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Diversity analysis in *Arachis* subspecies using phenotypic characterization and microsatellite (SSR) markers

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Abstract
Groundnut (*Arachis hypogaea* L.) is one of the important economic crops of the world. It is the world's fourth most important source of edible oil and third most important source of vegetable protein. A study was conducted at Department of Seed Science and Technology, B. A. College of Agriculture, AAU, Anand, to investigate morphological and molecular characterization in different *Arachis* spp. The experiment was laid out in randomized complete block design with two replications and evaluated for 28 quantitative and 20 qualitative characters. Genetic distances among the genotypes obtained from the analysis of 20 qualitative traits ranged from 1.00 between the pairs ICG1994 and AG2245 and upto 9.70 between pairs ICG6813 and JB1180. Dendrogram developed using average taxonomic distance matrix by UPGMA method formed four major clusters. The dendrogram could not establish any distinct relationship between different botanical groups. However, few genotypes belonging to spanish bunch group tend to cluster together exhibiting their common phylogenetic relationship. This was also evident in the case of Virginia bunch cultures where a common phylogenetic relationship was observed. Pooled SSR analysis of 50 groundnut genotypes using 23 SSR primers generated a total of 1293 scorable bands with 251 alleles. The average PIC value was 0.670. The average genetic similarity coefficient obtained by using SSR markers for different genotypes in this study was 0.27.

Keywords: Dendrogram, clusters, diversity, descriptors, characters

1. Introduction
Groundnut is also called as the "King" of oilseeds or "Wonder nut" and "Poor man's cashewnut". Groundnut (*A. hypogaea*) is classified into two subspecies, *viz*. ssp. *hypogaea* and ssp. *fastigiata* based on variation in morphology. Further, the ssp. *hypogaea* is bifurcated into var. *hypogaea* (Virginia bunch/runner) and var. *hirsuta* (Peruvian runner), and likewise ssp. *fastigiata* into var. *fastigiata* (Valencia), *Peruviana*, *aequatoriana* and var. *vulgaris* (Spanish bunch). These distinctions are made based on the presence (subsp. *fastigiata*) or absence (subsp. *hypogaea*) of flowers on the main axis and various other morphological traits such as growth habit, pod shape and pod reticulation. Only four botanical types namely, Virginia bunch (VB), Virginia runner (VR), Valencia (VL), and Spanish bunch (SB) are exclusively cultivated by the farmers owing to their agronomic attributes and market value. The characterization of diversity in germplasm collection is important to plant breeders to utilize and to the gene bank curators to manage the collection efficiently and effectively.

2. Materials and methods
The experimental material comprised of 50 *Arachis* subspecies genotypes. It included five different *Arachis* botanical types *viz*; spanish bunch, virginia bunch and valencia (cultivated) as well as the wild species *viz*; *peruviana* and *aequatoriana.* The experiment was laid out in randomized completely block design with two replications in two seasons. The groundnut germplasm was characterized morphologically for 20 qualitative and 28 quantitative traits using the descriptor for groundnut (IBPGR/ICRISAT, 1992) [4].
Data were recorded on five randomly selected plants of each entry per replication. The clustering was done on the basis of qualitative characters using UPGMA method. The molecular data were analyzed by using softwares NTSYSpc version 2.02, Alpha EaseFC4.0.0 and GenALEx 6.5.

2.1 Experimental materials

List of genotypes

Spanish Bunch				Virginia Bunch	
Sr. No.	**Genotype**	**Sr. No.**	**Genotype**	**Sr. No.**	**Genotype**
1.	J-68[a]	16.	GG-2[a]	1.	ICGV-00308[a]
2.	J -71[a]	17.	GG-5[a]	2.	ICGV-00309[a]
3.	J -73[a]	18.	GG-6[a]	3.	ICGV-00310[a]
4.	JB-1109[a]	19.	GG-7[a]	4.	ICGV-00321[a]
5.	JB-1137[a]	20.	TG-26[a]	5.	ICGV-00380[a]
6.	JB-1142[a]	21.	TAG-24[a]	6.	ICGV-00387[a]
7.	JB-1144[a]	22.	TPG-41[a]	7.	ICGV-00429[a]
8.	JB-1145[a]	23.	ICG 1122[b]	8.	ICGV-00440[a]
9.	JB- 1168[a]	24.	ICG 1173[b]	9.	ICGV-00441[a]
10.	JB- 1176[a]	25.	ICG 1323[b]	10.	ICGV-95070[a]
11.	JB-1180[a]	26.	ICG 1326[c]	11.	ICGV-99083[a]
12.	JB-1184[a]	27.	ICG 1994[b]	12.	ICGV-99213[a]
13.	AG-2240[a]	28.	ICG 3267[b]	13.	GG-20[a]
14.	AG-2245[a]	29.	ICG 4750[b]	14.	ICG 12370[b]
15.	AG-1[a]	30.	ICG 12697[b]	15.	ICG 14482[b]
		31.	ICG 9619[b]	16.	ICG 6813[b]
Valencia		*Peruviana*		*Aequatoriana*	
Sr. No.	Genotype	Sr. No.	Genotype	Sr. No.	Genotype
1.	ICG 7412[b]	1.	ICG 10933[b]	1.	ICG 12625[b]

Source: a-Regional Research Station, AAU, Anand, b-International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru (A.P),c-Main Oil Seed Research Station, JAU, Junagadh

2.2 Morphological characterization

Morphological characterization was done for two consecutive seasons under the field conditions. The qualitative traits included both binary (present or absent), ordinal (absent, slight, moderate, prominent) parameters.

