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Molecular Diversity Among Selected Groundnut (*Arachis hypogaea* L.) Genotypes I: RAPD Analysis

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ABSTRACT

The purpose of the present experiment were to study genetic diversity among eighteen selected groundnut genotypes following random amplified polymorphic DNA (RAPD) assay with twenty primers of a 10-mer. The number of amplicons varied from three to ten with an average of 7.5 per primer, of which three were polymorphic. Altogether the primers generated a sum of 150 amplified fragments with 42.32 percent polymorphism. The genetic similarity (S_{ij}) assessed by Dice similarity coefficient was ranged from 86 % to 99 %, with an average of 93 %, indicating very less diversity among genotypes. Clustering technique of unweighted pair group method with arithmetic averages (UPGMA) dendrogram revealed three distinct clusters at S_{ij} of 0.94. Among the genotypes TG 49 and ICG 13787 were found to span the extremes of the entire dendrogram with the remaining types distributed between them, whereas the resistant lines for *Aspergillus* seed colonization are distributed throughout the dendrogram. All germplasm except ICG 14985 (Spanish bunch) clustered distinctly away from cultivars and all the cultivars clustered together indicating narrow genetic diversity among the cultivars and germplasm.

Key words : *Aspergillus* seed colonization, Genetic diversity, Peanut, RAPD.

The gene pool of cultivated groundnut is classified based on difference in branching pattern and presence of reproductive nodes on the main axis into two subspecies, *fastigiata* and *hypogaea*. The subspecies *fastigiata* is sub-divided into four botanical varieties, *fastigiata*, *peruviana*, *aequatoriana* and *vulgaris,* whereas subspecies *hypogaea* includes varieties *hypogaea* and *hirsuta* (Krapovickas and Gregory, 1994). Extensive variation for morphological and physiological characteristics exists in both wild and cultivated groundnut (Halward *et al.,* 1992). Whereas, very low or no polymorphic variability in cultivated type to abundant polymorphic variability in wild diploid *Arachis* species have been reported (Halward *et al*., 1992). However, recent studies employing Amplified Fragment Length Polymorphism (AFLP) (He and Prakash, 1997), Random Amplified Polymorphic DNA (RAPD) (Bhagwat *et al.*, 1997; Dwivedi *et al.*, 2001) and Simple Sequence Repeats (SSR) (Hopkins *et al.*, 1999) revealed polymorphism in cultivated groundnut. The PCR-based RAPD approach offers quick screening of different regions of the genome for genetic polymorphisms using single 10-mer arbitrary primers with much less DNA and it is technically simple, cheaper and reliable method for the estimation of variability between different accessions as compared to other techniques. Hence an attempt has been made to know the diversity among 18 selected genotypes using RAPD assay.

MATERIAL AND METHODS

In the present study, a set of twenty RAPD markers were used for DNA profiling of a group of 18 selected genotypes consisting of different botanical types. The origin, pedigree and salient characters of 18 genotypes are described in Table 1. The random primers of 10-mer, each with at least 60 % G + C content were obtained from Operon technologies, Almedas, USA. The sequence details of the primers are presented in the Table 2. Ten seeds from each genotype were sown in pots and Young leaves from 2-week-old plants were bulk harvested for each genotype and immediately placed in liquid nitrogen. The Genomic DNA was extracted by following cetyltrimethylammonium bromide (CTAB) method (Saghai - Maroof *et al*., 1984) with some modifications.

