Indian J. Pl. Genet. Resources 12(2): 224-231, 1999

BIOCHEMICAL CHARACTERIZATION OF *Arachis* SPECIES OF THE SECTION *Arachis*

K. CHANDRAN AND S. M. PANDYA[1], National Research Centre for Groundnut, P.B. No. 5, Ivnagar Road, Junagadh, Gujarat 362 001; [1]Department of Biosciences, Saurashtra University, Rajkot, (Gujarat)

Thirty five accessions of 13 *Arachis* species belonging to the section *Arachis* were characterized for seed storage proteins and isozymes (peroxidase, esterase and glutamate oxaloacetate transaminase) banding pattern. The results of proteins and individual enzymes banding pattern were discussed and the cluster analysis based on cosine coefficient of similarity was done to ascertain the species relationship. High intraspecific variation was observed in banding pattern. Accessions of *A. monticola, A. batizocoi, A. kempf- meracadoi* and *A. correntina* were found closely related to *A. hypogaea* and two accessions in the collection were found as duplicates based on these studies.

Key Words : *Arachis*, characterization, seed storage proteins, isozymes, banding pattern, cluster analysis

Groundnut (*Arachis hypogaea*) is one of the major oil seed crop of India which is cultivated in about 8.1 million hectares with a production of 8.3 million tonnes and productivity of 1.02 tonnes/ha. The productivity is much lower than the world average (1.3 tonnes/ha). The major factors contributing to the poor productivity of groundnut include, biotic and abiotic stresses for which variability within the species is meagre. Hence, other species of the section *Arachis* are looked for incorporating the desirable genes to the cultivated species as the members of the section are relatively easier for gene transfer than species of the other sections. The major constraints to the utilization of the germplasm in crop improvement programmes are their poor characterisation and lack of knowledge about the phylogenetic relationship. Biochemical evaluation offers an effective way of finding inter- and intra-specific variability and relationship. The electrophoretic separation of proteins and isozymes are commonly used technique to study the relationship. The present studies were carried out on electrophoretic banding pattern of seed storage proteins and isozyme banding pattern of peroxidase, esterase and Glutamate oxaloacetatate (GOT) to find inter- and intra-specific variability among the *Arachis* species.

MATERIALS AND METHODS

Thirty five accessions belonging to 13 species of *Arachis* of the section *Arachis* were procured from International Crops Research Institute for Semi-Arid Tropics (ICRISAT) and grown in unreplicated plot (2.8 × 3.5 M^2) during 1996-1997. Seeds were sown in the month of June 96 and harvesting was done in May 97 except for the cultivated species where, the harvesting was done between 120-130 days after sowing. To avoid seed deterioration, the pods of cultivated species were stored under low temperature till the harvesting of other species were completed lists of the accessions studied (Table 1).

Table 1. List of accessions used in this study

Species Name (ICG No)
A. batizocoi (ICG Nos. 8124, 8209, 8210, 8958)
A.cardenasii (ICG No. 8216)
A.correntina (ICG No. 8132, 8918)
A.diogoi (ICG No. 4983)
A.duranensis (ICG Nos. 8123, 8139, 8196, 8200, 8201, 8205, 8207, 8208, 8956, 8957)
A.heodes (ICG No. 8955)
A.hoehnei (ICG No. 8190)
A. hypogaea. ssp. *hypogaea* var. *hypogaea* bunch type (ICG No. 5813)
A. hypogaea. ssp. *hypogaea* var. *hypogaea* runner type (ICG No. 5770)
A.hypogaea ssp. *fastifiata* var. *vulgaris* (ICG No. 287)
A.hypogaea ssp. *fastigiata* var. *fastigiata* (ICG No. 3704)
A.kempf-mercadoi (ICG No. 8164, 8959)
A.khulmanii (ICG No. 8954)
A.monticola (ICG No. 8197, 8198, 8135)
A.stenosperma (ICG No. 8125, 8126, 8137, 8906)
A.valida (ICG No. 11548)

