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Assessment of genetic diversity amongst twenty four genotypes of groundnut (*Arachis hypogaea* L.) with Inter simple sequence repeats

DHWANI SHARMA, ARUNABH JOSHI AND DEVENDRA JAIN

Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture & Technology, Udaipur-313 001, Rajasthan

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ABSTRACT

DNA-based markers provide a reliable means of estimating the genetic relationships between genotypes and taxonomic groups as compared to morphological markers. Inter simple sequence repeat (ISSR) markers are useful in detecting genetic polymorphisms among accessions by generating a large number of markers that target multiple microsatellite loci distributed across the genome. Twenty ISSR primers were used for PCR amplification out of which 18 primers get amplified. The Inter Simple Sequence Repeat (ISSR) analysis showed a high per-cent of polymorphism (85.71). A total of 77 amplified bands were obtained using 18 ISSR primers out of which 67 were found polymorphic. PIC values ranged from 0.07 to 0.37 with an average of 0.300 across all the genotypes. Jaccard's similarity coefficient values for ISSR primers ranged from 0.54-0.90 with an average of 0.72. Based on dendrogram generated through UPGMA method and PCA, most of genotypes could be divided into five major clusters. Cluster I, Cluster II, Cluster IV, included 2 genotypes each while cluster III included 9 genotypes. The cluster V included 5 genotypes. However, genotypes UG162 and UG158, UG162 and UG182 were found to be genetically diverse with a minimum similarity value of 0.59 followed bythe genotypes UG162 and UG182, PM2 and UG 158 having similarity value of 0.61.

Keywords: Groundnut PIC, UPGMA, similarity coefficient, ISSR, dendrogram.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important crop among oilseeds, as it can be consumed and utilized in diverse ways. It is an important source crop for edible oil and protein. It is important to identify the genetic diversity of groundnut genetic resources for cultivar development and evaluation of groundnut accessions. Knowledge about germplasm diversity and genetic relationships among breeding materials could be an invaluable aid in crop improvement strategies (Mohammadi and Prasanna, 2003). New molecular tools hold the promise of allowing the identification of genes involved in a number of traits including adaptive traits, and polymorphisms causing functional genetic variation. Conventional breeding techniques which are based on the processes of crossing, back-crossing and selection, proved to be time consuming. Therefore, molecular technology is increasingly becoming popular as a powerful tool for unambiguous authentication. Molecular techniques for detecting differences in the DNA of individual plants to examine variability in cultivar are useful for identification of potential parental lines. These differences in general are called molecular marker. These molecular markers used for characterization as well as phylogenic analysis in various plant species with reliable and authentic results (Behra *et al.*, 2008; Chandrika and Rai, 2009). DNA markers provide a direct measure of genetic diversity and go beyond diversity based on agronomic traits or geographic origin (Dreisigacker *et al.*, 2005), thus help in better germplasm management and develop more efficient strategies for crop improvement.

ISSR are arbitrary multiloci markers produced by PCR amplification with a micro satellite primer. They are advantageous because no primer genomic information is required for their use. Since 1994, a new molecular marker technique was discovered, called ISSR (Zietkiewicz *et al.* 1994). These are semi-arbitrary markers amplified by PCR in the presence of one primer complementary to target micro satellite. Like RAPDs, ISSR markers are quick and easy to handle and have been used in a number of plant species. ISSR markers overcome the short coming of the low reproducibility of RAPD; the high cost of AFLP, the complexity of SSR and represent a fast and a cost-efficient technique (Kurane *et al.* 2009). In

*Correspondence email: dhwaniisharma@gmail.com

the present study twenty ISSR primers were used for PCR amplification and diversity analysis carried out using UPGMA cluster analysis.

MATERIALS AND METHODS

The field studies were undertaken at CTAE (College of technology and engineering) Farm and Laboratory studies were taken at Rajasthan College of Agriculture, Udaipur and crop was raised during kharif 2016-17. The material comprised of 24 groundnut genotypes including three released varieties namely UG-5 (Pratap Raj Mungphali), PM-2 and GG-7 (Table 1). A field experiment was carried out with 24 groundnut genotypes in a randomized block design during *kharif,* 2016. The experimental material was planted in three replications. Each genotype was planted with row to row distance of 30 cm and plant to plant distance of 10 cm. Recommended agronomic practices were followed to raise a healthy crop.