All the observations were taken from five plants at random for each accession for both seasons and the data was pooled over the seasons for the analysis of quantitative characters.

The quantitative traits were recorded to check the genetic potential of the accessions for various yield related traits, which may be of useful for breeders for further utilization through hybridization and selection.

Pair wise genetic dissimilarities (E_{ij}) between genotypes were estimated by the SIMINT (similarity for interval data) function using Average taxonomic distance (E_{ij}). Clustering was done using the symmetric matrix of dissimilarity coefficient (DIST) and clusters obtained on Unweighted Pair Group Arithmetic Mean (UPGMA) using SAHN (Sequential, Agglomerative, Hierarchical Nested clustering method) module of NTSYS-pc version 2.02i (Rohlf, 1998) [11].

2.3 Molecular Characterization

Total DNA was extracted from the leaves by Cetyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980) with some modifications. A total of 31 Simple Sequence Repeat primers were used for molecular characterization of different accessions (Table 1).

Table 1: List of SSR Primers with their sequences

Sr. No	Marker	Expected product size (bp)	F/R	Primer sequences	*Tm*
1	PM3	100-253	F	GAA AGA AAT TAT ACA CTC CAA TTA TGC	57.4
			R	CGG CAT GAC AGC TCT ATG TT	57.3
2	PM15	177-250	F	CCT TTT CTA ACA CAT TCA CAC ATG A	58.1
			R	GGC TCC CTT CGA TGA TGA C	58.8
3	PM32	108-138	F	AGT GTT GGG TGT GAA AGT GGG GGA CT	66.4
			R	CGG AAC AGT GTT TAT C	46.6
4	PM35	142-162	F	TGT GAA ACC AAA TCA CTT TCA TTC	55.9
			R	TGG TGA AAA GAA AGG GGA AA	53.2
5	PM36	195-255	F	ACT CGC CAT AGC CAA CAA AC	57.3
			R	CAT TCC CAC AAC TCC CAC AT	57.3
6	PM42	122-200	F	ACG GGC CAA GTG AAG TGA T	56.7
			R	TCT TGC TTC TTT GGT GAT TAG C	56.5
7	PM45	50-100	F	TGA GTT GTG ACG GCT TGT GT	57.3
			R	GAT GCA TGT TTA GCA CAC TTG A	56.5
8	PM50	109-365	F	CAA TTC ATG ATA GTA TTT TAT TGG ACA	55.9
			R	CTT TCT CCT CCC CAA TTT GA	55.3
9	PM137	104-366	F	AAC CAA TTC AAC AAA CCC AGT	54.0
			R	GAA GAT GGA TGA AAA CGG ATG	55.9
10	PM183	110-166	F	TTC TAA TGA AAA CCG ACA AGT TT	53.5
			R	CGT GCC AAT AGA GTT TTA TAC GG	58.9
11	PM188	90-110	F	GGG CTT CAC TGC TTT TGA TT	55.3
			R	TGC GAC TTC TGA GAG GAC AA	57.3

12	PM210	190-240	F	CCG CAG ATC TTC TCC TGT GT	59.4
			R	CCT CCT CAT CCT CTA AAC TCT GC	62.4
13	PM375	90-110	F	CGG CAA CAG TTT TGA TGG TT	55.3
			R	GAA AAA TAT GCC GCC GTT G	54.5
14	PM377	130-164	F	ACG CTC ACA TGT TTG CTT TG	55.3
			R	GCT CGA TTT GAT TTG GGT GA	55.3
15	PM384	70-110	F	GGC GTG CCA ATA GAG GTT TA	57.3
			R	TGA AAA CCA ACA AGT TTA GTC TCT CT	58.5
16	PM402	240-280	F	CCG CCC TAA AAA CTG TAT TCG	57.9
			R	CCT AAG AGT ACA CGC GAC GA	59.4
17	PMc297	200-280	F	ATG CAC CTG CAA GTG AAG AG	57.3
			R	TCA AGG ATG CAG CAA GAC AC	57.3
18	PMc478	210-250	F	GTC GTG CAG GTC AAA GTG C	58.8
			R	TTA AGA TGG GTG CCT GCA AT	55.3
19	PMc588	140-200	F	CCA TTT TGG ACC CCT CAA AT	55.3
			R	TGA GCA ATA GTG ACC TTG CAT T	56.5
20	PGS15D03	290-370	F	CAT GCC ATC ATC ACA ACA CA	55.3
			R	GGA GGA AGC AAT GGT TTC AG	57.3
21	Ah-41	245-280	F	CGC CAC AAG ATT AAC AAG CAC C	60.3
			R	GCT GGG ATC ATT GTA GGG AAG G	62.1
22	Ah-6-125	225-280	F	TCG TGT TCC CGA TGC C	54.3
			R	GCT TTG AAC ATG AAC ATG CC	55.3
23	Ah-191	480-570	F	TTG TTG AGG AGG TGA TGC TGG T	60.3
			R	CCC AAA GGC CGG TAA ATG AAT C	60.3
24	Ah-193	122-472	F	CTT GCT GAA GGC AAC TCC TAC G	62.1
			R	TCG GTT TGT CTC TTT GGT CAC TC	60.6
25	Ah-229	193-293	F	GCA AAC ATC TTC CTT CCC AAC A	58.4
			R	ATT GAC GTA AGC TGC CAA GAG G	60.3
26	Ah-522	140-180	F	GTC AAT GCC GAA CCT CAA CGT A	60.3
			R	TTC ACC ATC ATC TCC AAC GCT T	58.4
27	PM-106	145-180	F	TGC ATT GTG CTT GAA CTT CC	55.3
			R	TGC AAG CAA GCA GAG AGC AAA GAG A	63.0
28	PM-179	70-100	F	CTG ATG CAT GTT TAG CAC ACT T	56.5
			R	TGA GTT GTG ACG GCT TGT GT	57.3
29	IPAHM-689	240-300	F	GAT GAC AAT AGC GAC GAG CA	57.3
			R	GTA AGC CTG CAG CAA CAA CA	57.3
30	pPGPseq4G2	280-300	F	TCA ACT TTG GCT GCT TCC TT	55.3
			R	TCA ACC GTT TTT CAC TTC CA	53.2
31	pPGPseq12F7	270-280	F	TGT CGT TGT AAG ACC TCG GA	57.3
			R	TTG GTT TCC TTA AGG CTT CG	55.3