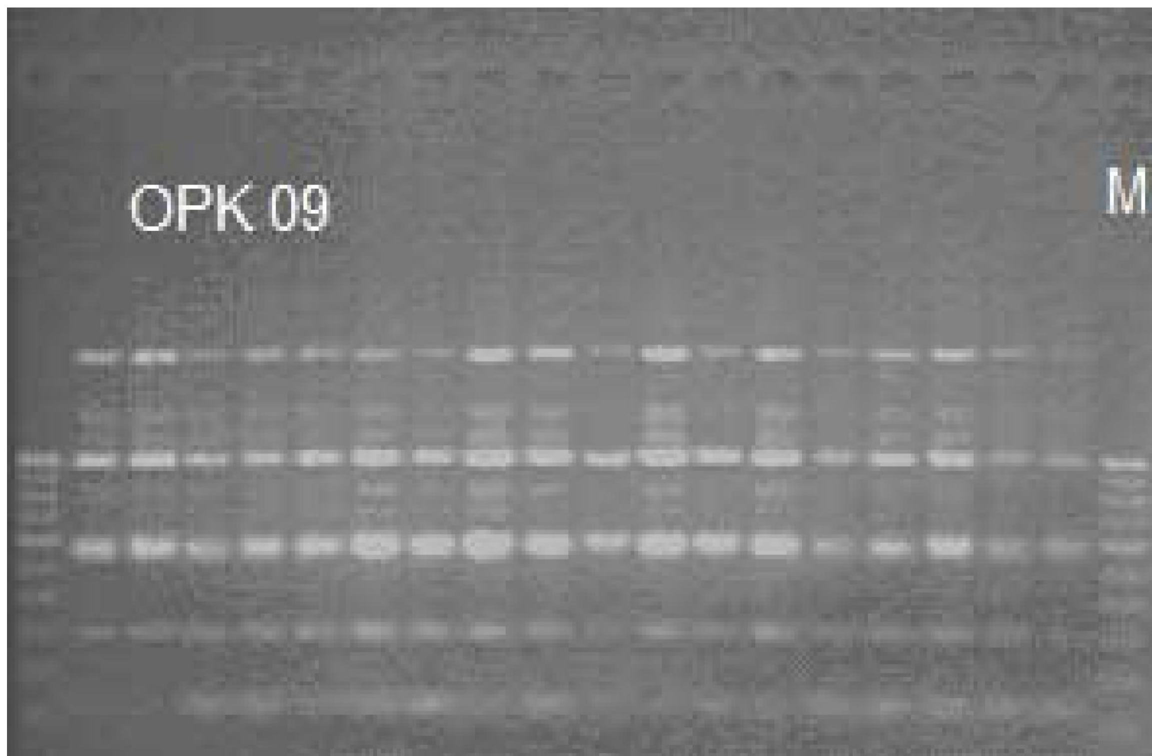
The Polymerase chain reaction (PCR) mixture (20 µl) consisted of 1.0 µl (5 ng) of genome DNA, 2 µl of 10 X PCR buffer, 1.0 µl of 10 mMd NTPs, 2 µl of 5 pM RAPD primers, 13.67 µl of nanopure water and 0.33 µl of Taq DNA polymerase. The amplification reaction was carried out in 0.2 ml tubes placed in Thermal cycler (Eppendorf 2231, Hamburg, Germany). The amplification conditions were maintained at 94^0C for 5 min and 36 cycles of

Table 1. Pedigree of selected genotypes of groundnut.

Sl. No.	Genotypes	Botanical group	Pedigree / origin	IVSCAF*	Remarks
1	ICG 14985	Spanish bunch	Unknown	1.08	Germplasm
2	ICG 8760	Virginia runner	Sudan	1.00	Germplasm
3	ICG 13787	Virginia bunch	Niger	1.11	Germplasm
4	ICG 6027	Valencia	Sudan	1.10	Germplasm
5	ICGV 86155	Spanish bunch	ICGS 30 × (TMV10 × Chico F6)	2.10	Germplasm
6	ICGV 86699	Virginia bunch	(*Arachis batizocoi* × *A. duranensis*) × *A. hypogaea* (NC2 × CS29)	1.20	Germplasm
7	GPBD 5	Spanish bunch	TG 49 × D 39d, 10-1	2.25	Breeding lines
8	GPBD 6	Valencia	Mutant × NcAc 343-1-35	1.35	Breeding lines
9	TG 19	Virginia bunch	TG 17 × TG1	1.00	Breeding lines
10	TG 49	Spanish bunch	TG 28A × TG 26	1.00	Breeding lines
11	TG 41	Spanish bunch	TG 28 × TG 22	1.38	Cultivar
12	TGLPS 3	Valencia	TAG 24 × TG 19	2.03	Cultivar
13	M 28-2	Valencia	EMS treated mutant derived from VL-1 (2002)	1.45	Cultivar
14	GPBD 4	Spanish bunch	Cross between KRG-1 and ICGV 86855	4.00	Cultivar
15	TAG 24	Spanish bunch	TGS2 × TGE1	4.00	Cultivar
16	JL 24	Spanish bunch	Selection from EC94943 (1878)	3.70	Cultivar
17	TMV 2#	Spanish bunch	Mass selection from Gudhiatham bunch (1940)	4.00	Cultivar
18	J 11##	Spanish bunch	Ah 4218 × Ah 4354	3.95	Cultivar

- Resistant check   *- *in vitro* seed colonization by *A. flavus*

Plate 1. RAPD profile in selected groundnut genotypes.



