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Molecular Diversity Analysis of Peanut Mini Core Collection using RAPD Markers

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ABSTRACT

Twenty nine accessions of groundnut minicore collection belonging to different botanical types along with five cultivars viz., GPBD-4, M 28-2, TAG 24, JL 24 and MN 1-35 were selected for RAPD assay to assess the molecular diversity through twenty primers. Out of the twenty primers used, the primers namely OPK 14, OPA 19, OPC 15, OPC 09, OPC 13, OPB 11, OPF 09, OPJ 06, OPV 16, OPA 15, OPA 20, OPA 12 and OPF 10 have shown high polymorphism across all four botanical types. The polymorphism per primer ranged from 57.14% to 100%. The dendrogram revealed six distinct clusters but the accession in each cluster could not associate with subspecies or botanical types, even cluster did not show any association with geographical origin. This indicated independence of molecular diversity with morphological diversity. The similarity coefficient ranged from 0.63 to 0.93 indicating substantial diversity present in the mini core collection. Accessions with the most distinct DNA profiles are likely to contain greater number of novel alleles as revealed by RAPD assay. Substantial genetic diversity exists in the mini core that could be exploited in crop improvement programme.

Key words : Dendrogram, Groundnut, Mini Core collection, RAPD polymorphism, Similarity Coefficient.

Groundnut is one of the principal economic oilseed crop of the world. It is the world's fourth most important source of edible oil and third most important source of vegetable protein. Presence of genetic variability is a pre-requisite to sustain the crop improvement that allows identification and incorporation of divergent alleles in the germplasm collection to develop promising cultivars. Utilization of the germplasm collection could be enhanced by the development of more efficient evaluation techniques. A core collection is a subset of accessions from the entire collection that captures most of the available genetic diversity of the species (Frankel and Brown, 1984). Upadhyaya and Ortiz, (2001) suggested that mini core subset contains about one percent of total accessions but it is representative of the entire diversity of the collection.

A variety of molecular and morphological descriptors are used to characterize the genetic diversity among and within the crop species. Substantial diversity exists among cultivated peanut genotypes for various morphological, physiological and agronomic traits (Stalker, 1992). Very low polymorphism for cultivated type and abundant polymorphism in wild *Arachis* (Kochert *et al*., 1991: Halward *et al*., 1992 and Paikro *et al*., 1992) was revealed in Peanut through Random Amplified Polymorphic DNA (RAPD) and Restriction Fragment Length Polymorphism (RFLP) techniques. The low level of polymorphic variation in groundnut is attributed to its recent origin from single polyploidization event in the evolutionary time scale (Young *et al*., 1996). This urges the need for studying more germplasm to assess diversity.

DNA based markers provide a reliable means of estimating the genetic relationships between genotypes and taxonomic groups as compared to morphological markers (Nalini *et al*., 2005). So far significant morphological variation in groundnut was revealed but the information on molecular diversity of gene pool of peanut was not much studied (Norden *et al*., 1982). Hence, the present investigation reports about molecular divergence of mini core collection of peanut.

MATERIAL AND METHODS

Twenty nine accessions of mini core collection of groundnut representing Valencia (9), Spanish Bunch (7), Virginia Runner (6), and Virginia

Bunch (7) of different geographical origin and five cultivars namely, GPBD-4, M 28-2, TAG 24, JL 24 and MN 1-35 were selected for RAPD assay (Table 1).

Plant Material and DNA Extraction

DNA from tender leaflets of 21 days age old seedlings was extracted by following Cetyl Trimethyl Ammonium Bromide (CTAB) method (Saghai-Maroof *et al*., 1984). The quality and concentration of DNA was assessed by a Spectrophotometer and by Gel Electrophoresis using 0.8% Agarose with known concentration of Uncut Lambda DNA.

