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Characterization of Groundnut (*Arachis Hypogaea* L.) through Biochemical Markers

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ABSTRACT: *The cultivated peanut,* Arachis hypogaea *L. is an important source crop for edible oil and protein. It is important to identify the genetic diversity of peanut genetic resources for cultivar development and evaluation of peanut accessions. The aim of the present study was to study and evaluate isozymes and protein profiling for varietal identification of 12 groundnut genotypes. Four enzyme systems* viz. *esterase, peroxidase, polyphenol oxidase and superoxide dismutase and protein profiles were studied at 6 and 9 days after germination (DAG). The average polymorphism revealed by the isozymes and protein profiles was 50.58%. The polymorphic index content varied from 0.621 to 0.875 with an average of 0.713. Isozymes and protein profiling indicated that GG-11 and GG-13 exhibited highest similarity (97.3%), followed by GG-2 and GG-5 (97.1%), while GG-13 and BAU-13 showed minimum similarity (65.9%). These result revealed information of variation among the different genotypes, thus indicating the usefulness of electrophoretic variation in varietal identification.*

Keywords: *isozymes, electrophoresis, peanut, polymorphism*

INTRODUCTION

Cultivated groundnut or peanut (*Arachis hypogea L.*) is a highly self pollinated, allotetraploid annual legume with 2n=4x=40 with a basic chromosome number of x=10 (Stalker, 1997). It is an important crop for edible oil and protein, which is grown mainly in tropical, sub-tropical and temperate areas of the world. It is the 13th most important food crop and the 4th most important oilseed crop of the world. Groundnut is grown on nearly 25.44 million ha worldwide with the total production of 45.22 million tons and an average yield of 1777 kg/ha in 2013 (FAO, 2013). Groundnut is commercially popular due to its superior edible oil quality and protein in the meal. As an important oil crop, groundnut is grown in about 35% of the area and accounts for 40% of the production of total oilseeds in India. Among the major groundnut growing states in India, Gujarat ranks first in production and second in the area among the states in the country. Groundnut can be consumed and utilized in diverse ways which makes it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries. Groundnut kernels are also used in a variety of culinary preparations like peanut candies, butter, peanut milk and chocolates (Desai *et al*., 1999). Cake left after extraction of the oil is an excellent feed for livestock. Vegetative parts of groundnut like leaf and stem are good source of nutritionally high quality fodder for farm animals.

For many years, cultivars with narrow morphological diversity were evaluated with reference to yield and quality (Guan, *et al*., 2010; Wang, *et al*., 2010), using traditional field plot techniques. However, these techniques are tedious and time consuming. Furthermore, the morphological characters may be unstable and influenced by environmental conditions (Badr, *et al*., 2002). Therefore, cultivars did not assure the accurate determination. Because of that, biochemical and molecular markers were used for cultivars identification to achieve more exact identification (Hamoud, *et al*., 1994). Biochemical genetic markers offer specific advantage in assessment of genetic diversity and trait-specific crop improvement. Such markers can facilitate appropriate choice of parents for crosses to mapping/tagging of gene blocks associated to economically important traits and in turn permits marker-assisted selection (MAS) in backcross, pedigree and population improvement programs (Mohan *et al*., 1997).

Isozymes are multiple molecular form of an enzyme with similar or identical catalytic activities occurring within the same organism (Market and

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Moler, 1959). Although, isozymes are independent of pleiotropic and epistatic interaction (Brown and Weir, 1983), in some cases however, significant quantitative and heritable variation can occur due to some biotic and abiotic factors/stresses (Cullis, 1981). The analysis of the seed proteins and isozymes by polyacrylamide gel electrophoresis is the quick, precise and reliable technique. The technique is helpful to ensure the genetic purity of plant cultivars and their parental lines in commercial seed production of hybrids. The electrophoretic protein profiles and their high stability and independence of the ecological conditions were used as cultivar markers (Sammour, *et al*., 2012). Cultivar identification is useful for describing a new cultivar, testing genotype purity and speeding up-distinctness uniformity stability (DUS) test for candidate cultivar (Sammour, *et al*., 1993). It is also useful for the registration of new varieties, pedigree analysis studied and plant variety rights applications. Thus, the present study has been undertaken with the objective of assessing the genetic diversity and characterization of important groundnut genotypes of Saurashtra region using biochemical markers.