*T*m – melting temperature

2.4 Master mix preparation

All the PCR reactions were carried out in 200 µl thin walled PCR tubes. Cocktail for PCR reaction was prepared by adding 10× Standard Taq Buffer with $MgCl_2$ (BioLabs, UK) followed by forward and reverse primers, dNTPs, Taq DNA polymerase (BioLabs, UK) and template DNA. The reagents were mixed by gently tapping against the tube and brief spin (~3,000 rpm for 30 seconds). The tubes were then placed in the Thermal Cycler (Applied BiosystemsVeriti, Foster City, CA, USA) for cyclic amplification. The amplified products of SSR were analyzed electrophoretically using 2.8% agarose gel.

Components for PCR mixture for SSR

Sr. No.	Reagents	Volume
1	10× Standard *Taq* Buffer with $MgCl_2$ (BioLabs, UK)	2.5 µl
2	Forward Primer (10 p moles/µl) (MWG biotech, Germany)	0.5 µl
3	Reveres Primer (10 p moles/µl) (MWG biotech, Germany)	0.5 µl
4	dNTPs (2.5mM each) (BioLabs, UK)	0.5 µl
5	*Taq* DNA Polymerase (5U/ µl) (BioLabs, UK)	0.2 µl
6	Template DNA (20ng/µl)	2.0 µl
7	Sterile distilled water	18.8 µl
	Total volume	25.0 µl

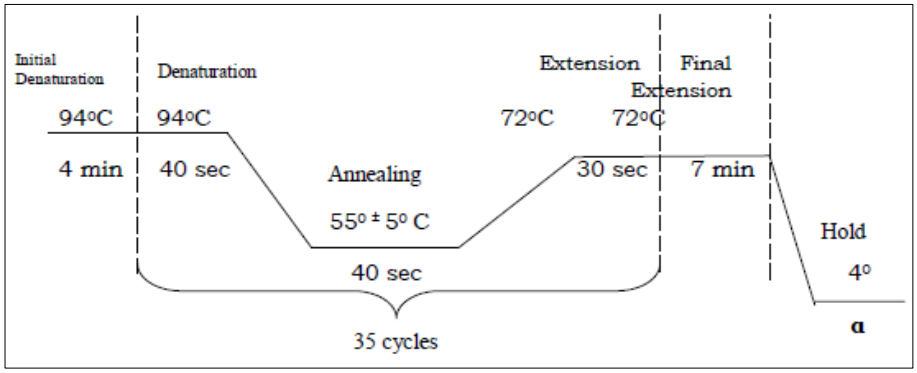

Fig 1: Steps in PCR amplification reaction conditions for SSR primers

2.5 Data analysis

Clear and distinct bands amplified by SSR primers were scored for the presence and absence of the corresponding band among the genotypes. The scores 1 and 0 indicates the presence or absence of bands, respectively. The softwares used for the analysis of the scored data were NTSYSpc version 2.02 (Rholf 1994). The size of the amplified product was calculated on the basis of its mobility relative to molecular mass of marker (100bp, MBI, Fermentas). The molecular weight of the PCR products was estimated by Alpha EaseFC4.0.0 software (Alpha Innotech Corporation, USA) for each primer to analyze alleles range.

Polymorphism Information content (PIC) was calculated according to formula described by Bootstein *et al*., (1980) [3] and Anderson *et al*. (1995) [1].

$$PIC = 1 - \sum_{i=1}^{k} Pi^2$$

Where *k* is total number of alleles detected for a given marker locus and P*i* is the frequency of the *i*th allele in the set of genotypes investigated.

2.6 Genetic similarity and cluster analysis

Coefficients of similarity were calculated by using Jaccard's similarity coefficient by SIMQUAL function and cluster analysis was performed by agglomerative technique using the UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) method by SAHN clustering function of NTSYSpc. Relationships among the genotypes were expressed in the form of dendrograms and genetic similarity matrix.