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

Name of genotypes	Pedigree	Source	Name of genotypes	Pedigree	Source
UG-158	J 63 × TPG 41	DGR, Junagarh	UG-173	GG 2 × ICGV 91114-1	DGR, Junagarh
UG-160	GG 2 × B 95	DGR, Junagarh	UG-174	TG 40 × ICGV 86325	DGR, Junagarh
UG-161	GG 8 × TKG 19 A	DGR, Junagarh	UG-175	PBS 24030 × TG 37 A	DGR, Junagarh
UG-162	GG 2× TPG 41	DGR, Junagarh	UG-177	J 11 × TPG 41	DGR, Junagarh
GG 20 × PBS 24030	DGR, Junagarh	DGR, Junagarh	UG-178	ICGV 76 × ICGV 86305	DGR, Junagarh
ICGX 090018	ICRISAT	ICRISAT	UG-179	ICGV 86564 × TPG 41	DGR, Junagarh
GG 21 × R-2001-3	DGR, Junagarh	DGR, Junagarh	UG-181	ICGV 86590 × PBS 24030	DGR, Junagarh
GG 2 × TG 26	DGR, Junagarh	DGR, Junagarh	UG-182	UG 20 × ALR-3	DGR, Junagarh
GG 20 × TAG 24	DGR, Junagarh	DGR, Junagarh	UG-184	GG 5 × TPG 41	DGR, Junagarh
GG 20 × ICGV 86325	DGR, Junagarh	DGR, Junagarh	PM -2	ICGV- 86055 × ICG- (FDRS 10)	Junagarh
GG-7 × R-2001-3	DGR, Junagarh	DGR, Junagarh	UG-5	Selection from ICGV-98223	DGR, Junagarh
TG-37 A × GG 20	DGR, Junagarh	DGR, Junagarh	GG-7	S 206 × FEFR 81-1-9-B-B	DGR, Junagarh

DNA isolation, purification and quantification

In groundnut genotypes, genomic DNA was extracted from young leaves of 21-28 days old seedlings following CTAB (Cetyl trimethyl ammonium bromide) extraction method (Doyle and Doyle 1987). The quantification of DNA was done by measuring OD at the wavelengths of 260 nm and 280 nm on a spectrophotometer, quality of DNA checked on Agarose Gel Electrophoresis (Sambrook *et al.*, 1989) in 0.8 percent (w/v) agarose gel and visualized.

Polymerase Chain Reaction (PCR) Amplification

The primers used in analysis were anchored at 3′ end to ensure that perfect annealing of the primer occurs at the 3′ end of the microsatellite motif, thus obviating internal priming and smear formation. ISSR was carried out in 20 µl of reaction mix containing 25 ng genomic DNA, 2 µl of 10X Taq DNA polymerase buffer, 1.5 mM $MgCl_2$, 200 µM each dNTPs, 0.03 µM of primer and 1 unit of Taq DNA polymerase in an 200 µl Eppendorf. PCR condition for ISSR analysis included an initial predenaturation step of 4 minutes at 94^0C and following 35 cycles of amplification.

Denaturation	94^0C	45sec
Annealing*	28.9^0-55.5^0C	1min
Extension	72^0C	2min

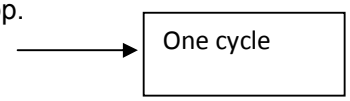
Final extension was carried out at 72^0C for 10 minutes and a hold temperature of 4^0C at the end. Primer annealing temperature depends on its Tm (Thermal melting temperature) value, which can be calculated. Annealing temperature varying from 28.9^0-55.5^0C were used for different primers. Separation of amplified products is carried out by agarose gel electrophoresis.