MATERIAL AND METHODS

Plant Material, Seedling Protein and Enzyme Extraction

In the present investigation seeds of twelve groundnut genotypes were obtained from the Main Oilseeds Research Station, Junagadh Agricultural University, Junagadh. The habit type, pedigree, origin, and year of release are described in Table 1. Five seeds of each genotype were sown in pots and young leaves were collected from each plant. For analysis of isozymes and protein profiling 6 and 9 days old seedling were raised in the pots under natural condition. The seedling protein extraction and electrophoresis were done according to the procedure of Agarwal *et al.,* (Agarwal, *et al*., 1998). The following isozymes were assayed, Esterase (EC 3.1.1.6), Peroxidase (EC 1.11.1.x), polyphenol oxidase (EC 1.14.18.1) and superoxide dismutase (EC 1.15.1.1). The standard staining and de-staining procedures were used for visualization of clear protein bands. The isozymes were analyzed using standard protocols as described by Sadasivam and Manikam (Sadasivam and Manickam, 1996).

Scoring and Analysis of Bands

Clear and distinct bands amplified by isozymes and protein were scored as present (1) or absent (0) of a band, and the data obtained were used to generate a rectangular matrix. The data matrix was then used to generate a genetic similarity index (Nei and Li, 1979), using NTSYS 2.1 (Rohlf, 2000). Cluster analysis was carried out based on genetic distance, using UPGMA (unweighted pair-group method using arithmetic averages) (Sneath and Sokal, 1973). The resulting clusters were represented as dendrograms and viewed in the program Tree View 1.5. Estimates of the differences between the dendrograms based on isozymes and protein analyses were obtained by computing the cophenetic values and constructing the relative cophenetic matrices for each marker type. These cophenetic matrices were compared using Mantel's test for matrix correspondence (Mantel, 1967**)**. A polymorphic index (PIC) was calculated as PIC = 1 – S Pi^2, where *Pi* is the band frequency of the i^{th} allele (Smith, et al., 1997).

RESULTS AND DISCUSSION

Banding pattern of isozymes *viz.* esterase, peroxidase, polyphenol oxidase and superoxide dismutase and protein profiling has been shown in Figure 1 (a, b, c, d, e and f). The data of the four Isozyme systems and protein profiling were collectively pooled for the construction of a dendrogram using UPGMA method. Cluster analysis performed by combining data of markers generated a dendrogram that separated the varieties into two distinct clusters, cluster A and B (Figure 2). The polymorphic index content varied from 0.621 to 0.875 with an average of 0.713 (Table 2). The Jaccard's similarity coefficient ranged from a minimum of 0.659 to a maximum of 1.000 (Table 3). The first major cluster A comprised 10 varieties viz., GG-2, GG-5, GG-21, GG-11, GG-12, GG-13, GG-16, GG-20, TG-26 and JL-24. The cluster B comprised of 2 varieties BAU-13 and Kadiri-3. Cluster A included two Sub-clusters A1 and A2, in which sub-cluster A1 comprised of groundnut genotypes which exhibited much similarity i.e. GG-11 and GG-13 (97.3%) as well as GG-2 and GG-5 (97.1%). Sub-cluster A2 had two genotypes TG-26 and JL-24 which showed similarity of 96.6%. Cluster B isolated two distinct genotypes namely, BAU-13 and Kadiri-3, showed ample similarity in between them.