Pair-wise comparisons of the cultivars based on the ratio of exceptional to common alleles were used to calculate the genetic similarity by Dice coefficients using SIMQUAL sub-program in similarity routine of software NTSYS-pc version 2.2 (Exeter Software, Setauket, NY, U.S.A.) software package (Rohlf, 2005) [10]. Estimates of genetic similarity were considered among all pairs of the genotypes according to Nei and Li (1979) [8]. Dendrogram was constructed using UPGMA to calculate genetic associations among peanut accessions by the protocol earlier described by Rohlf, 2005 [10]. The PIC for each SSR was determined according to Anderson *et al*. (1993).

2.7 A summary of the statistics used in analysis of SSR markers

The analysis was performed using GenALEx 6.5 software and calculated manually in Microsoft *Excel* 2007 (Peakall and Smouse, 2006) [9].

- Allele Frequency (Codominant Data)

$$FreqAllele\ x = \frac{2Nxx+Nxy}{2N}$$

Calculated for a single locus. Determined for each allele.*Nxx*= # of XX homozygous individuals, and *Nxy*= # of XY heterozygous individuals, where Y can be any other allele. *N* = the number of samples can also be determined simply by direct count of the proportion of different alleles (Nei M, 1979) [8].

- Observed Heterozygosity (H_0) (Codominant Data)

$$Ho = \frac{No\_of\_Hets}{N}$$

This was calculated on a per locus basis. GenAlEx also provides the arithmetic mean across loci. Where the number of heterozygotes is determined by direct count and *N* = the number of samples (Nei M, 1987).

- Expected Heterozygosity (H*e*) or Genetic Diversity (Codominant Data)

$$He = 1 - \sum p_i^2$$

Calculated on a single locus basis. GenAlEx also provides the arithmetic mean across loci. Here, *pi* is the frequency of the *i*th allele.

3. Results and discussion

In any crop improvement programme, assessment of parental divergence is an important and foremost objective. The threat to genetic erosion has led to a significant interest in the assessment of genetic diversity in germplasms collections (Manifesto *et al*., 2001) [6]. Molecular markers are useful complement to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects and allow cultivar identification early in plant development. Simple Sequence Repeats (SSR) is used as a primer to amplify regions between the microsatellites. This marker reveals a much larger number of fragments per primer than RAPD analysis (Bajpai *et al*., 2008) [2].

3.1 Morphological characterization

The analysis of variance revealed highly significant differences among genotypes for all the characters studied. This indicated the presence of sufficient variability in the

experimental material. The results showed various range (Table 2) for different yield attributing characters *viz*; days to 50% flowering (28.25-34.50), days to maturity (103.00-136.25), number of mature pods/plant (12.00-47.75), pod yield/plant (g) (11.51-52.05), kernel yield/plant (g) (7.95-34.49), hundred pod mass (g) (62.42-153.41), shelling% (42.92-84.03), hundred seed weight (g) (34.43-76.57), sound mature kernel (%) (53.24-96.34), oil content (%) (42.51-52.57) and protein content (%) (25.14-31.14).

Table 2: Range of different quantitative characters

S.N.	Characters	*kharif*	Summer	Pooled
1	Days to initiation of germination	4.50-9.50	5.00-11.50	4.75-10.25
2	Days to initiation of flowering	22.00-27.00	21.50-32.50	21.80-29.00
3	Days to 50 % flowering	27.00-34.50	28.00-35.50	28.25-34.50
4	Days to maturity	108.50-140.00	97.50-132.50	103.00-136.25
5	Plant height (cm)	23.00-65.50	18.05-63.95	20.53-64.73
6	Length of primary branch (cm)	26.00-43.55	19.55-42.05	23.03-42.80
7	Number of primary branches	5.50-8.50	3.50-7.50	4.50-8.00
8	Number of secondary branches	3.00-7.50	2.00-6.00	2.75-6.75
9	Leaf length (cm)	3.95-7.00	3.55-6.74	3.75-6.82
10	Leaf width (cm)	2.18-3.35	1.96-3.15	2.12-3.20
11	Leaf length/leaf width	1.56-2.55	1.48-2.50	1.52-2.51
12	Number of mature pods / plant	13.00-48.50	10.50-47.00	12.00-47.75
13	Number of immature pods / plant	1.00-8.00	2.50-10.50	1.75-9.25
14	Pod yield/plant (g)	12.58-53.54	10.45-50.56	11.51-52.05
15	Kernel yield/plant (g)	8.58-36.42	6.98-32.56	7.95-34.49
16	Number of one seeded pod (%)	1.50-43.00	3.50-45.50	2.50-44.25
17	Number of two seeded pod (%)	57.00-97.50	54.50-96.50	55.75-97.00
18	Number of three seeded pod (%)	0.05-14.99	0.05 -13.96	0.05 -14.22
19	Pod length (cm)	1.45-3.54	1.35-3.40	1.40-3.47
20	Pod width (cm)	1.04-1.64	0.96-1.59	1.01-1.61
21	Hundred pod mass (g)	64.50-115.59	60.34-152.34	62.42-153.41
22	Shelling per cent (S %)	44.62-84.67	41.22-83.39	42.92-84.03
23	Seed length (cm)	1.02-2.07	0.78-1.99	0.90-2.03
24	Seed width (cm)	0.46-1.48	0.43-1.23	0.44-1.26
25	Hundred seed weight (g)	35.05-77.30	33.80-75.94	34.43-76.57
26	Sound mature kernel (SMK %)	54.62-97.34	51.86-95.75	53.24-96.34
27	Oil content (%)	43.45-53.62	41.32-51.52	42.51-52.57
28	Protein content (%)	25.56-31.40	24.71-30.89	25.14-31.14