RESULTS AND DISSCUSSION

The amount of DNA isolated from various genotypes of groundnut ranged from 529 to 5904 ng/µl (Table 2). The genotype UG-172 yielded the highest amount of DNA (5904 ng/µl), whereas the lowest amount (599 ng/µl) was obtained from genotype UG-158.The ratio of absorbance (A260/A280) ranging from 1.70 to 1.96 revealed that the DNA obtained was free from contaminants like polysaccharides, protein and RNA. The quality of DNA was also checked by gel electrophoresis revealed a single discrete band in all genotypes

Molecular Marker Analysis of RAPD

Twenty ISSR primers were used in the present investigation, out of which eighteen primers showed amplification a total of 77 amplified bands out of which 67 were found polymorphic in 24 genotypes except 2 (UBC-872 and UBC-878) . The 18 ISSR primers that yielded the total number of bands observed for each primer was recorded separately and polymorphic bands percentage was calculated subsequently (Table 2). The total number of amplified bands varied between 2 (UBC-813) and 6 (UBC-817, UBC873 and UBC854) with an average of 5 per primer. The polymorphism percentage ranged from as low as 20% (UBC-815) to as high as 100% in 14 primers (UBC-810, UBC-811, UBC-813, UBC-814, UBC817, UBC818, UBC-820, UBC-822, UBC-826, UBC-834, UBC836, UBC-840, UBC-845 and UBC-873) and the average polymorphism was 87%. The overall size of PCR amplified products ranged between 100 bp to 2500 bp. PIC values ranged from 0.07 to 0.37 with an average of 0.300 across all the genotypes. One unique band was detected in PM2 genotype with UBC-817 ISSR primer. The size of this unique band was 300 bp.



Genetic Relationship and Cluster Tree analysis:

ISSR primers were further used to construct similarity matrix of 24 groundnut genotypes using 'Simqual' sub-programmeme of software NTSYS-pc. Dendrogram were constructed using these similarity matrix values as determined from ISSR data for 24 groundnut genotypes using unweighted pair group method with arithmetic average (UPGMA) sub-programmeme of NTSYS-pc software.

Table 2: Quality and quantity of total genomic DNA of groundnut

S. No.	Genotype code	Genotype	Quality (A260/A280)	Quantity (ng/µl)	S. No.	Genotype code	Genotype	Quality (A260/A280)	Quantity (ng/µl)
1.	G_1	UG158	1.93	599	13.	G_{13}	UG174	1.83	1571
2.	G_2	UG160	1.94	2757	14.	G_{14}	UG 175	1.85	3770
3.	G_3	UG161	1.93	1775	15.	G_{15}	UG177	1.96	907
4.	G_4	UG162	1.92	3061	16.	G_{16}	UG178	1.89	3483
5.	G_5	UG163	1.90	2103	17.	G_{17}	UG179	1.80	4250
6.	G_6	UG164	1.93	1433	18.	G_{18}	UG181	1.88	1863
7.	G_7	UG165	1.88	2767	19.	G_{19}	UG182	1.91	3627
8.	G_8	UG167	1.76	4900	20.	G_{20}	UG184	1.84	5250
9.	G_9	UG168	1.87	4532	21.	G_{21}	UG5	1.85	3501
10.	G_{10}	UG169	1.87	5257	22.	G_{22}	GG7	1.85	1953
11.	G_{11}	UG 170	1.78	5064	23.	G_{23}	UG172	1.87	5904
12.	G_{12}	UG173	1.8	1620	24.	G_{24}	PM2	1.82	1469

Table 3: DNA amplification profile and polymorphism generated in groundnut using 18 ISSR primers

S. No.	Primer Code	Ta* (°C)	Molecular weight range (bp)	Total no. of bands amplified	Polymorphic bands		PIC
					Number	Frequency (%)	
1.	UBC-810	42.9	400-900	4	4	100.0	0.218
2.	UBC-811	43.3	500-900	3	3	100.0	0.239
3.	UBC-813	43.3	500-1000	2	2	100.0	0.31
4.	UBC-814	41.3	300-1500	4	4	100.0	0.392
5.	UBC-815	44.9	200-1000	5	1	20.0	0.079
6.	UBC-817	52	300-2000	4	4	100.0	0.353
7.	UBC-818	52	400-900	4	4	100.0	0.328
8.	UBC-820	50	100-1000	5	5	100.0	0.369
9.	UBC-822	45	300-1000	4	2	50.00	0.076
10.	UBC-824	43.3	100-2000	5	4	80.0	0.392
11.	UBC-826	52	300-800	6	6	100.0	0.462
12.	UBC-834	49.9	400-1500	4	4	100.0	0.379
13.	UBC-836	43.3	500-1500	4	4	100.0	0.228
14.	UBC-840	45	200-900	4	4	100.0	0.178
15.	UBC-845	47.7	500-2500	3	3	100.0	0.229
16.	UBC-848	55.5	200-600	4	3	70.62	0.163
17.	UBC-854	48.0	400-2500	6	4	66.6	0.255
18.	UBC-873	45	400-1000	6	6	100.0	0.348
	Total			77	67	87.00	0.300