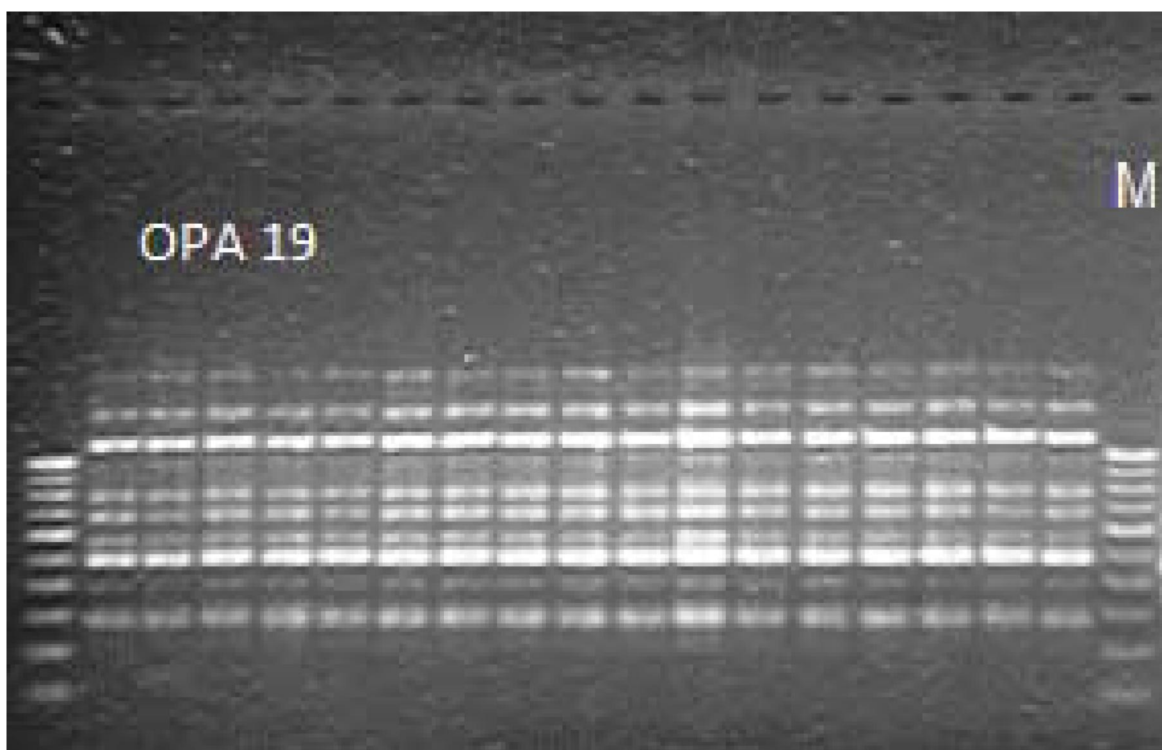


Table 2. List of primers used for RAPD analysis.

Sl. No	Primers	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	ACGCCAGTTC
16	OPC 03	GGGGGTCTTT
17	OPV 15	AGTCGCCCTT
18	OPC 06	GAACGGACTC
19	OPF 10	GCAAGCTTGG
20	OPA 17	GACCGCTTGT

94^0C for 2 min (denaturation), 37^0C for 1 min (annealing) and 72^0C for 2 min (extension) followed by 10 min at 72^oC. PCR products were separated by electrophoresis in 1.4 % gel stained in ethidium bromide (10 mg/ml).The gel was visualized and photographed by using UV transilluminator. The amplified fragments were scored as '1' for presence and '0' for absence of a band from higher to lower molecular weight. Pair wise genetic similarity (S_{ij}) between genotypes was estimated using Dice similarity coefficient. Clustering was done using symmetric matrix of similarity coefficient. A dendrogram was constructed based on S_{ij} values using clustering technique of unweighed pair group arithmetic mean (UPGMA) using SHAN module of NTSYSpc version 2.0(5). The polymorphic information content (PIC) (Bolstein. 1980) of the primer was estimated by,

$$PIC = 2 \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} P_iP_j (1- P_iP_j)$$