Genetic diversity analysis through phenotypic markers

In the present investigation genetic diversity analysis was conducted in 50 groundnut genotypes based on 20 qualitative traits or phenotypic characters by developing dissimilarity matrices as per UPGMA method. Genetic dissimilarities ranged from 1.00 between the pairs ICG 1994 and AG2245 and up to 9.70 between pairs ICG6813 and JB1180. The groundnut genotypes were clustered into mainly two groups such as A and B based on average taxonomic distance by UPGMA method. The dendrogram developed (Figure 2) on the basis of phenotypic characters could not establish any distinct relationship between different botanical groups. However, few genotypes belonging to spanish bunch group tend to cluster together exhibiting their common phylogenetic relationship. This was also evident in the case of virginia bunch cultures where a common phylogenetic relationship was observed.

3.2 Molecular characterization

In the present study, molecular characterization of *Arachis ssp*. was done using microsatellite (SSR) markers (Figure 4 to 9). Pooled SSR analysis of 50 groundnut genotypes using 23 SSR primers generated a total of 1293 scorable bands with 251 alleles. The average PIC value was 0.670. The maximum scorable bands (96) were generated by primer PM377, whereas primer PM15 generated only 30 scorable bands. Maximum alleles (17) were generated by primer PGS15D03 whereas minimum (6) was observed in primer Ah522, and pPGPseq4G2. The highest PIC value (0.835) was exhibited by primer PM3 while lowest PIC (0.424) was exhibited by PM15 with an average of 0.670.

The data of microsatellite markers for 50 *Arachis ssp.* genotypes were utilized for calculating genetic similarity coefficients and constructing dendrogram. The maximum similarity index of 0.94 was obtained between the genotypes AG-1 and GG-2. The average genetic similarity coefficient obtained by using SSR markers for different genotypes in this study was 0.27.Clustering pattern of dendrogram (Figure 3) generated by using the pooled molecular data of 23 SSR loci indicated eleven clusters namely I to XI, which were formed at a similarity coefficient of 0.27. Cluster I to III included only spanish bunch type. Cluster IV included all genotypesfrom virginia bunch type. Cluster V included only one genotype ICG1326 i.e., J11. Cluster VI included ICG3267, ICG4750 and ICG12697. Cluster VII included genotypes TG26, ICG1122, ICG1173, ICGV95070, GG7, ICGV99083 ICGV99213, TAG24 and TPG41. Cluster VIII includes genotypes ICG12370, ICG6813, ICG14482, ICG7412 (*valencia*), ICG10933 (*peruviana*) and ICG12625 (*aequtoriana*). Cluster IX, X and XI included only one genotype each *viz*., JB1137, GG20 and JB1109 respectively.

Genetic diversity studies in cultivated groundnut using SSR markers were reported by various authors. Twenty three SSRs were screened across 22 groundnut genotypes with differing levels of resistance to rust and LLS by Mace *et al*. (2006) [5] and they reported that twelve of the 23 SSRs (52 per cent) showed a high level of polymorphism with PIC values ≥0.5. Thirty four SSR markers were used to assess the genetic

variation of four sets of twenty-four accessions each from the four botanical varieties of the cultivated peanut were as reported by Tang *et al*. (2007) [14].

Molecular diversity and association of simple sequence repeat (SSR) markers with rust and late leaf spot (LLS) resistance were detected in a set of 20 cultivated groundnut genotypes differing in resistance against both diseases and were reported by Mondal and Badigannavar (2009) [7].

From the experimental findings, following conclusion could be drawn

1. Considerable variation existed in *Arachis* germplasm under investigation.
2. Refinement is required if UPGMA method is to be employed as a tool for diversity analysis of phenotypic characters. However, diversity analysis can produce a clear picture if much more efficient bioinformatics tools can be used.
3. The diversity analysis of phenotypic characters formed two major clusters in which most of the genotypes were included except for two genotypes *viz*., ICG6813 and ICG12625 which uprooted from the rest.
4. Diversity analysis using SSR primers revealed that the *Arachis* genotypes tend to cluster separately according to genetic orientation.
5. The Spanish bunch genotype J68 out rooted from the rest of the cultures.