Ta=Annealing temperature*

Similarity Matrix values based on ISSR data:

Based on ISSR, the values of similarity coefficient ranged from 0.54 to 0.90 *i.e.,* 54-90% or genetic diversity ranged from 10 to 46% (Table3 & Plates of ISSR). Similar results were obtained by Singh *et al.* (2014) and Das *et al.* (2014). The average similarity coefficient value across all the genotypes was found out to be 0.72, showing that genotypes were moderately similar. Maximum similarity value of 0.90 was observed between genotypes UG181 and UG163 followed by UG184 and UG181 (similarity coefficient of 0.89). Genotypes UG162 and UG158, UG162 and UG182 were found to be genetically diverse with a minimum similarity value of 0.59 followed by UG162 and UG182, PM2 and UG158 similarity coefficient of 0.61.

ISSR Marker based Cluster Tree Analysis

The ISSR data were used to obtain a similarity matrix. The similarity coefficient was found to lie between 0.54-0.90. The observations showed that they could be divided into 5 major clusters at a similarity coefficient of 0.77 (Fig.1). Cluster I included two genotypes *viz.,* UG172 and UG167found similar at a similarity coefficient of 0.78. Cluster II also included two genotypes UG5 and PM2 that were similar at similarity coefficient of 0.80. Cluster III included nine genotypes *viz.,* UG182, UG169, UG170, UG168, UG179, UG184, UG181, UG164, and UG163. It could be divided into 5 sub-clusters. In sub-cluster I, one genotype UG169 was present at 0.92 similarity coefficient. In sub-cluster II, two genotypes UG170 and UG168 were related to each other at 0.87 similarity coefficient. In sub-cluster III, genotype UG179 was present at 0.92 similarity coefficient. In sub-cluster IV, two genotypes UG184 and UG181 were related to each other at 0.90 similarity coefficient. In sub-cluster V, two genotypes UG164 and UG163 were related to each other at 0.92 similarity coefficient. Genotype UG182 out-grouped from the sub-cluster analysis.

The cluster IV included 2 genotypes, UG178 and UG173. Both the genotypes were related to each other at 0.88 similarity coefficient. The cluster V included 5 genotypes namely UG177, UG175, UG174, UG161 and UG160. Cluster V could be subdivided into two sub-groups, sub-group I including two genotypes UG175 and UG174 were related to each other at 0.91 similarity coefficient. Sub-group II including two genotypes UG161 and UG160 were related to each other at 0.85 similarity coefficient. Genotypes UG177 was out-grouped from the sub-cluster. Also genotypes UG158 and UG165 were farthest from cluster analysis. Similar results were reported by Kanimozhi (2009).

Table 4: Jaccard's similarity coefficient for ISSR profile generated by agarose gel electrophoresis