Where, P_i = Frequency of ith allele
K = Number of alleles

***Aspergillus* seed colonization:** Twenty matured seeds (weighing 4-10 g depending on the seed size) with intact seed coat and free from any damage were selected from each genotype for *in vitro* inoculation by *A. flavus*. Seeds were surface sterilized with 0.1 % (w/v) aqueous solution of mercuric chloride for 2 minutes and subsequently washed in two changes of distilled sterilized water to remove any traces of mercuric chloride. Each seed was uniformly wounded by pricking with a sterile needle to facilitate the invasion by *A. flavus* spores. Seeds were placed in a sterilized Petri dish (9 cm diameter) and spray inoculated with *A. flavus* spore suspension (1 × 10^6 spores/ml) using an automizer under strict aseptic conditions. The Petri dishes were shaken vigorously to roll the seeds allowing uniform distribution of inoculum on the seeds. The experiment was conducted in four replications with 10 seeds per replication. The Petri dishes were placed at high humidity (>95 % RH) in semi-rigid plastic boxes lined with cotton wool and blotting paper with closely fitting lids and incubated at 25 $\pm$ 1^0 C in dark for 7 - 10 days. Individual seeds were

Table 3. Analysis of RAPD banding pattern in varieties.

	No of amplified bands	No of polymorphic bands	Per cent Polymorphism	PIC values
OPA 12	5	5	100.00	0.19
OPA 15	3	2	66.66	0.10
OPA 17	7	0	0.00	0.00
OPA 19	10	0	0.00	0.00
OPA 20	6	3	50.00	0.07
OPB 11	7	4	57.14	0.13
OPC 03	8	5	62.50	0.11
OPC 06	6	2	33.33	0.04
OPC 09	10	4	40.00	0.12
OPC 13	8	5	62.50	0.15
OPC 15	9	2	22.22	0.07
OPF 07	7	3	42.85	0.05
OPF 09	8	7	87.50	0.17
OPF 10	8	7	87.50	0.20
OPJ 06	7	0	0.00	0.00
OPJ17	7	1	14.28	0.03
OPK 09	8	4	50.00	0.12
OPK 14	8	1	12.50	0.05
OPV 15	10	2	20.00	0.04
OPV 16	8	3	37.50	0.06
Total	**150**	**60**	**-**	**-**
Average	**7.5**	**3.0**	**42.32**	**0.0840**

scored for surface colonization by *A. flavus* and for colonization severity following rating scale given by Thakur *et al*., (2000).

RESULTS AND DISCUSSION

Twenty primers generated a total of 150 amplified fragments out of which 60 showed polymorphic bands. The polymorphism percentage for primers ranged from zero (OPA-19) to 100 per cent (OPA-12) with an overall average of 42.32 per cent. Number of amplified fragments ranged from 3 to 10 with an average of 7.5 bands per primer among which 3.00 bands per primer were polymorphic. Primer OPA-17, OPA-19 and OPJ-06 showed monomorphic bands in all varieties. Primer OPK-14, OPJ-17, OPA-12, OPF-09 and OPF-10 showed polymorphism (plat 1). The band profile obtained from 20 primers is summarized in Table 3. PIC (polymorphic information content) values were calculated to identify most polymorphic primer. PIC values ranged from 0 (OPA-19) to 0.2 (OPF-10) with mean PIC of 0.08 per primer. OPF-10 had high PIC values followed by OPA-12 (0.19) and OPF-09 (0.18). In spite of using primers which were polymorphic (Dwivedi *et al*., 2002 and Ajay, 2006), only moderate level of polymorphism was observed, which is in accordance with Dwivedi *et al.,* 2001; Varsha kumari *et al*., 2009 who had observed limited polymorphism among different accessions of cultivated groundnut.

