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ASSESSMENT OF DIVERSITY IN GROUNDNUT GENOTYPES USING ISOZYME MARKERS

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ABSTRACT

Groundnut (*Arachis hypogaea* L.) is an annual warm-season plant of the legume family that originated in South America. The metabolic role of isozymes is regulatory in nature.Alleles coding for slightly modified proteins as a subclass of the isozymes are called allozymes. In the present study two isozymes, i.e. peroxidase (POX) and superoxide dismutase (SOD) were studied, and a total of 9 alleles were generated by the two isozymes *viz*., POX and SOD. Isozymes of SOD exhibited a maximum of five activity zones followed by POX (2). Average polymorphism in both isozymes was 75%. PIC values ranged from 0.24 to 0.26 with an average of 0.25.Cluster tree analysis using UPGMA method based on genetic distance revealed similarity coefficient values that ranged from 0.65 to 1.00 between the 24 genotypes and classified in to three major clusters.

Key words : *POX, SOD, PIC, UPGMA, Arachis hypogaea and similarity coefficient.*

Groundnut (*Arachis hypogaea*) is an annual warm-season plant of the legume family originated in South America. Characterization of germplasm using biochemical parameters (profiles) has received attention because of the increased recognition of germplasm resources in crop improvement and in selection of desirable genotypes to be used in biochemical traits based breeding programmes. Genetic markers are useful for screening germplasm with the minimum expenditure of time and labour. Seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary relationships among crops and their wild relatives (1). They are generally made up of subunits, and the assemblies of various subunits give rise to enzymes with the same catalytic activity.

The isozyme analyses have several advantages compared to morphological markers. The alleles (allozyme) at most loci are co-dominant. This co-dominance causes no deleterious changes in plant phenotype through recessiveness or pleiotrophy and allows heterozygous to be distinguished from homozygous. It is also possible to screen plants at seedling stage and retains only desirable genotypes, therefore, saving time and money. Isozymes are widely used as molecular markers in saturated linkage mapping, lack of pleiotropic and/or epistatic interactions and resilience to environmental influence (2).

In the present study, the enzymes POX and SODwere used for their isozyme profiling. The amounts of total soluble protein of A.hypogea L. genotypes were also estimated by Lowry's method.

MATERIALS AND METHODS

Twenty-four genotypes of A. hypogea were sown by using germination paper. The changes in the protein profiles for isozymes activities based on banding pattern for Peroxidase and Superoxide dismutase were recorded from young leaves at 28 days after sowing (28 DAS). Details of the source and pedigree of material used are given in Table 1.

Enzyme Extraction : Preliminary experiments were conducted to optimize the extraction condition with respect to pH, molarity and type of buffer, concentration of stabilizing agent(s) and others constituents of extraction medium according to (3) with minor modifications.

Peroxidase : Staining : Peroxidase activity was localized on the gel according to (4). The 10% resolving gel was stained in solution of 25% acetic acid containing 0.3% benzidine and 0.5% H_2O_2. Within 2 min, blue coloured bands appeared which turned brown after 10-15 min.

Superoxide dismutase

Staining : Superoxide dismutase activity was localized on the gel according to (5) with minor modifications.

Scoring of Gels : Bands with dark to very light intensities were scored and used to construct the zymograms. Rm (Rm=Relative mobility) value of each band was calculated using the following formula (6).

$$Rm = \frac{\text{Distance travelled by the band}}{\text{Distance travelled by the tracking dye}}$$

Bands were numbered on the basis of increasing Rm value or according to the distance travelled in the gel.

Table-1: List of genotypes used in present study and their pedigree.