Genotypes	UG 158	UG 160	UG 161	UG 162	UG 163	UG 164	UG 166	UG 167	UG 168	UG 169	UG 170	UG 173	UG 174	UG 175	UG 177	UG 178	UG 179	UG 181	UG 182	UG 184	UG 5	GG 7	UG 172	
UG158	1.00																							
UG160	0.75	1.00																						
UG161	0.71	0.85	1.00																					
UG162	0.59	0.74	0.83	1.00																				
UG163	0.68	0.80	0.74	0.70	1.00																			
UG164	0.66	0.77	0.71	0.62	0.92	1.00																		
UG166	0.70	0.76	0.80	0.68	0.72	0.72	1.00																	
UG167	0.61	0.75	0.79	0.62	0.66	0.66	0.70	1.00																
UG168	0.68	0.80	0.76	0.75	0.87	0.84	0.70	0.63	1.00															
UG169	0.68	0.70	0.63	0.54	0.84	0.81	0.59	0.63	0.79	1.00														
UG170	0.68	0.72	0.74	0.70	0.81	0.79	0.54	0.63	0.87	0.76	1.00													
UG173	0.74	0.64	0.74	0.64	0.68	0.68	0.62	0.68	0.74	0.79	0.76	1.00												
UG174	0.70	0.84	0.80	0.68	0.77	0.77	0.76	0.72	0.88	0.75	0.77	0.77	1.00											
UG175	0.66	0.85	0.79	0.67	0.79	0.79	0.70	0.76	0.84	0.74	0.74	0.74	0.90	1.00										
UG177	0.63	0.75	0.74	0.67	0.71	0.68	0.72	0.74	0.81	0.66	0.71	0.71	0.85	0.79	1.00									
UG178	0.72	0.76	0.85	0.71	0.77	0.75	0.74	0.75	0.83	0.77	0.80	0.88	0.87	0.80	0.80	1.00								
UG179	0.71	0.70	0.68	0.62	0.84	0.81	0.67	0.61	0.84	0.84	0.79	0.76	0.77	0.74	0.76	0.83	1.00							
UG181	0.64	0.71	0.77	0.76	0.90	0.83	0.71	0.67	0.83	0.77	0.83	0.72	0.76	0.72	0.70	0.81	0.83	1.00						
UG182	0.64	0.68	0.67	0.61	0.83	0.80	0.61	0.59	0.75	0.77	0.80	0.70	0.68	0.67	0.64	0.71	0.75	0.81	1.00					
UG184	0.70	0.71	0.75	0.66	0.88	0.85	0.71	0.72	0.85	0.83	0.83	0.70	0.79	0.75	0.75	0.79	0.88	0.89	0.79	1.00				
UG5	0.59	0.71	0.72	0.61	0.77	0.83	0.79	0.67	0.80	0.75	0.72	0.70	0.76	0.72	0.72	0.76	0.80	0.76	0.71	0.84	1.00			
GG7	0.64	0.55	0.64	0.58	0.62	0.67	0.61	0.57	0.67	0.70	0.72	0.77	0.61	0.54	0.57	0.74	0.67	0.68	0.68	0.68	0.79	1.00		
UG172	0.62	0.76	0.72	0.68	0.64	0.62	0.63	0.77	0.67	0.62	0.64	0.62	0.71	0.70	0.72	0.68	0.57	0.63	0.66	0.63	0.66	0.63	1.00	
PM2	0.61	0.64	0.63	0.51	0.63	0.71	0.70	0.63	0.63	0.63	0.66	0.68	0.70	0.66	0.61	0.64	0.63	0.64	0.67	0.70	0.80	0.72	0.64	1.00