The Dice similarity coefficient was generated from pooled data of all primers and presented in Table 4. The genetic similarity of genotypes ranged from 0.86 to 0.99 indicating limited diversity among the genotypes. The low level of polymorphic variation (Bhagwat *et al*., 1997, Dwivedi *et al* 2001) in cultivated groundnut is attributed to recent origin of the crop by single event of polyplodization. Among all the genotypes ICG 13787 and GPBD 4 exhibited the highest genetic diversity. The dendrogram revealed three distinct clusters at S_{ij} of 0.94 (Figure. 1). TG 49 and ICG 13787 were found to span the extremes of the entire dendrogram with the remaining types distributed between them. All germplasm except ICG 14985 (Spanish bunch) clustered distinctly away from cultivars and all the cultivars clustered together indicating narrow genetic diversity among the cultivars.

Table 4. Dice similarity coefficient matrix of eighteen genotypes of groundnut based on RAPD banding pattern.

	TG 49	ICG 14985	TMV 2	TAG 24	GPBD 4	JL 24	GPBD 6	GPBD 5	M 28-2	ICG 6027	ICG 8760	TGLPS 3	TG 19	ICGV 86699	J 11	TG 41	ICGV 86155	ICG 13787
TG 49	1.000																	
ICG 14985	0.983	1.000																
TMV 2	0.972	0.969	1.000															
TAG 24	0.962	0.966	0.954	1.000														
GPBD 4	0.936	0.940	0.935	0.946	1.000													
JL 24	0.951	0.955	0.957	0.947	0.934	1.000												
GPBD 6	0.969	0.973	0.969	0.965	0.953	0.961	1.000											
GPBD 5	0.986	0.983	0.966	0.976	0.950	0.951	0.983	1.000										
M 28-2	0.983	0.986	0.969	0.972	0.947	0.962	0.979	0.990	1.000									
ICG 6027	0.928	0.925	0.912	0.916	0.902	0.926	0.938	0.935	0.932	1.000								
ICG 8760	0.936	0.940	0.935	0.953	0.933	0.949	0.946	0.943	0.947	0.895	1.000							
TGLPS 3	0.980	0.983	0.972	0.969	0.957	0.965	0.990	0.986	0.990	0.935	0.957	1.000						
TG 19	0.986	0.983	0.972	0.969	0.943	0.951	0.976	0.986	0.990	0.928	0.950	0.986	1.000					
ICGV 86699	0.983	0.980	0.962	0.965	0.940	0.947	0.972	0.990	0.986	0.924	0.940	0.983	0.983	1.000				
J 11	0.983	0.986	0.976	0.966	0.954	0.969	0.986	0.983	0.986	0.932	0.954	**0.997**	0.983	0.980	1.000			
TG 41	0.986	0.990	0.973	0.976	0.951	0.958	0.983	0.993	**0.997**	0.936	0.951	0.993	0.993	0.990	0.990	1.000		
ICGV 86155	0.944	0.947	0.943	0.939	0.919	0.920	0.961	0.958	0.954	0.918	0.904	0.951	0.951	0.954	0.947	0.958	1.000	
ICG 13787	0.900	0.889	0.875	0.894	**0.864**	0.889	0.902	0.907	0.896	0.949	0.879	0.900	0.892	0.896	0.896	0.900	0.888	1.000

The performance of genotypes for *in vitro* seed colonization by *A. flavus* (IVSCAF) revealed that TG 19 (1), TG 49 (1), ICG 8760 (1), ICG 14985 (1.08), ICG 6027 (1.10), ICG 13787 (1.11) and ICGV 86699 (1.20) had high level of resistance conforming the observations of Harish Babu *et al.,* (2005) and Yugandhar (2005). GPBD 6 (1.35), TG 41 (1.38) and M 28-2 (1.48) were the other genotypes that were superior followed by TGLPS 3 (2.03), ICGV 86155 (2.10) and GPBD-5 (2.25) showing moderate level of resistance to IVSCAF. The resistant lines (TG 19, TG 49 and ICG 8760) for *Aspergillus* seed colonization are distributed throughout the dendrogram. The limited level of polymorphism among the genotypes indicating a need to apply more primers or advanced technique like SSR for better understanding of diversity and resistance at molecular level.

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