PCR with random primers

Commercial kits of random decamer DNA primers were obtained from Operon Tenchnologies Inc., Alamedas, USA. A total of twenty random primers were used for the assay. The sequence details of the primers were furnished in Table 2. PCR reaction was carried out with 20 µl reaction mixture containing 1 mg/µl of template (Genomic DNA), 10mM of dNTPs (M/s Bangalore Genei Pvt.Ltd., Bangalore), and 0.33U of *Taq* DNA Polymerase (M/s Bangalore Genei Pvt.Ltd., Bangalore) and 5 pM of primer in 1.25x reaction buffer with 15mM Mgcl, (M/s Bangalore Genei Pvt.Ltd., Bangalore). The amplification reaction was performed in the Eppendorf master cycler with an initial denaturation for 5 min at 94°C, then 37 cycles for 2 min denaturation at 94°C, then 1 min for annealing at 36°C, and 2 min extension at 72°C. Final extension was carried out at 72°C for 10 minutes. After the completion of the PCR, the products were stored at -4°C until the gel electrophesis was done. The PCR product was mixed with 2ml of loading dye (Bromophenol Blue) and was loaded in 1.2% Agarose Gel of 1xTAE buffer containing Ethidium Bromide and the gel was run at 90 volts. The gel was photographed by using documentation system (Uvitech, Cambridge, England).

Data analysis

The amplified fragments were scored as '1' for the presence and '0' for the absence of band generating the '0' and '1' matrix. Based on the analysis of the profile of the amplified fragments pair wise genetic similarities (S_{ij}) between genotypes were estimated using Jaccard similarity coefficient. Clustering was done by using the symmetric matrix of similarity coefficient in SPSS software.

RESULTS AND DISCUSSION

Twenty nine genotypes of mini core collection and five elite cultivars were subjected to RAPD assay to assess the molecular diversity through twenty primers. The band profiles obtained with twenty primers in four botanical groups were summarized in Table 3. Out of twenty primers used, thirteen primers Viz., OPK 14, OPA 19, OPC 15, OPC 09, OPC 13, OPB 11, OPF 09, OPJ 06, OPV 16, OPA 15, OPA 20, OPA 12, and OPF 10 have shown high polymorphism across all four botanical types. Number of amplified fragments ranged from three to eleven with a given primer. On an average, six to seven bands per primer were amplified and all the bands were polymorphic. The polymorphism per primer ranged from 57.14% to 100%. The high level of polymorphism was observed among the accessions and may be due to large number of accessions representing wide genetic base and use of primers with potential for depicting polymorphism in groundnut.

The Dendrogram revealed six distinct clusters at S_{ij} value of 0.63 (Fig 1). The accession ICG 2381 clustered distinctly from all other accessions. The accessions in each cluster could not be associated with respect to sub-species or botanical types and the clusters did not showed any association with geographical origin. This indicated the independence of molecular diversity with morphological diversity. These findings were in conformity with the reports of Dwivedi *et al*., (2001) and reported an average of 86.2 % polymorphism among 26 accessions which were grouped into six clusters.

The similarity coefficient ranged from 0.63 to 0.93 indicating substantial diversity in the mini core collection of groundnut. The highest genetic diversity was observed between the accessions ICG 76 (Virginia runner from India) and ICG 14118 (Spanish bunch from United Kingdom); the cultivar GPBD-4 (Spanish bunch from India) and ICG8760 (Virginia runner from Zambia); M28-2(Valencia cultivar from India) and ICG 14475 (Virginia bunch from Nigeria); TAG 24 (Spanish bunch cultivar from

Table1. Accessions selected for RAPD assay in minicore collection of groundnut.

