same location due to differential selection pressure and micromutation, it is exhibited in their protein profiles.

Genetic variation in production of certain biochemical components, mav be responsible for variation in some morphological features (leaflet size, color), and possibly also for differences in resistance potential of the accessions as postulated above. In the light of these observations, the present study emphasizes the need for detailed information about the accessions of a species used in an evaluation study. The study also suggests that conclusions regarding such important traits as resistance to diseases should not be drawn only on the basis of reaction of few accessions but on the basis of the reaction of a range of accessions, especially those collected from different eco-geographic regions. This would be significant not only in breeding programs for selecting the right parent and variation studies but also in future pointed collections to capture the variability for a specific trait.

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