ICG No. = ICRISAT germplasm collection number

Protein Electrophoresis

The seeds were crushed in a mortar and defatted the cake using hexane by changing the solvent 3 to 4 times till it becomes fine powder. The defatted powder was extracted with 0.05M Tris-HCl buffer (pH 7.5) by keeping overnight. The mixture was then centrifuged at 13000 rpm in eppendorf tube and supernatant collected and estimated for protein using Bradfords method (Bradford, 1976). The extract then mixed with sample buffer containing glycerol and bromophenol blue. Sample containing approximately 100 µg of proteins were loaded in each wells of 15 per cent T Polyacrylamide gel and electrophoresis was conducted at the constant current of 1.5 mA per well at 15°C using a BIO-RAD Protean II electrophoresis system. Protein molecular weight markers were also loaded in one well in each gel for calculating the molecular weight. The run was continued till the dye front reached the bottom of the gel. Two gel were run simultaneously to accommodate 35 accessions. The experiment was repeated three times. The separated bands were scanned against the molecular weight markers for studying the polymorphism.

Isozyme Banding Pattern

Immature and un-opened leaves were collected from 110 days old field grown plants in an ice box and kept for freezing at 80°C for 1 hr. The frozen tissue were ground with neutral sand and extraction buffer in a chilled mortar. Tris-HCl buffer (0.05M) pH 8.6 was used for extracting esterase and peroxidase enzymes and Arulsekhar's buffer (Arulsekhar, 1986) was used for GOT. The sample were centrifuged at 13000 rpm at 4°C for 30 min and the supernatant was collected and estimated for protein. The extract was then mixed with sample buffer containing sucrose and bromophenol blue as tracking dye. Electrophoresis was done in a basic buffer system at 4°C by circulating cryo-fluid between the plates. Electrophoresis was continued till the tracking dye reached the bottom of the gel and activity staining was imparted for Peroxidase (E.C. 1.11.1.7), Esterase (E.C.3.1.12) and GOT (E.C. 2.6.1.1). The polymorphism is studied on relative movement and number of bands for each enzyme. Activity staining procedure is given below:

Peroxidase: 8 mg of O-dianisidine was dissolved in 1 ml of methyl formamide and added 50 ml of 0.4M sodium acetate buffer (pH 5.6). 0.02 ml of water was added just before use and incubated the gel in dark till golden brown bands appeared. The gels were fixed and preserved in 7 per cent acetic acid.

Esterase: 20 mg of alpha naphthyl acetate was dissolved in 0.5 ml of acetone and added 0.5 ml of water. 50 ml of 0.2 M potassium phosphate buffer (pH 6.2) was added followed

by 38 mg of fast blue BB salt. Incubation of gel was carried out till dark bands appeared and fixing was done in 7 per cent acetic acid.

GOT : 10 mg of α-ketogltarate and 200 mg of L-aspartic acid dissolved in 100 ml of 0.1M tris-HCl (pH 8.5). 10 mg of pyridoxal-5-phosphate and 150 mg of fast blue BB salts added just before use. The gel was incubate in dark at 30°C, till blue bands appeared. The gel was fixed in 7 per cent acetic acid.

Cluster analysis is performed based on cosine coefficient of association (SPSS 6.0). Multi state characters were converted into binary characters (Sokal and Sneath, 1963) prior to analysis.

RESULTS AND DISCUSSION

I. Seed protein electrophoresis

A total of eighteen bands stained with coomassie blue reagent. The molecular weight of separated proteins ranged from 2.7 kD to 116 kD. Fourth and fifth bands are common in all the accessions. First three bands are seen only in ICG 8132 (*A. correntina*). Three accessions of *A. duranensis* (ICGs 8201, 8205, 8139) and ICG 4983 (*A. diogoi*) showed maximum number (11) of bands (Table 2). Cent per cent similarity in banding pattern is observed among *A. duranensis* accessions (ICGs 8205, 8201, 8139), ICG 8123 and 8200 and *A. stenosperma* accessions (ICGs 8126 and 8906). Among the interspecific relationships, *A. hoehnei* shared similar bands with ICG 8135 (*A. monticola*) and *A. valida* with *A. khulmanii, A. stenosperma* (ICGs 8125 and 8126) and ICG 8210 (*A. batizocoi*). Singh *et al* (1991) observed similarity in proteins profiles between synthetic amhiploid species and of diploid species *A. duranensis* and *A. batizocoi* and suggested the two haploid species as progenitors of cultivated species.