S.No.	Accession	Botanical type	Origin
1	ICG 76	Virginia runner	India
2	ICG 81	Spanish bunch	Unknown
3	ICG 115	Valencia	India
4	ICG 532	Virginia bunch	Unknown
5	ICG 1470	Valencia	Cameroon
6	ICG 1862	Spanish bunch	Unknown
7	ICG 2381	Virginia runner	Brazil
8	ICG2857	Virginia runner	Argentina
9	ICG 3027	Virginia bunch	India
10	ICG 3053	Virginia bunch	India
11	ICG 3673	Valencia	Korea
12	ICG 3681	Valencia	USA
13	ICG 4716	Spanish bunch	Unknown
14	ICG 6027	Valencia	Sudan
15	ICG 6057	Virginia bunch	USA
16	ICG 6646	Valencia	Unknown
17	ICG 7963	Virginia bunch	USA
18	ICG 8760	Virginia runner	Zambia
19	ICG 11144	Valencia	Argentina
20	ICG 11219	Virginia runner	Mexico
21	ICG 12370	Virginia runner	India
22	ICG 12625	Valencia	Ecuador
23	ICG 12682	Spanish bunch	India
24	ICG 12697	Spanish bunch	India
25	ICG 13787	Virginia bunch	Niger
26	ICG 13856	Valencia	Uganda
27	ICG 14118	Spanish bunch	United Kingdom
28	ICG 14475	Virginia bunch	Nigeria
29	ICG 14985	Spanish bunch	Unknown
30	GPBD-4	Spanish bunch	India
31	M 28-2	Virginia runner	India
32	TAG 24	Spanish bunch	India
33	JL 24	Spanish bunch	India
34	MN 1-35	Virginia runner	India

India) and ICG 11219 (Virginia runner from Mexico); JL 24 (Spanish bunch cultivar from India) and ICG 8760 (Virginia runner from Zambia); MN 1-35 (Valencia breeding line from India) and ICG 12682 (Spanish bunch from India). The dendrogram constructed from the molecular data revealed distinct cluster of accession ICG 2381 (Virginia runner from Brazil) away from the rest at 0.63 similarity coefficient. Besides being potential donors of multiple traits, these accessions are also useful in widening the genetic base of existing cultivars. The accessions ICG 6027 (Valencia from Sudan) and ICG 6057 (Virginia bunch from USA) were found to be most similar at Sij value of 0.92. The cultivar JL 24 was grouped along with the accessions ICG 13787, ICG 3027 and ICG 8760 thus, appeared to be distinct from the others at Sij value of 0.69. The accessions ICG 12625 (*Aequatoriana* from Ecuador), ICG 12370 (Virginia runner from India), ICG 12697 (Spanish

Table 2. The base sequence of primers utilized for RAPD assay of groundnut minicore collection.

SI.No.	Primer	Sequence (5'-3')
1	OPK 09	CCCTACCGAC
2	OPK 14	CCCGCTACAC
3	OPA 19	CAAACGTCGG
4	OPC 15	GACGGATCAG
5	OPC 09	CTCACCGTCC
6	OPC 13	AAGCCTCGTC
7	OPB 11	GTAGACCCGT
8	OPF 09	CCAAGCTTCC
9	OPJ 06	TCGTTCCGCA
10	OPV 16	GGGCCAATGT
11	OPA 15	TTCCGAACCC
12	OPA 20	GTTGCGATCC
13	OPF 07	CCGATATCCC
14	OPA 12	TCGGCGATAG
15	OPJ 17	ACGCCAGTTT
16	OPC 03	GGGGGTCTTT
17	OPV 15	AGTCGCCCTT
18	OPC 06	GAACGGACTC
19	OPF 10	GCAAGCTTGG
20	OPA 17	GACCGCTTGT

Fig 1. Dendrogram depicting genetic diversity of potential Accessions in the minicore collection of groundnut