Sr. No.	Name of genotypes	Pedigree	Source
1.	UG-158	J 63 × TPG 41	DGR, Junagarh
2.	UG-160	GG 2 × B 95	DGR, Junagarh
3.	UG-161	GG 8 × TKG 19 A	DGR, Junagarh
4.	UG-162	GG 2× TPG 41	DGR, Junagarh
5.	UG-163	GG 20 × PBS 24030	DGR, Junagarh
6.	UG-164	ICGX 090018	ICRISAT
7.	UG-165	GG 21 × R-2001-3	DGR, Junagarh
8.	UG-167	GG 2 × TG 26	DGR, Junagarh
9.	UG-168	GG 20 × TAG 24	DGR, Junagarh
10.	UG-169	GG 20 × ICGV 86325	DGR, Junagarh
11.	UG-170	GG-7 × R-2001-3	DGR, Junagarh
12.	UG-172	TG-37 A × GG 20	DGR, Junagarh
13.	UG-173	GG 2 × ICGV 91114-1	DGR, Junagarh
14.	UG-174	TG 40 × ICGV 86325	DGR, Junagarh
15.	UG-175	PBS 24030 × TG 37 A	DGR, Junagarh
16.	UG-177	J 11 × TPG 41	DGR, Junagarh
17.	UG-178	ICGV 76 × ICGV 86305	DGR, Junagarh
18.	UG-179	ICGV 86564 × TPG 41	DGR, Junagarh
19.	UG-181	ICGV 86590 × PBS 24030	DGR, Junagarh
20.	UG-182	UG 20 × ALR-3	DGR, Junagarh
21.	UG-184	GG 5 × TPG 41	DGR, Junagarh
22.	PM -2	ICGV- 86055 × ICG- (FDRS 10)	DGR, Junagarh
23.	UG-5	Selection from ICGV-98223	DGR, Junagarh
24.	GG-7	S 206 × FEFR 81-1-9-B-B	DGR, Junagarh

Table-2: Protein profiling and polymorphism generated in *A. hypogaea* L. using two isozyme markers

S. No.	Isozyme markers	Total no. of bands	No. of polymorphic bands	% polymorphism	PIC
1.	SOD	5	5	100.0	0.26
2.	POX	4	2	50.00	0.24
	Average	4.5	3.5	75	0.25

Total Soluble Protein : The amounts of total soluble proteins were calculated by the method of (7).

RESULTS AND DISCUSSION

POX (E.C.1.11.1.7) : Electrophoretic profiles of peroxidase isozyme showed four activity zones having Rm value of 0.11, 0.29, 0.31 and 0.44 . Two bands were present in all genotypes, the difference was only found in terms of intensity of bands (Plate-1). In genotypes viz., UG172, UG161, UG184, UG5, PM2 and UG164 the intensity was high at a Rm value of 0.29 and 0.44 and less in remaining genotypes. High intensity bands of each genotype lay more towards cathodic side, possibly having a net positive charge and high molecular weight, while rest of the bands were towards anode indicating a net negative charge on them and correspondingly lower molecular weights. The unique bands were present in G5, G20, G22, G24 and G14 genotypes at Rm value 0.11. At Rm value 0.31 almost all genotypes show bands of similar intensity.

SOD (E.C. 1.15.1.1) : Reactive O_2 species (ROS) are produced in both unstressed and stressed cells. However, during times of environmental stress (e.g. UV or heat exposure) ROS level can increase dramatically, which can result in significant damage to cell structures. This leads a situation known as oxidative stress. Within a cell, the SOD constitutes the first line of defense against ROS and is present in all subcellular locations (8). Isozyme profiles as observed for SOD for *A. hypogaea* L. genotypes are presented in Plate-2. Corresponding SOD zymogram in all genotypes indicated five bands having the Rm value 0.14, 0.23, 0.31, 0.41 and 0.58, respectively (Fig. 2). Bands having Rm value of 0.14 were present in UG170, UG 173, UG175 and GG7. Bands having Rm value of 0.23 were present in G2, G7, G8, G11, G12 and G14. Bands having Rm value of 0.31 were present in UG158, G2, G5, G6, G7, G8, UG11, UG13, G14, G19, G20, G21, G22 and G24. Bands of Rm values 0.41 and 0.48 were present almost in all the genotypes with variations in band intensity except in UG174. Similar reports was published by Patra and Chawala (2010) in basmati rice, where they analysed using five isozymes.

Genetic Relationship and Cluster Tree Analysis : The differences in isozyme patterns are usually directly related to the organism's metabolic activity.In the present study, through observations leading to discovery of genetic diversity in the 24 genotypes of *A. hypogaea* L., a

Table-2 : Jaccard's similarity for ISOZYME profile generated by agarose gel electrophoresis.