PLATES OF DNA FRAGMENT AMPLIFIES BY ISSR PRIMERS

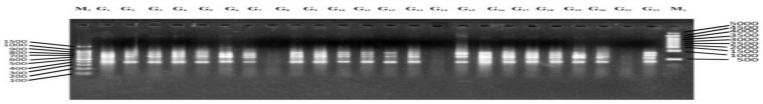


Plate 13 : ISSR profile generated through UBC-810 {5'(GA)$_8$T3'}

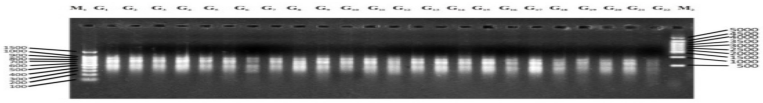


Plate 14 : ISSR profile generated through UBC-811 {5'(GA)$_8$C3'}

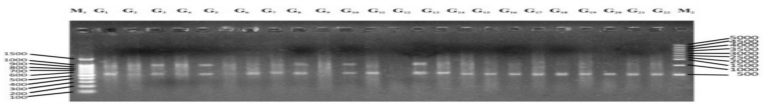


Plate 15 : ISSR profile generated through UBC-813 {5'(CT)$_8$T3'}

M1 – 100 bp DNA Ladder M2 – 500 bp DNA Ladder

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

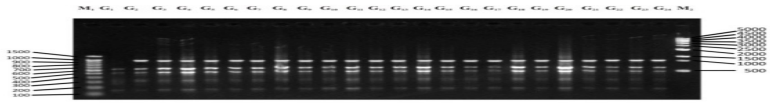


Plate 16 : ISSR profile generated through UBC-815 {5'(CT)$_8$G3'}

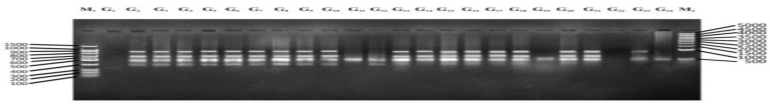


Plate 17 : ISSR profile generated through UBC-818 {5'(CA)$_8$A3'}

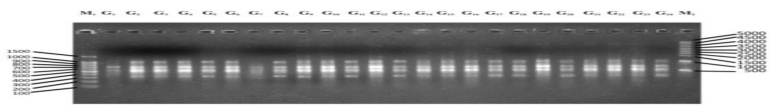


Plate 18 : ISSR profile generated through UBC-822 {5'(TC)$_8$A3'}

M1 – 100 bp DNA Ladder M2 – 500 bp DNA Ladder

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

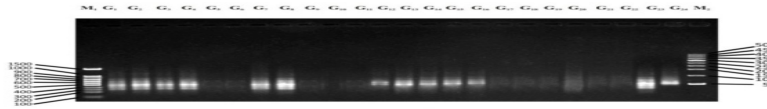


Plate 19 : ISSR profile generated through UBC-826{5'(AC)$_8$C3'}

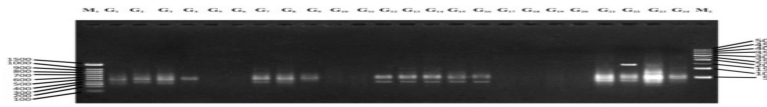


Plate 20 : ISSR profile generated through UBC-834 {5'(AC)$_8$Y*T3'}

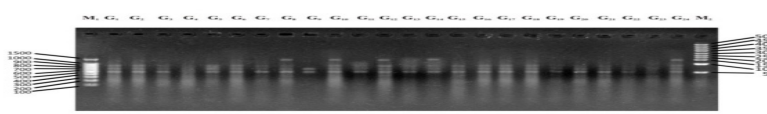


Plate 21 : ISSR profile generated through UBC-836 {5'(AG)$_8$YA3'}

M1 – 100 bp DNA Ladder M2 – 500 bp DNA Ladder

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

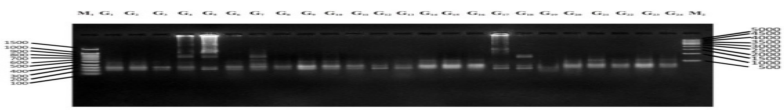


Plate 22 : ISSR profile generated through UBC-840 {5'(GA)$_8$YT3'}

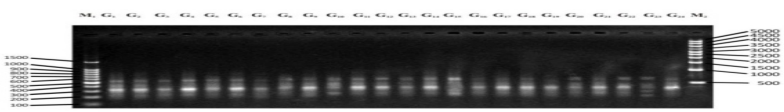


Plate 23 : ISSR profile generated through UBC-848 {5'(CA)$_8$RG3'}