Cherry & Ory (1973a, b) also carried out work on variation among cultivars of *Arachis hypogaea* from different areas, working with seven enzymatic systems, such as INT oxidase, catalase, leucine aminopeptidase, acid phosphatase, alcohol dehydrogenase, besides esterase, and peroxidase utilized in the present research. Similar work was also carried out by Jyosthna *et al*., (2004), Phongyuth

Table 1
The Pedigree, Develop Location of 12 Genotypes of Groundnut Evaluated

Sr. no.	*Genotype*	*Habit Type**	*Pedigree*	*Origin*	*Year of release*
1	BAU-13	HYB	BAU 6 x MI 3	BAU, Kanke	1993
2	GG-11	HYR	M13 x GAUG 10	GAU, Junagadh	1984
3	GG-12	HYR	Shulamit x GAUG 10	GAU, Junagadh	1991
4	GG-13	HYR	GAU 10 x TMV 10	GAU, Junagadh	1994
5	GG-16	HYR	JSP 14 × JSSP 4	JAU, Junagadh	2006
6	GG-2	VUL	J 11x EC 16659	GAU, Junagadh	1983
7	GG-20	HYB	GAUG 10 x Robut 33-1	GAU, Junagadh	1991
8	GG-21	HYB	Somnath x NCAc 2232	JAU, Junagadh	2005
9	GG-5	VUL	27-5-1 x JL 24	GAU, Junagadh	1996
10	JL-24	VUL	Selection from EC 94943	MPKV, Jalgaon	1978
11	KADIRI-3	HYB	Selection from Robut 33-1	ANGRAU, Hyderabad	1978
12	TG-26	VUL	BARCG 1 x TG 23	BARC, Mumbai	1995

* HYB=Virginia bunch; HYR=Virginia runner; VUL=Spanish

Table 2
Number of Amplified Bands, Polymorphism Percentage and PIC Obtained by Isozymes and Protein Assay of Groundnut Genotypes

Isozymes	*Day After Germination (DAG)*	*Total no. of allele (A)*	*Polymorphic Band (B)*	*Polymorphic % (B/A)*	*PIC Value*
Esterase	6	3	3	100	0.621
Esterase	9	3	2	66.67	0.662
Peroxidase	6	8	5	62.50	0.838
Peroxidase	9	6	2	33.33	0.811
Polyphenol Oxidase	6	3	2	66.67	0.580
Polyphenol Oxidase	9	5	3	60.00	0.672
Superoxide Dismutase	6	2	0	0.00	0.500
Superoxide Dismutase	9	6	3	50.00	0.803
Protein	6	6	4	66.67	0.769
Protein	9	8	0	0.00	0.875
Total	50	24	—-	—-	
Mean	5	2.4	50.584	0.713	

Table 3
Genetic Distance Estimated among the Groundnut Genotypes using Jaccard's Coefficient based on the Pooled Isozyme and Protein Data

	GG-2	*GG-5*	*TG-26*	*JL-24*	*GG-11*	*GG-12*	*GG-13*	*GG-16*	*GG-20*	*GG-21*	*BAU-13*	*KADIRI-3*
GG-2	1.000											
GG-5	0.971	1.000										
TG-26	0.786	0.762	1.000									
JL-24	0.829	0.805	0.951	1.000								
GG-11	0.895	0.868	0.833	0.878	1.000							
GG-12	0.825	0.800	0.814	0.814	0.923	1.000						
GG-13	0.821	0.795	0.727	0.767	0.872	0.897	1.000					
GG-16	0.846	0.821	0.750	0.791	0.897	0.923	0.973	1.000				
GG-20	0.825	0.800	0.814	0.857	0.829	0.854	0.850	0.875	1.000			
GG-21	0.865	0.838	0.721	0.762	0.868	0.800	0.795	0.821	0.800	1.000		
BAU-13	0.674	0.690	0.711	0.711	0.682	0.667	0.659	0.682	0.744	0.690	1.000	
KADIRI-3	0.732	0.707	0.727	0.767	0.738	0.682	0.674	0.698	0.762	0.795	0.780	1.000