Geno-type	UG158	UG160	UG161	UG162	UG163	UG164	UG166	UG167	UG168	UG169	UG170	UG173	UG174	UG175	UG177	UG178	UG179	UG181	UG182	UG184	UG5	GG7	UG172	PM2
UG158	1.00																							
UG160	0.1	1.00																						
UG161	0.88	0.88	1.00																					
UG162	0.88	0.88	1.00	1.00																				
UG163	0.88	0.88	0.77	0.77	1.00																			
UG164	1.00	1.00	0.88	0.88	0.88	1.00																		
UG166	0.88	0.88	0.77	0.77	0.77	0.88	1.00																	
1UG167	0.88	0.88	0.77	0.77	0.77	0.88	1.00	1.00																
UG168	0.77	0.77	0.88	0.88	0.66	0.77	0.66	0.66	1.00															
UG169	0.88	0.88	1.00	1.00	0.77	0.88	0.77	0.77	0.88	1.00														
UG170	0.77	0.77	0.66	0.66	0.66	0.77	0.88	0.88	0.55	0.66	1.00													
UG173	0.77	0.77	0.66	0.66	0.66	0.77	0.88	0.88	0.55	0.66	1.00	1.00												
UG174	0.66	0.66	0.77	0.77	0.55	0.66	0.55	0.55	0.66	0.77	0.44	0.44	1.00											
UG175	0.55	0.55	0.44	0.44	0.66	0.55	0.66	0.66	0.55	0.44	0.77	0.77	0.22	1.00										
UG177	0.77	0.77	0.88	0.88	0.66	0.77	0.66	0.66	1.00	0.88	0.55	0.55	0.66	0.55	1.00									
UG178	0.77	0.77	0.88	0.88	0.66	0.77	0.66	0.66	1.00	0.88	0.55	0.55	0.66	0.55	1.00	1.00								
UG179	0.88	0.88	1.00	1.00	0.77	0.88	0.77	0.77	0.88	1.00	0.66	0.66	0.77	0.44	0.88	0.88	1.00							
UG181	0.88	0.88	1.00	1.00	0.77	0.88	0.77	0.77	0.88	1.00	0.66	0.66	0.77	0.44	0.88	0.88	1.00	1.00						
UG182	1.00	1.00	0.88	0.88	0.88	1.00	0.88	0.88	0.77	0.88	0.77	0.77	0.66	0.55	0.77	0.77	0.88	0.88	1.00					
UG184	0.77	0.77	0.77	0.77	0.88	0.77	0.77	0.77	0.77	0.88	0.55	0.55	0.66	0.55	0.77	0.77	0.88	0.88	0.77	1.00				
UG5	0.88	0.88	0.77	0.77	0.77	0.88	0.77	0.77	0.88	0.77	0.66	0.66	0.55	0.66	0.88	0.88	0.77	0.77	0.88	0.66	1.00			
GG7	0.66	0.66	0.55	0.55	0.77	0.66	0.77	0.77	0.44	0.55	0.88	0.88	0.33	0.55	0.44	0.44	0.55	0.55	0.66	0.66	0.55	1.00		
UG172	0.77	0.77	0.88	0.88	0.66	0.77	0.66	0.66	1.00	0.88	0.55	0.55	0.66	0.55	1.00	1.00	0.88	0.88	0.77	0.77	0.88	0.44	1.00	
PM2	0.77	0.77	0.66	0.66	0.88	0.77	0.66	0.66	0.77	0.66	0.55	0.55	0.44	0.77	0.77	0.77	0.66	0.66	0.77	0.77	0.88	0.66	0.77	1.00

Table-4 : Total soluble protein (fresh weight basis) in 24 genotype of *A. hypogaea* L.