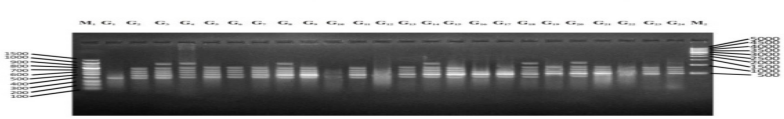


Plate 24 : ISSR profile generated through UBC-873 {5'(GATA)$_4$3'}

M1 – 100 bp DNA Ladder M2 – 500 bp DNA Ladder

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

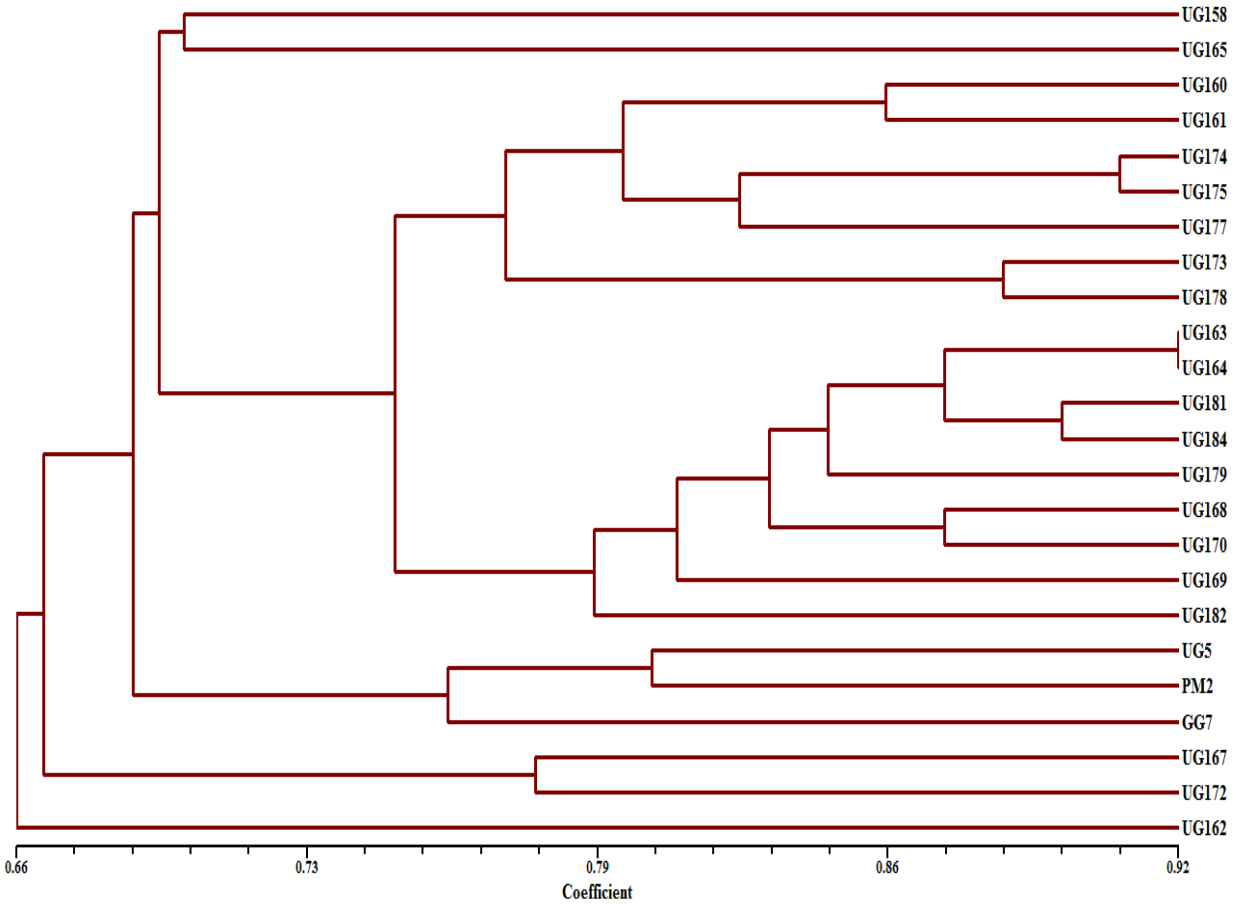


Fig. 1 Dendrogram generated for ISSR

PCA Based on ISSR data-

Two and three dimension principal component analysis based on ISSR data showed similar clustering of 24 genotypes as evident from cluster tree analysis. Dice similarity coefficients ranged from 0.78 to 0.92, indicative of an average level of variation among the genotypes. As visible in the dendrogram, the genotypes that were closer were more similar than those that were lying apart. Similar observations were recorded with PCA as well. Cluster I included seven genotypes *viz.,* UG5, UG170, UG179, UG164, UG181, UG163 and UG184; and the cluster II included 2 genotypes *viz*., UG162 and UG167 that lay closer to each other; and cluster III included 3 genotypes *viz*., UG175, UG160, and UG161; and cluster IV included UG174, UG178 and UG168; and cluster V included two genotypes UG165 and G177.Genotype GG7 was laying far apart followed by four other genotypes UG182, UG173, UG158 and PM2.

The study showed that 24 genotypes used for study had significant level of diversity and genotypes those are falling in different clusters are genetically more diverse; and can be correlated with morphological data and can be used in crop improvement program.

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