II. Isozyme Electrophoresis

1. Peroxidase (E.C. 1.11.1.7)

Peroxidases catalyses the dehydrogenation of large number of organic compounds like phenols, aromatic amines and hydroquinones. Totally eleven bands were resolved for peroxidase with an Rf value ranging from 0.02 to 0.38 (Table 3) The maximum number of bands expressed by a genotype was eight. All *A. hypogaea* accessions (5813, 5770, 287, 3704), ICG 8132 (*A. correntina*), ICG 8209 (*A. batizocoi*), ICGs 8123, 8201, 8205, 8956 (*A. duranensis*), ICG 8955 (*A. helodes*), ICG 8959, (*A. kempf-mercadoi*), ICGs 8198, 8135 (*A. monticola*) showed similar banding pattern. In other accessions polymorphism was found for different loci. Lu and pickergsill (1993) observed that the tetraploid species were, identical for Prx1, Prx2 and Prx4 but differed for Prx3. In the present study such variations could not be observed among tetraploid species. *A. monticola* reported to show close similarity in banding pattern with ssp. *hypogaea* was found true only with two accessions of *A monticola* (ICGs 8198, 8135) and the other accession showed difference in the position of slow moving bands.

2. Esterase (E.C.3.1.1.2)

Eleven bands have been observed for esterase with an Rf value ranging from 0.08 to 0.48 (Table 3). Mass *et al.* (1993) reported 11 bands for esterase and 2nd and 11th bands as monomorphic in *A. pintoi* accessions. However, none of the bands were monomorphic among the accessions studied. Three accessions (ICGs 8958, 8124, 8123) could not give activity staining for esterase and these accessions were excluded from the cluster analysis. Similar banding pattern was observed among ICGs 8207, 8200 (*A. duranensis*) and ICG 4983 (*A. diogoi*). Cent per cent similarity was also shown by ICG 5770 (*A. hypogaea*) with ICG 8954 (*A. khulmanii*), ICG 8133 (*A. duranensis*) with 8137 (*A. stenosperma*), and ICG 8197 (*A. monticola*) with ICG 8164

Table 2. Seed storage proteins banding pattern

ICG	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
287	-	-	-	+	+	-	-	-	-	-	-	+	+	-	-	+	+	+
8125	-	-	-	+	+	-	-	-	-	-	+	+	+	-	-	+	+	-
8126	-	-	-	+	+	-	-	-	-	-	+	+	+	-	-	+	+	-
8196	-	-	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	-
8197	-	-	-	+	+	-	-	-	-	+	-	+	+	-	-	-	-	+
8209	-	-	-	+	+	-	-	-	-	-	+	+	+	-	-	-	+	-
8210	-	-	-	+	+	-	-	-	-	-	+	+	+	-	-	+	+	-
8957	-	-	-	+	+	-	-	-	-	-	+	-	+	-	-	-	+	-
8958	-	-	-	+	+	-	-	-	+	+	-	-	+	-	-	+	+	+
8959	-	-	-	+	+	-	-	-	-	-	+	+	-	-	+	-	-	+
8956	-	-	-	+	+	-	-	-	+	+	+	-	+	+	+	+	+	-
8216	-	-	+	+	+	-	-	-	-	-	+	+	+	-	-	+	+	-
8208	-	-	-	+	+	-	-	-	-	-	+	+	+	+	-	+	+	+
8198	-	-	-	+	+	-	+	+	+	-	+	+	+	-	-	+	+	-
8190	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	+	+	-
8132	+	+	+	+	+	-	-	-	-	-	+	+	+	-	-	+	-	-
8124	-	-	-	+	+	-	-	-	+	+	-	-	+	-	-	+	+	-
3704	-	-	-	+	+	-	-	-	-	-	+	+	+	+	-	+	+	-
5813	-	-	-	+	+	-	-	+	-	-	+	+	-	-	-	-	+	-
8123	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-
8135	-	-	-	+	+	-	-	-	-	-	-	+	+	-	-	+	+	-
8164	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-
8200	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-
8207	-	-	-	+	+	-	-	+	+	-	+	-	+	-	-	+	+	-
8906	-	-	-	+	+	-	-	-	-	-	+	+	+	-	-	+	+	-
8955	-	-	-	+	+	-	-	-	-	+	+	+	+	-	+	-	+	-
11548	-	-	-	+	+	-	-	-	-	-	+	+	+	-	-	+	+	-
5770	-	-	-	+	+	-	-	-	-	-	-	+	+	-	-	-	+	-
8954	-	-	-	+	+	-	-	-	-	-	+	+	+	-	-	+	+	-
8918	-	-	-	+	+		-	+	+	-	-	+	+	-	-	+	+	-
8205	-	-	-	+	+	-	-	+	+		-	+	+		+		+	-
8201	-	-	-	+	+	-	-	+	+	+	-	+	+	+	+	+	+	-
8139	-	-	-	+	+	-	-	+	+	+	-	+	+	+	+	+	+	-
8137	-	-	-	+	+	-	-	-	-	-	-	+	+	-	-	-	+	-
4983	-	-	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	-