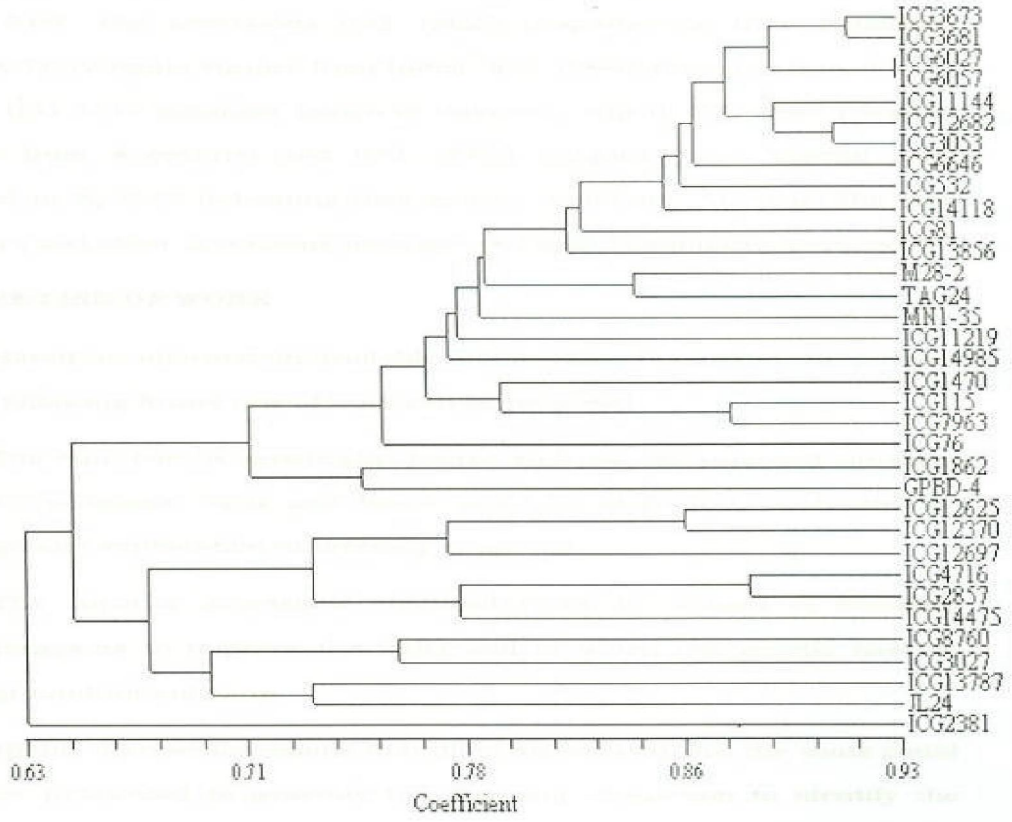

Table 3. Analysis of RAPD banding pattern in minicore collection of groundnut.

Primer	Valencia			Spanish bunch			Virginia runner			Virginia bunch		
	No. of amplified fragments	No. of polymorphic fragments	Per cent polymorphism	No. of amplified fragments	No. of polymorphic fragments	Per cent polymorphism	No. of amplified fragments	No. of polymorphic fragments	Per cent polymorphism	No. of amplified fragments	No. of polymorphic fragments	Per cent polymorphism
OPK 09	11	11	100	11	11	100	11	11	100	11	11	100
OPK 14	8	8	100	8	8	100	8	8	100	8	8	100
OPA 19	3	3	100	3	3	100	3	3	100	3	3	100
OPC 15	8	8	100	8	8	100	8	8	100	8	8	100
OPC 09	6	6	100	6	6	100	6	6	100	6	6	100
OPC 13	8	8	100	8	8	100	8	8	100	8	8	100
OPB 11	7	7	100	7	7	100	7	7	100	7	7	100
OPF 09	8	8	100	8	8	100	8	8	100	8	8	87.5
OPJ 06	7	7	100	7	7	100	7	7	100	7	7	100
OPV 16	8	8	100	8	8	100	8	8	100	8	8	100
OPA 15	7	7	100	7	7	100	7	7	100	7	7	100
OPA 20	5	5	100	5	5	100	5	5	100	5	5	100
OPF 07	6	5	83.33	6	4	66.66	6	4	66.66	6	6	83.3
OPA 12	6	6	100	6	6	100	6	6	100	6	6	100
OPJ 17	7	7	100	7	7	100	7	7	100	7	7	71.42
OPC 03	5	5	80	5	5	100	5	4	80	5	5	100
OPV 15	5	5	80	5	4	80.00	5	4	80	5	5	60
OPC 06	5	5	100	5	5	100	5	4	80	5	5	60
OPF 10	8	8	100	8	8	100	8	8	100	8	8	100
OPA 17	7	4	57.14	7	7	100	7	4	57.14	7	7	85.71
Total Bands	135	129		135	132		135	130		135	126	
Bands per primer	6.75	6.45		6.75	6.6		6.75	6.5		6.75	6.3	