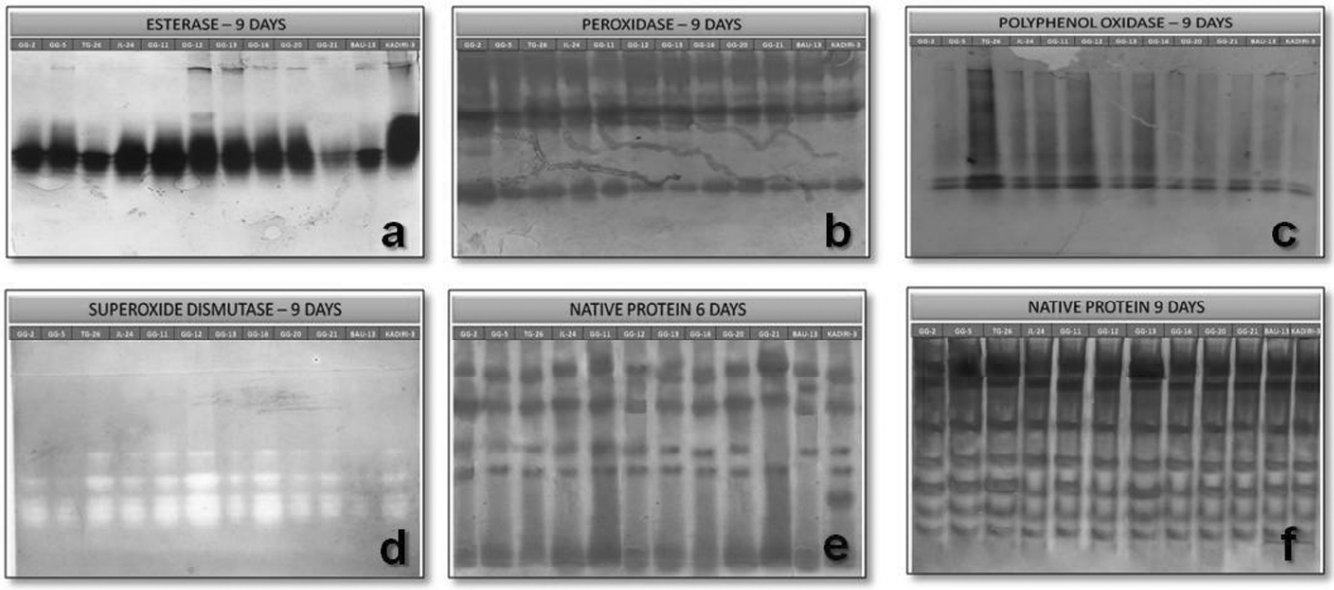

Figure 1: Banding pattern of 12 groundnut genotypes. (a) esterase-9 days, (b) peroxidise-9days, (c) polyphenol oxidase-9 days, (d) superoxide dismutase, (e) protein-6 days and (f) protein-9 days

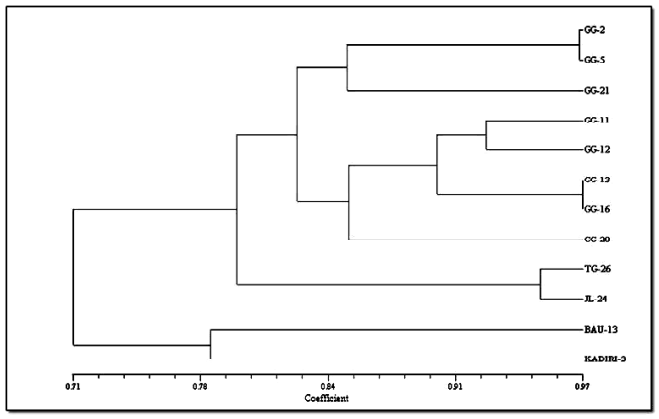

Figure 2: Dendrogram depicting the Genetic relationship among the groundnut genotypes based on combined Isozyme and protein data

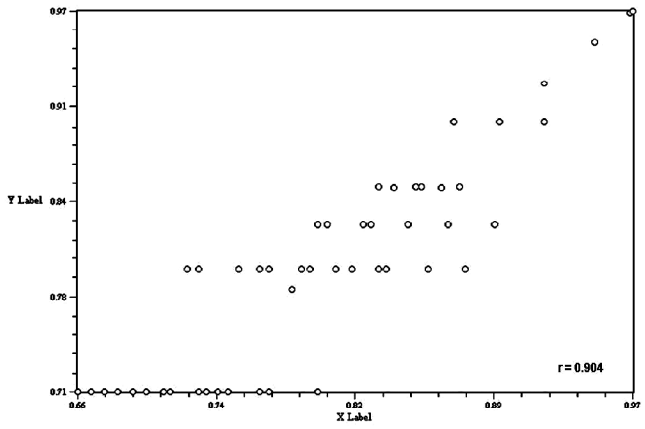

Figure 3: Cophenetic values against Jaccard's similarity coefficients from combined Isozyme and protein data