S. No.	Genotype	Total soluble protein concentration (mg/g)
1.	UG158	9.2
2.	UG160	13.6
3.	UG161	15.2
4.	UG162	12.9
5.	UG163	15.0
6.	UG164	14.9
7.	UG165	15.1
8.	UG167	14.5
9.	UG168	12.6
10.	UG169	9.2
11.	UG170	11.9
12	UG172	17.4
12.	UG173	13.1
13.	UG174	14.2
14.	UG175	11.8
15.	UG177	14.7
16.	UG178	14.3
17.	UG179	15.0
18.	UG181	15.4
19.	UG182	12.8
20.	UG184	14.8
21.	PM2	16.4
23.	UG5	14.9
24.	GG7	12.9

total of 9 alleles were detected by the two isozymes (Table 2). In scoring the bands obtained, only easily resolved and bright isozyme bands were counted. POX and SOD isozymes showed 50 and 100% polymorphism, respectively. In the same line of works, Cluster tree analysis was carried out by UPGMA method based on genetic distance. Similarity coefficient ranged from 0.44 to 1.00 between 24 *A. hypogaea* L. (Table-3). The average similarity across all the genotypes was found out to be 0.72, showing that genotypes were genetically similar. All genotypes could be placed into three clusters at a similarity coefficient of 0.77 (Fig. 3). Cluster-I included six genotypes i.e., UG165, UG167, UG170, UG173,UG175 and GG7at a similarity coefficient of 0.78. Cluster I was further divided into two subclusters. Subcluster I included two genotypes viz., UG175 and GG7at a similarity coefficient of 0.89.

Subcluster I divide into two subgroups, subgroup A posses one genotype i.e.,UG 175 at a similsrity coefficient 1.00. Subgroup B posses one genotype i.e.,GG7 at a similsrity coefficient 1.00. Subcluster II included four genotypes viz., UG165, UG167, UG170 and UG173 at a similarity coefficient of 0.89. Subcluster I divide into two subgroups, subgroup A posses two genotype i.e.,UG170 and UG163 at a similarity coefficient 1.00. Subgroup B also posses two genotype i.e., UG165 and UG167 at a similarity coefficient 1.00.

One genotype UG174 which is far apart from all 24 genotypes genotypes at a similarity coefficient of 1.0.

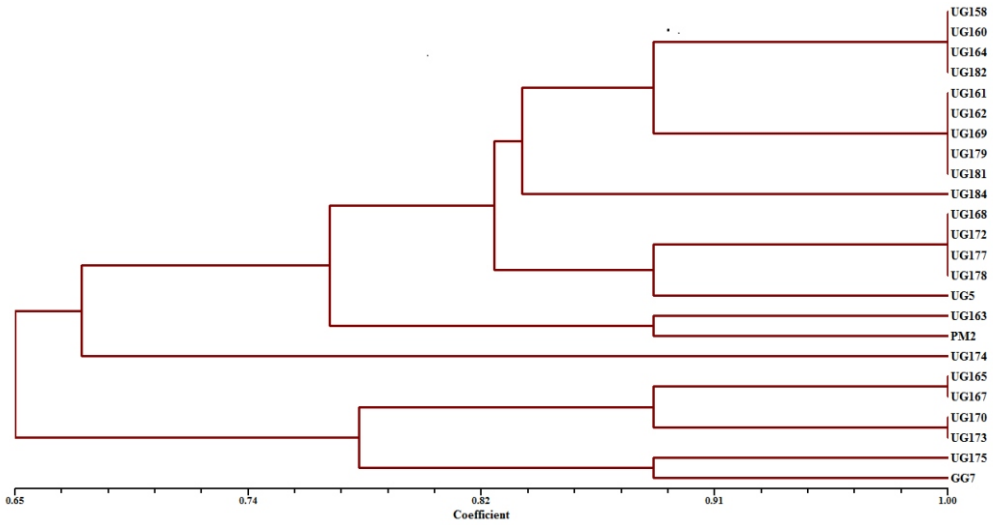

Fig-3 : Dendrogram constructed for *Arachis hypogaea L.* genotypes for Isozymes using UPGMA cluster analysis based on Jaccard Similarity Coefficients.