Rf Value: 1 = 0.08, 2= 0.08, 3= 0.10, 4= 0.13, 5= 0.14, 6= 0.17, 7= 0.19, 8= 0.21, 9= 0.24, 10= 0.27, 11= 0.29, 12= 0.32, 13= 0.43, 14= 0.47, 15= 0.50, 16= 0.58, 17= 0.67, 18= 0.75;
'+' and '-' stands for presence and absence of bands respectively.

Table 3. Isozymes banding pattern of peroxidase, esterase and Glutamate oxaloacetate transminase (GOT)

ICG	Peroxidase											Esterase											GOT		
No.	1	2	3	4	5	5	7	8	9	10	11	1	2	3	4	5	6	7	8	9	10	11	1	2	3
287	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	+	+
8125	+	+	+	-	+	+	+	+	-	+	-	+	-	+	-	-	+	+	-	-	-	-	+	-	+
8126	+	+	+	-	+	+	+	+	-	+	-	+	-	+	-	-	+	+	-	-	-	-	-	+	-
8196	+	+	+	-	+	+	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	-	+	-	+
8197	-	+	+	-	+	-	-	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	+	+	-
8209	+	+	+	-	-	-	-	-	+	+	+	+	-	+	-	-	-	+	+	+	-	-	+	-	+
8210	+	+	+	-	+	-	-	-	+	+	+	+	-	+	-	-	-	+	+	+	-	-	-	+	-
8957	-	-	+	-	-	-	-	-	+	+	+	+	-	+	-	-	-	+	+	-	-	-	-	-	+
8958	+	+	-	+	-	-	-	-	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	-	+	-
8959	+	+	+	-	-	-	-	-	+	+	+	+	-	+	+	-	-	+	+	-	+	+	-	+	-
8956	+	+	+	-	-	-	-	-	+	+	+	+	-	+	+	-	+	+	-	-	+	+	-	+	+
8216	+	+	+	-	-	-	+	-	-	-	-	+	-	+	+		-	+	+	+	+	-	+	+	+
8208	-	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	-
8198	+	+	+	-	-	-	-	-	+	+	+	-	-	-	+		-	+	-	+	-	+	-	+	-
8190	+	+	+	-	-	-	+	+	+	+	+	+	-	-	+		-	-	+	-	+	+	+	-	+
8132	+	+	+	-	-	-	-	-	+	+	+	+	-	+	+	-	+	+	+	+	-	+	-	+	-
8124	+	+	-	+	-	-	-	-	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	-	+	-
3704	+	+	+	-	-	-	-	-	+	+	+	-	-	+	+	+	-	+	+	+	+	+	-	+	+
5813	+	+	+	-	-	-	-	-	+	+	+	-	-	+	+	+	-	+	+	+	+	+	-	+	+
8123	+	+	+	-	-	-	-	-	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	-	+	-
8135	+	+	+	-	-	-	-	-	+	+	+	-	-	+	+	+	-	+	+	-	+	+	-	+	+
8164	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-
8200	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-	+	+	-	-	+
8207	+	+	+	-	-	-	-	-	+	+	-	-	-	+	-	+	-	+	+	-	+	+	-	-	+
8906	+	+	+	-	-		-	-	-	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	+
8955	+	+	+	-	-	-	-	-	+	+	+	+	-	-	+	-	+	-	-	-	+	-	-	+	-
11548	+	+	-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	+	+	-	-	+	+	+	-
5770	+	+	+	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	-
8954	+	-	+	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	-	+	+	+	+	-
8918	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	-	+	-
8205	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-	-	+	+	+	-
8201	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-	-	+	+	+	-
8139	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	+	-	+	+	+	+	-
8137	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	+
4983	+	+	+	-	-	-	-	-	+	+	+	-	-	+	-	+	-	+	+	-	+	+	-	+	-