(1999), Sulochana and Venkaiah (1988). Isozymes in plants have generally been studied either on seeds or seedlings under controlled conditions. The objectives in most of these studies were varietal identification (Mcleod *et al*., 1979), measuring genetic variability in plant population (Brown & Weir, 1983), and identification of alien genetic material introgressed from wild species into cultivated varieties (Tanksley, 1983). Isozyme patterns are more complicated in tetraploid than in diploid plants, since a diploid plant has a maximum of two alleles per locus, while a tetraploid plant may contain four different alleles per locus. (Nielson, 1980).

Clustering methods will create clusters of the data, no matter whether there are true clusters in the data or not, so a check must be made for the existence of true clusters. To test the goodness of fit of the clustering of isozyme and protein data, matrix of cophenetic values were also computed using the tree matrix produced by SAHN to calculate the cophenetic values of similarity or dissimilarity by the program COPH (cophenetic values). The cophenetic matrices were compared to the original tree matrices produced by SIMQUAL. The plots of one matrix against the other and the association statistics were made and calculated by MxComp (Rohlf, 2000). The plot and statistics of 12 groundnut varieties included in the present study were shown in Figure 3. As the cophenetic correlation coefficient is positively correlated to the mantel test statistics. The program MXCOMP plots the cophenetic value matrix against

the original tree matrix, and computes the cophenetic correlation coefficient (r) and the Mantel test statistic (Z). In the present investigation the Mantel test statistics Z was normalized and degree of goodness of fit for a cluster analysis (Matrix correlation r = 0.904) as categorized by Rohlf (Rohlf, 2000) was found to fall under the category of "Very Good Fit".

Allelic variation or allozymic polymorphism at iosozymic loci are of significant importance in plant breeding as this is a major mechanism through which plants can adapt to environmental changes (Weeden, 1983). Since the two allozymes possessing the same electrophoretic mobility may not necessarily be identical in structure or in many of their physiological properties (Gottlieb and Greve, 1981). Therefore variation in allozyme number can play an important role in studies of genetic diversity and in detection of inter and intraspecific differences in different crop plants. Such studies have been made in rice (Farooq *et al*., 1996), maize (Brewbaker *et al*., 1985), and sunflower (Kahler and Lay, 1985).

Electrophoretic differences in protein banding pattern of different genotypes enable us to identify a particular genotype with the presence or absence of specific position of band and also the intensity of band which could be used as 'genetic marker' (Liang *et al*., 2004). These specific protein bands migrating to the same distance gave some evidence of homology in molecular structure and function (Bianchi *et al*., 1994; Javaid *et al*., 2004). From these results it is clear that there is enough variation in protein profiles among genotypes and could be considered as a tool for varietal identification and screening for various traits. To a wide extent, proteins profiling, has been used concerning genetic investigations from the sixties to the nineties. Several books and book chapters have been written about this topic, e.g. Ferguson (1980), Richardson *et al.,* (1986), Pasteur *et al.,* (1989), Whitmore (1990) and Murphy *et al.,* (1990).

From above study it can be concluded that a wide range of variation existed at morphological and biochemical level. Protein profiling and isozymes analysis proved helpful for estimating the magnitude of genetic diversity at biochemical level and establishing genetic relatedness among genotypes. Besides the high homozygosity commonly observed in self-pollinated specie as *A. hypogaea,* the intensive selections in the breeding programs may have contributed to the high uniformity exhibited by the peanuts cultivars (Galgaro and Lopez, 1994).

In Conclusion, the results of the present study indicated that there is enough variation in protein profile among genotypes and could be considered as a tool for varietal identification and screening for various traits. This opens up a possibility for developing tools to genetic enhancement of cultivated groundnut.

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