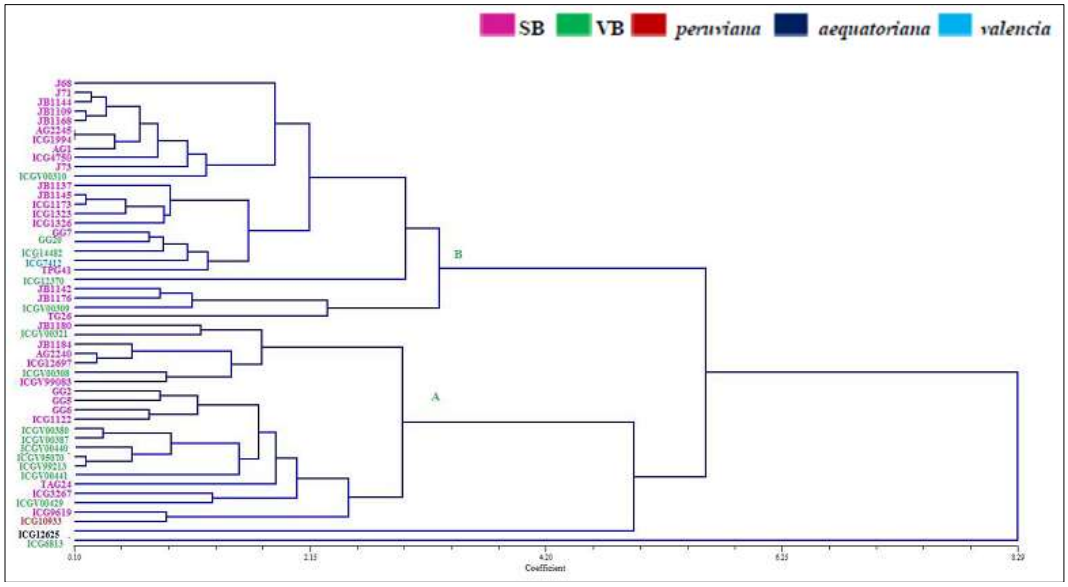

Fig 2: Dendrogram showing clustering of 50 groundnut genotypes constructed using UPGMA based on Jaccard's coefficient based on phenotypic traits

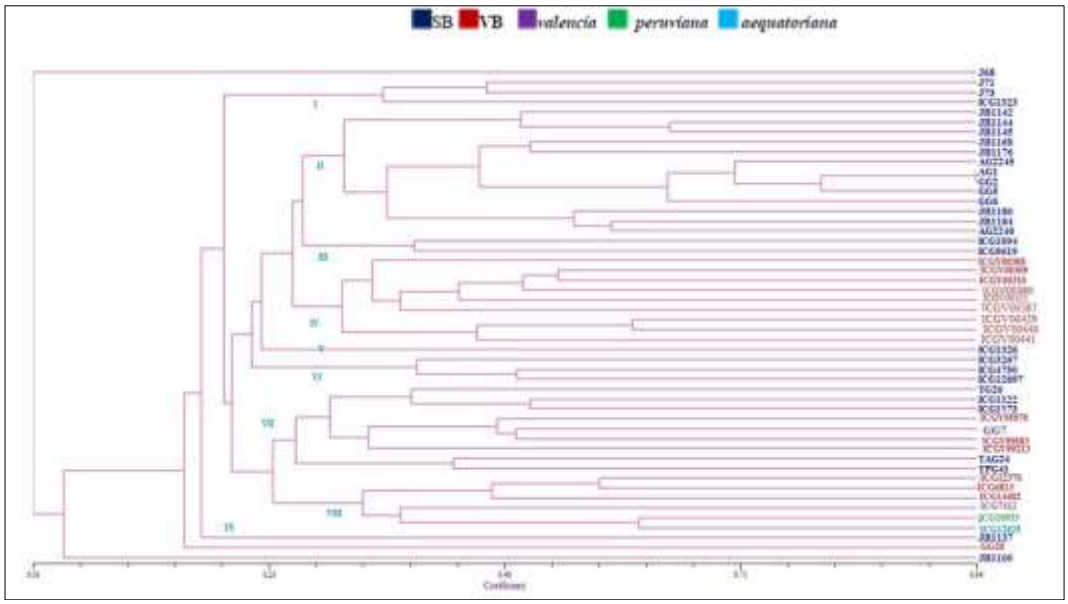

Fig 3: Dendrogram showing clustering of 50 groundnut genotypes constructed using UPGMA based on Jaccard's coefficient using SSR primers