Peroxidase, Rf value: 1 = 0.02, 2 = 0.04, 3 = 0.09, 4 = 0.12, 5 = 0.16, 6 = 0.17, 7 = 0.19, 8 = 0.21, 9 = 0.31, 10 = 0.34, 11 = 0.38
Esterase, Rf value: 1 = 0.08, 2 = 0.13, 3 = 0.17, 4 = 0.21, 5 = 0.25, 6 = 0.28, 7 = 0.31, 8 = 0.33, 9 = 0.37, 10 = 0.43, 11 = 0.48
GOT, Rf value : 1 = 0.12, 2 = 0.16, 3 = 0.19
'+' and '-' indicate the presence and absence of bands respectively.

Table 4. Cosine Similarity Coefficient Matrix of Biochemical characters

Label	8209	8210	8216	8132	8918	4983	8139	8196	8200	8201	8205	8207	8208	8956	8957	8955	8190	5813	5770	287	3704	8164	8959	8954	8197	8198	8135	8125	8126	8137	8906
ICG 8210	0.878																														
ICG 8216	0.774	0.746																													
ICG 8132	0.802	0.822	0.724																												
ICG 8918	0.635	0.651	0.657	0.594																											
ICG 4983	0.757	0.730	0.638	0.667	0.792																										
ICG 8139	0.651	0.667	0.622	0.609	0.776	0.870																									
ICG 8196	0.651	0.715	0.622	0.609	0.517	0.609	0.727																								
ICG 8200	0.669	0.633	0.737	0.577	0.858	0.818	0.754	0.553																							
ICG 8201	0.683	0.699	0.565	0.681	0.759	0.851	0.934	0.756	0.688																						
ICG 8205	0.683	0.699	0.565	0.681	0.759	0.851	0.934	0.756	0.688	**1.000**																					
ICG 8207	0.732	0.700	0.699	0.639	0.759	0.822	0.715	0.572	0.896	0.699	0.699																				
ICG 8208	0.720	0.738	0.639	0.722	0.572	0.722	0.754	0.603	0.556	0.737	0.737	0.633																			
ICG 8956	0.742	0.716	0.667	0.735	0.582	0.776	0.768	0.768	0.660	0.792	0.792	0.760	0.660																		
ICG 8957	0.817	0.837	0.613	0.709	0.519	0.655	0.570	0.570	0.504	0.613	0.613	0.657	0.630	0.695																	
ICG 8955	0.801	0.718	0.622	0.702	0.501	0.656	0.685	0.734	0.487	0.718	0.718	0.564	0.649	0.826	0.675																
ICG 8190	0.714	0.732	0.728	0.713	0.635	0.668	0.651	0.558	0.566	0.683	0.683	0.586	0.720	0.611	0.583	0.651															
ICG 5813	0.837	0.763	0.756	0.740	0.672	0.783	0.682	0.546	0.754	0.667	0.667	0.810	0.704	0.725	0.684	0.685	0.744														
ICG 5770	0.884	0.763	0.756	0.783	0.672	0.783	0.727	0.636	0.704	0.756	0.756	0.715	0.704	0.768	0.741	0.783	0.791	0.818													
ICG 287	0.732	0.800	0.699	0.730	0.597	0.685	0.620	0.667	0.633	0.653	0.653	0.650	0.580	0.760	0.717	0.718	0.683	0.715	0.763												