bunch from India), ICG 4716 (Spanish bunch of unknown origin), ICG 2857 (Virginia runner from Argentina) and ICG 14475 (Virginia bunch from Nigeria) were grouped at Sij value of 0.72 indicating their genetic similarity. As usual, four cultivars and other accessions were grouped at 0.71 similarity coefficient.

Based on information available and results obtained in the present study, it is clear that the mini core is genetically diverse and possess potential variation for economic traits, hence it could be extensively evaluated for greater exploitation in breeding programs to improve the traits and to widen the genetic base of groundnut cultivars. The highly diverse accessions that differ significantly for the traits could be hybridized to generate the mapping population to identify the markers for use in crop improvement programmes through marker-assisted selection. The substantial molecular diversity existed in the mini core could be further screened with more number of RAPD primers and advanced markers like SSRs and AFLPs in order to identify an adequate number of polymorphic primers to enhance its scientific exploitation of genetic improvement of groundnut.

LITERATURE CITED

Dwivedi S L, Gurtu S, Chandra S, Yuejin W and Nigam S N 2001 Assessment of genetic diversity among selected groundnut Germplasm by RAPD Analysis. *Plant Breeding,* 120: 345-349.

Frankel O H and Brown A H D 1984 Genetic perspectives of germplasm conservation. *In*: W. K. Arer *et al*. (eds), *Genetic Manipulation:Impact on Man and Society*, Cambridge University Press, Cambridge, UK, 161-170.

Halward T, Stalker H T, Larue E and Kochert G 1992 Use of single primer DNA amplification in genetic studies of peanut (*Arachis hypogaea* L.). *Plant Molecular Biology,* 18: 315-325.

Kochert G, Halward T, Branch W D and Simpson C E 1991 RFLP variability in peanut (*Arachis hypogaea* L.) cultivars and wild species. *Theoretical and Applied Genetics* 81**:** 565-570.

Nalini M, Maheedhar G and Subhash Chandra 2005 Genetic diversity among *Arachis* species based on RAPDs. *Indian Journal of Genetics and Plant Breeding,* 65(1): 5-8.

Norden A J, Smith O D and Gorbet E W 1982 Breeding of the cultivated peanut. *In*: Pattee, H.E. and Young C.T., *Peanut Science and Technology*, American Peanut Research and Education Society, Inc., Yoakum, TX, 95122.

Paik-ro O G, Smith R L and Knauft D A 1992 Restriction fragment length polymorphism evaluation of six peanut species within the *Arachis* section. *Theoretical and Applied Genetics,* 84**:** 201-208.

Saghai-Maroof M A, Soliman K M, Jorgensen R A and Allard R W 1984 Ribosomal DNA spacer length polymorphism in barley. Mendelian inheritance, chromosomal location and population dynamics. *Proceedings of National Academy of Sciences,* USA 81**:** 8014- 8018.

Stalker H T 1992 Utilizing *Arachis* germplasm resources. In: Nigam S.N., *Groundnut – A Global Perspective*, ICRISAT, Patancheru, India, 281-296.

Upadhyaya H D and Ortiz R 2001 A mini core subset for capturing Diversity and promoting utilization of chickpea genetic resources in crop improvement. *Theoretical and Applied Genetics* 102: 1292-1298.

Young N D, Weeden N F and Kochert G 1996 Genome mapping in legumes (Fam: Fabaceae). *In:* Paterson A.H., *Genome Mapping in Plants*, Lander Co., Austin, Tx, .211-227.

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