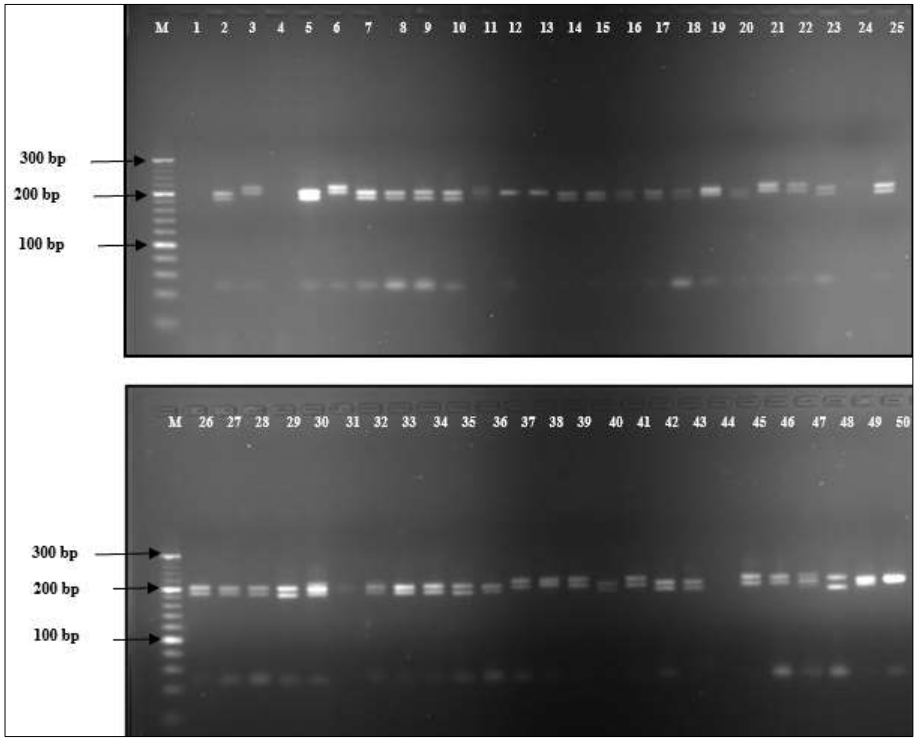

Fig 4: SSR profile of groundnut genotypes generated by primer PM3

M- 20 bp ladder

1. J-68	11. JB-1180	21. TAG-24	31.ICG 9619	41. ICGV 95070
2. J -71	12. JB-1184	22. TPG-41	32. ICGV 00308	42. ICGV 99083
3. J -73	13. AG-2240	23. ICG 1122	33. ICGV 00309	43. ICGV 99213
4. JB-1109	14. AG-2245	24. ICG 1173	34. ICGV 00310	44. GG-20
5. JB-1137	15. AG-1	25. ICG 1323	35. ICGV 00321	45. ICG 12370
6. JB-1142	16. GG-2	26. ICG 1326	36. ICGV 00380	46. ICG 14482
7. JB-1144	17. GG-5	27. ICG 1994	37. ICGV 00387	47. ICG 6813
8. JB-1145	18. GG-6	28. ICG 3267	38. ICGV 00429	48. ICG 7412
9. JB- 1168	19. GG-7	29. ICG 4750	39. ICGV 00440	49. ICG 10933
10. JB- 1176	20. TG-26	30. ICG 12697	40. ICGV 00441	50.ICG 12625