ICG 3704	0.846	0.822	0.809	0.792	0.693	0.833	0.740	0.653	0.770	0.724	0.724	0.822	0.818	0.817	0.709	0.702	0.757	0.914	0.827	0.776											
ICG 8164	0.642	0.717	0.669	0.600	0.908	0.764	0.798	0.570	0.819	0.780	0.780	0.717	0.567	0.588	0.571	0.552	0.583	0.627	0.627	0.657	0.655										
ICG 8959	0.810	0.830	0.728	0.802	0.635	0.757	0.698	0.605	0.669	0.683	0.683	0.732	0.720	0.786	0.758	0.751	0.762	0.837	0.837	0.830	0.846	0.642									
ICG 8954	0.781	0.700	0.839	0.730	0.705	0.685	0.667	0.620	0.738	0.653	0.653	0.700	0.633	0.716	0.657	0.667	0.683	0.715	0.858	0.650	0.776	0.657	0.781								
ICG 8197	0.655	0.727	0.521	0.612	0.546	0.612	0.693	0.640	0.471	0.730	0.730	0.503	0.707	0.550	0.668	0.631	0.600	0.586	0.693	0.671	0.612	0.601	0.655	0.503							
ICG 8198	0.744	0.763	0.667	0.740	0.776	0.827	0.727	0.591	0.704	0.756	0.756	0.763	0.704	0.725	0.627	0.685	0.744	0.818	0.727	0.715	0.827	0.741	0.744	0.667	0.586						
ICG 8135	0.810	0.781	0.774	0.757	0.741	0.846	0.744	0.605	0.823	0.728	0.728	0.830	0.720	0.786	0.700	0.701	0.762	0.884	0.884	0.830	0.935	0.700	0.857	0.781	0.655	0.791					
ICG 8125	0.732	0.800	0.699	0.685	0.597	0.593	0.572	0.763	0.580	0.606	0.606	0.600	0.527	0.671	0.657	0.667	0.634	0.572	0.667	0.700	0.639	0.657	0.683	0.700	0.559	0.620	0.634				
IC 8126	0.732	0.800	0.699	0.685	0.597	0.593	0.572	0.763	0.580	0.606	0.606	0.600	0.527	0.671	0.657	0.667	0.634	0.572	0.667	0.700	0.639	0.657	0.683	0.700	0.559	0.620	0.634	**1.000**			
ICG 8137	0.642	0.598	0.725	0.546	0.713	0.655	0.741	0.627	0.819	0.669	0.669	0.717	0.630	0.588	0.500	0.552	0.583	0.684	0.741	0.598	0.709	0.714	0.642	0.717	0.601	0.570	0.758	0.538	0.538		
ICG 8906	0.751	0.718	0.813	0.656	0.723	0.702	0.636	0.636	0.865	0.574	0.574	0.821	0.649	0.642	0.552	0.526	0.601	0.783	0.685	0.616	0.843	0.675	0.701	0.770	0.459	0.685	0.801	0.667	0.667	0.797	
ICG 11548	0.720	0.685	0.737	0.722	0.743	0.674	0.704	0.653	0.667	0.737	0.737	0.685	0.667	0.660	0.567	0.649	0.720	0.704	0.804	0.633	0.770	0.693	0.720	0.843	0.589	0.754	0.772	0.685	0.685	0.693	0.703

(*A. kempf- mercadoi*). Between two accessions of *A. duranensis* (ICGS 8205 and 8201), *A. stenosperma* (ICGs 8125 and 8126) also showed cent percent similarity in banding pattern.