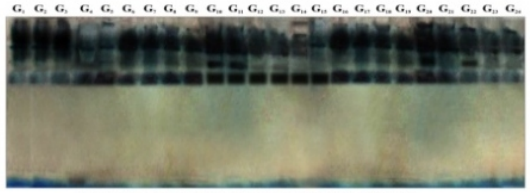

Plate 1: Zymogram showing isozymic bands of Peroxidase

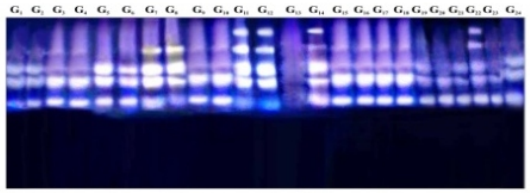

Plate 2: Zymogram showing isozymic bands of Superoxide dismutase

G_1-G_{24} represent following *Arachis hypogaea* L. genotypes

G_1 –UG158	G_2 -UG160	G_3 -UG161	G_4 -UG162	G_5 -Ug163	G_6 -UG164
G_7 –UG165	G_8 -UG167	G_9 -UG168	G_{10} -UG169	G_{11} -UG170	G_{12} -UG173
G_{13}–UG174	G_{14}-UG175	G_{15}-UG177	G_{16} -UG178	G_{17} -UG179	G_{18}-UG181
G_{19}–UG182	G_{20} -UG184	G_{21}-UG5	G_{22} -GG7	G_{23} -UG172	G_{24} -PM2

Cluster-II included two genotypes i.e., PM2 and UG174 at a similarity coefficient 0.89. Cluster II divide into two subcluster, subcluster I posses one genotype i.e.,UG 174 at a similarity coefficient 1.00. Subcluster II posses one genotype i.e.,PM2 at a similarity coefficient 1.00.

Cluster III comprised of 15 genotypes viz., UG158, UG160, UG164, UG182, UG161, UG162, UG169, UG184, UG179, UG181, UG168, UG172, UG177, UG178 and UG5 at a similarity coefficient 0.77. It was further divided into two subclusters at a similarity coefficient 0.82. Subcluster I included five genotypes viz., UG168, UG172, UG177,UG178 and UG5 at a similarity coefficient of 0.83. Subcluster II further divided into two subgroups at a similarity coefficient 0.89. Sub group A consisted only one genotype i.e., UG5 at a similarity coefficient 1.00. Subgroup B consisted four genotypes i.e., UG168, UG172, UG177 and UG178 at a similarity coefficient 1.00

Subcluster II included ten genotypes viz., UG158, UG160, UG164, UG182, UG161, UG162, UG169, UG184, UG179 and UG181 at a similarity coefficient of 0.82. Subcluster II further divided into two subgroups at a similarity coefficient 0.83, subgroup A comprises only one genotype i.e., UG184 at a similarity coefficient 1.00. Subgroup B consists of nine genotypes viz., UG158, UG160, UG164, UG182, UG161, UG162, UG169, UG179 and UG181, at a similarity coefficient 0.89. In subgroup B genotypes UG158, UG160, UG164 and UG182 present on same scale at a similarity coefficient 1.00, while genotypes, UG161, UG162, UG169, UG179 and UG181 on the same scale at a similarity coefficient of 1.00. Genetic diversity and distance derived from isozyme analysis were very low due to small number of polymorphic alleles. This has also been reported by (9) in Arachis species who studied 4 isozymes systems.

Researchers can use information on genetic similarity to make decisions regarding selection of superior genotypes for improvement or for use as parents for the development of future cultivars through hybridization

Total soluble protein : The total soluble protein of *A. hypogaea L. genotypes* as estimated by Lowry's method reveals the most soluble protein content found in genotypes UG-172 (17.4 mg/g) followed by PM2 (16.4 mg/g) and UG181 (15.4 mg/g), whereas the least soluble protein content was observed in genotypes UG169 and UG158 (9.2 mg/g), respectively as shown in Table-4.

CONCLUSION

Genetic diversity of twenty four genotypes of *A. hypogaea* L. was investigated for, biochemical variations by using 2 isozymes and significant variation found in term activity of enzymes for all 24 genotypes. So these data can be used for improvement of breeding lines. As these enzyme indicative of stress in plant so genotypes having major activity zones can be further used in stress analysis.

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