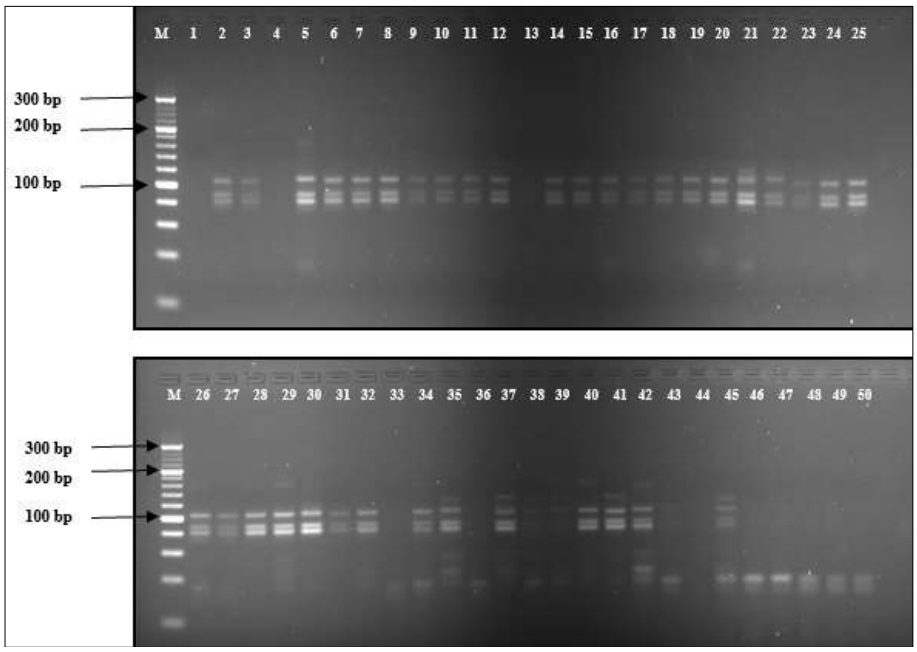

Fig 5: SSR profile of groundnut genotypes generated by primer PM179

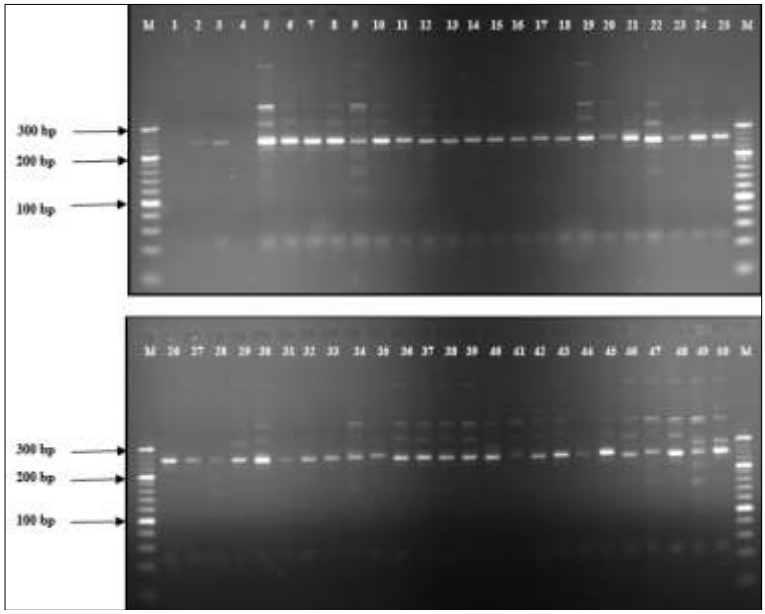

Fig 6: SSR profile of groundnut genotypes generated by primer. IPAHM689

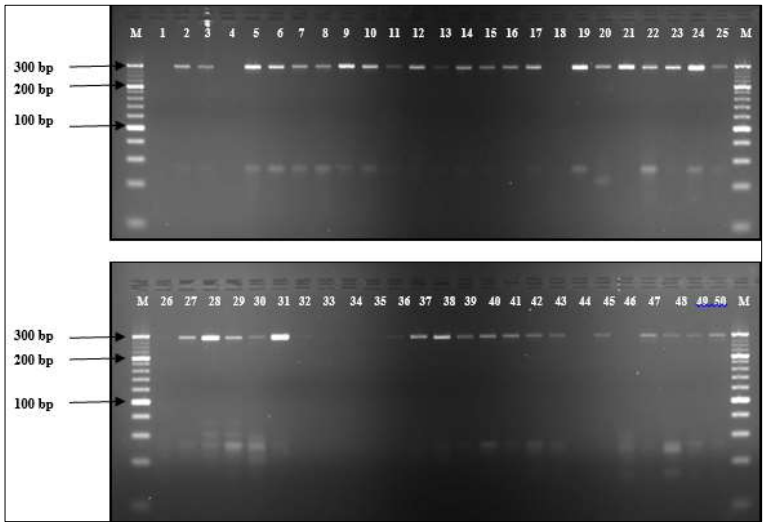

Fig 7: SSR profile of groundnut genotypes generated by primer pPGp seq4G2

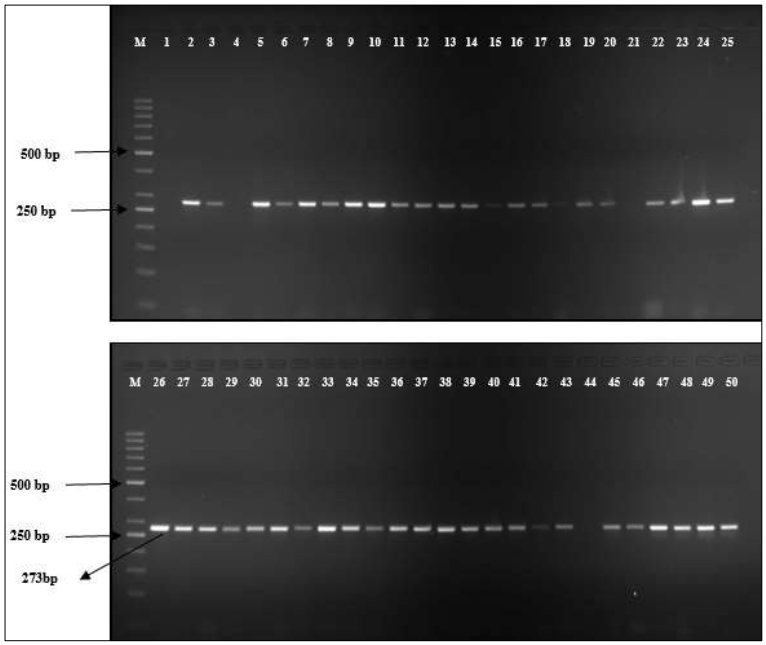

Fig 8: SSR profile of groundnut genotypes generated by primer Lipoxygenase

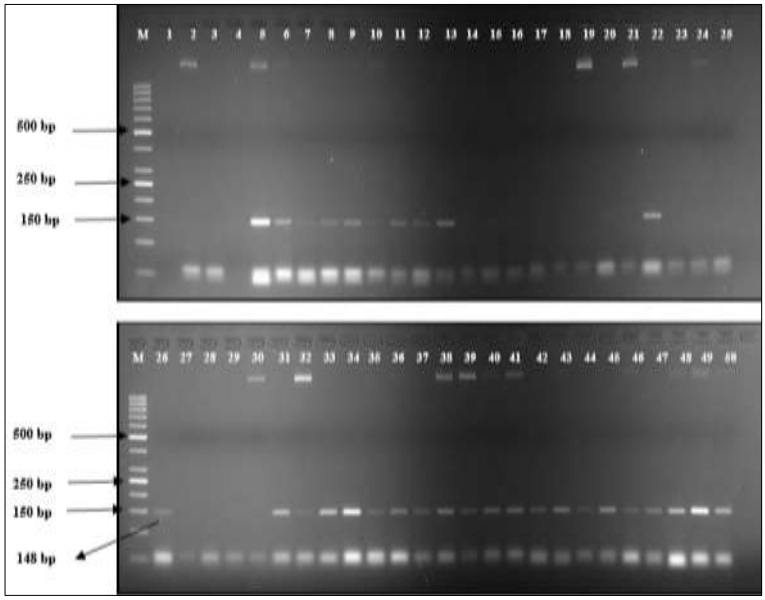

Fig 9: SSR profile of groundnut genotypes generated by primer Trypsin inhibitor

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