3. Glutamate oxalo acetate tansaminase (E.C. 2.6.1.1)

Glutamate oxalo acetate tansaminase, also known as aspartate amino transferase catalyses the reversible transmination of glutamate and oxaloacetate to 2-oxoglutarate and aspartate. The other role assigned to it include biosynthetic, in nitrogen metabolism and transport of reducing equivalents among sub cellular compartments (Newton, 1983). Three to four bands have been reported for GOT in plant system (Gottlieb, 1982). The maximum number of bands resolved in this study was three (*A. cardensaii*) in all other accessions either only one or two bands could be resolved (Table 3). All accessions was *A. batizocoi* showed only one band with an Rf value of 0.16. *A. diogoi*, ICG 8918, (*A. correntina*), 8123, 8957 (*A. duranensis*), *A. helodes, A. kempf-mercadoi*, 8198 (*A. monticola*), 8125, 8126 (*A. stenosperma*) share the banding pattern of *A. batizocoi*. Among *A hypogaea* runner type accession was different from other accessions in position of the bands. Greishammer and Wynne (1990) and Lacks and Stalker (1993) reported two different banding pattern for GOT which are consistent among *Arachis* species and their interspecific hybrids. In the present study one more type of banding pattern was observed with three bands in *A. cardensaii. A. duranensis* accessions showed cent per cent similarity for seed protein banding pattern also showed similarity in GOT and Esterase banding patterns.

Cluster Analysis

Combined cluster analysis of both seed proteins and isozymes banding pattern showed cent per cent similarity among ICG 8125 with 8126 (*A. stenosperma*) and ICG 8201 with 8205 (*A. duranensis*) (Table 4). Probably one of the accessions of the above species may be duplicate in the collection. One accessions each of *A. batizocoi, A. correntina, A.kempf-mercadoi* and *A. monticola* clustered with three accessions of *A. hypogaea* showing similarity of the species with the cultivated species. The spanish bunch accession of *A. hypogaea* remain unclustered (Fig. 1). The following groupings are formed at 13 cluster stage.

Cluster no.	List of accessions
I	ICG 287 (*A. hypogaea*)
II	ICGs 8125,8126 (*A. stenosperma*)
III	ICGs 8196 (*A. duranensis*)
IV	ICG 8197 (*A. monticola*)
V	ICGs 8209 (*A. batizocoi*) 8132 (*A. correntina*)
VI	ICGs 8210 (*A. batizocoi*), 8957 (*A. duranensis*),
VII	ICGs 8956 (*A. duranensis*), 8955 (*A. helodes*)
VIII	ICGs 8216 (*A. cardenasii*), 11548 (*A. valida*), 8954 (*A. khulmanii*)
IX	ICG 8208 (*A. duranensis*)
X	ICG 8198 (*A. monticola*)
XI	ICG 8190 (*A. hoehnei*)
XII	ICGs 4983 (*A. diogoi*), 8139, 8201, 8205 (*A. duranensis*), 8164, (*A. kempf-mercadoi*), 8918 (*A. correntina*)
XIII	ICGs 8200, 8207 (*A. duranensis*), 8906, 8137 (*A. stenosperma*)

Very high intraspecific variability was observed for banding pattern for isozymes as well as seed storage proteins. This leads to the clustering of different accessions of the same species with other species. Species having high morphological variability especially the annual and perennial species found to be homogeneous in biochemical characterization. Hence, it rules out the possibility of using these traits in delimiting taxa at the species level. However, these characters will be much useful in ascertaining intraspecific variability to a large extent and for finding duplicates in the germplasm collection.

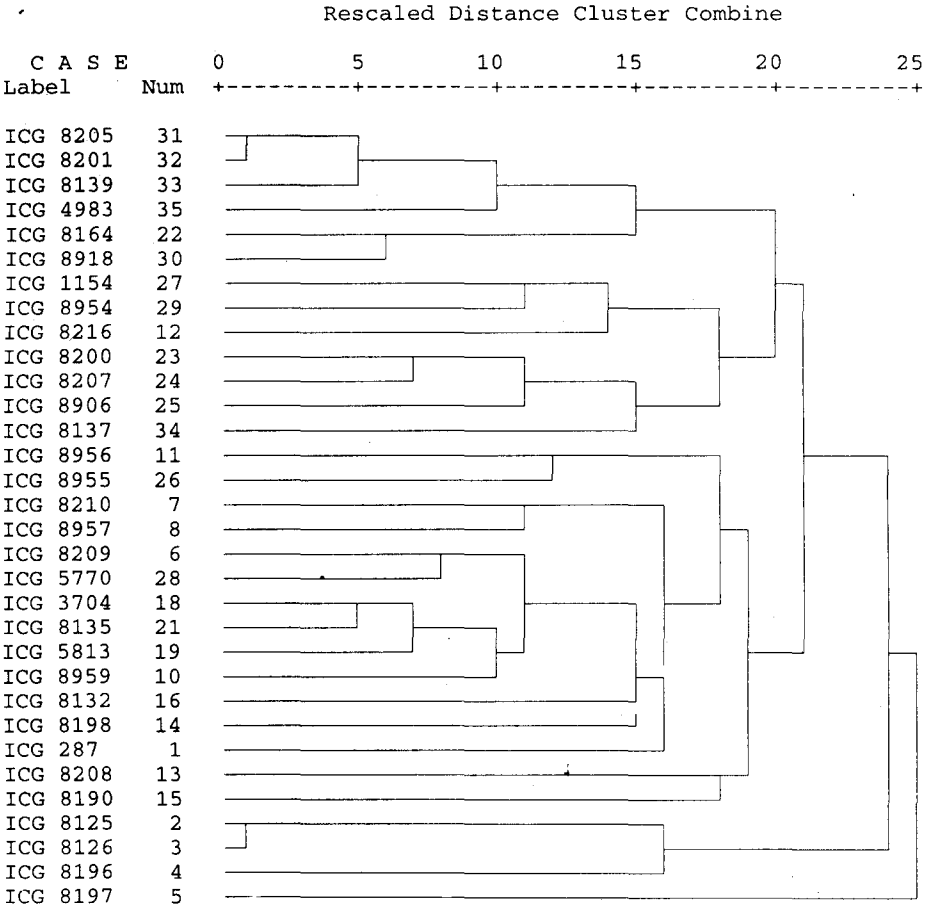

Fig. 1. Dendrogram based on biochemical characters

ACKNOWLEDGEMENTS

Authors are thankful to Dr. A.K. Singh, erstwhile Senior Scientist, ICRISAT, Patancheru and currently Head, Germplasm Conservation Division, NBPGR, New Delhi for providing the germplasm and Director, NRCG, Junagadh for facilities.

REFERENCES

Arulsekhar, S. and D.R. Paraffit. 1986. Isozyme analysis procedure for stone fruits, almond, grape, walnut, pistachio and fig. *Hort. Sci.* 21: 928-933.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantitaties of proteins utilizing the principle of protein dye binding. *Anal. biochem.* 72: 248.

Gottlieb, L.D. 1982. Conservation and duplication of isozymes in plants. *Science* 216: 373-380.

Grieshammer, V. and J.C. Wynne. 1990. Isozyme variability in mature seeds of U.S. Peanut cultivars and collection. *Peanut Sci.* 18(2): 72-75.

Lacks, G.D. and H.T. Stalker. 1993. Isozyme Analyses of *Arachis* Species and Interspecific hybrids. *Peanut Sci.* 20(2): 76-81.

Lu, J. and B. Pickersgill. 1993. Isozyme variation and species relationship in peanut and its wild relatives (*Arachis* L.-Leguminosae). *Theor. Appl. Genet.* 85: 550-560.

Mass, B.L., A.M. Torres and C.G. Ocampo. 1993. Morphological and isozyme characterization of *Arachis pintoi* Krap.et Greg.nom.nud. *Euphytica.* 70(1-2): 43-52.

Newton, K.J. 1983. Genetics of mitochondrial isozymes. *In*: S.D. Tanskley and T.J. Orton (eds), Isozyme in plant genetics and breeding, Part A. Elseview Science publishers B.V. Amsterdam. p 150-176.

Singh, A.K., S. Sivramakrishnan, M.G. Mengesha and C.D. Ramaiah. 1991. Phylogenetic relations in section *Arachis* based on seed protein profile. *Theor. Appl. genet.* 82: 593-597.

Sokal, R. and P.H.A. Sneath. 1963. Principles of numerical taxonomy. W.H. Freeman & Co. Sanfrancisco, London.