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Research Article

RAPD based assessment of genetic diversity in groundnut (*Arachis hypogaea* L.)

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Abstract
Genetic diversity was assessed among 12 genotypes of groundnut using RAPD markers. Out of 60 RAPD markers screened, seven markers were found to be polymorphic. In total, 58 amplification products were realized out of which 48 alleles were polymorphic (82.9%). Among the markers, OPJ 6 was highly polymorphic with 11 alleles with nine being polymorphic. Least number of five alleles were amplified with OPJ 4 marker. The analysis of Jaccards similarity co-efficients among genotypes revealed that the genotypes, TCGS 645 and Tirupati 4 were distant and Narayani and Kalahasti were highly similar (92.9%). Genotype-specific alleles were identified with markers, OPA3 for Tirupati 4 and TCGS 888, OPA 19 for Tirupati 3, TG 47 and TCGS 913, OPJ4 for Tirupati 3 and Prasuna and OPJ 6 for Kalahasti and TCGS 750. The study indicated the scope and usefulness of RAPD markers for diversity analysis and for identification of genotypes in groundnut.

Key words:
Groundnut, RAPD markers, diversity

Introduction

The molecular tools such as DNA markers are becoming increasingly important as effective tools in crop breeding programmes but their application in genetic enhancement of groundnut is lagging behind due to limited knowledge of the genome. Wide variation for morphological and physiological characteristics exists in both wild and cultivated groundnut (Halward *et al.*, 1993). Since 1993, many studies revealed considerable amount of polymorphism using molecular markers (RAPD, SSR, RFLP and AFLP) (Dwivedi and Gurtu, 2002,Ferguson *etal*.,2004,He *etal*.,2005, Mace *etal*,2006 and Jiang *etal* .2010). The present study has been planned to assess genetic diversity using RAPD markers in groundnut.

Material and methods

The experimental material consisted of 12 genotypes (Table 1) selected based on information from D^2 analysis of 29 genotypes taking morphological and yield data (Suneetha *et al*., 2013). RAPD markers were used for detection of polymorphism among 12 selected genotypes. The genotypes were sown in pots under sterile conditions. After 15 days, healthy leaf samples were used for DNA extraction as per modified CTAB (Cetyl trimethyl ammonium bromide) method given by Murray and Thompson (1980). The standardized DNA amplification assay was as follows: Template DNA 25 ng, Taq DNA polymerase (*Genei*) 0.5 units, $MgCl_2$ 5mM; dNTP (Genei) 100 µl each, primer (Operon technology, USA) 1 µM, Buffer (Genei) 10x in a reaction volume of 25 µl. Amplification was carried out on Corbett research thermocycler using 60 primers from OP series (Operon Technology, USA) with the following temperature profile. The initial denaturation of template DNA at 94°C for 5 min followed by 45 cycles of denaturation at 92°C for 1 min, annealing at 37°C for 1 min, extension at 72°C for 2 min with final elongation at 72°C for 5 minutes. Out of 60 primers screened, only seven primers gave scorable bands with high percentage of polymorphism (Table2). To the amplified product obtained after the PCR reaction (25 µl), 5 µl gel loading dye (Bromophenol Blue) was added and loaded into individual wells of 1% agarose gel. 1 kb ladder (Fermantas) was loaded in first lane as marker in 1 x TBE buffer. Electrophoresis was carried out at 100 v for 3 h and the gel was stained with ethidium bromide (1 µg/ml). After electrophoresis, the gel was observed under U.V. light using Alpha Innotech corporation Geldoc system. Each amplified product was considered as an allele. The RAPD pattern of each genotype was evaluated, assigning character state '1' to all the bands that could be reproducible and detected in the gel and '0' for the absence of band. The data matrix thus generated was used to calculate Jaccard's similarity coefficient for each pair-wise comparison (Jaccard 1908). The similarity co-efficients were subjected to Unweighted Pair-Group Method of Arithmetic Average (UPGMA) cluster analysis to group the genotypes based on their

overall similarities using Statistical Package for Social Sciences (SPSS).

Results and discussion

The selected seven primers gave a total of 58 amplification products out of which 48 amplification products were polymorphic (82.75%) in 12 selected genotypes. Number of amplified products obtained were specific to each primer and ranged from 5 (OPJ 4) to 11 (OPJ 6). The maximum polymorphism (82.75%) was observed with OPJ-6 (11 alleles), OPA-3 (10 alleles), OPH-7 (10 alleles) followed by OPH-20 (8 alleles), OPA-19 (7 alleles), OPJ-1 (7 alleles) and OPJ 4 (5 alleles). The alleles obtained were polymorphic with respect to size of the fragments amplified which ranged from 100 to 7000 bp (Fig 1-4).

Similarity index and grouping of cultivars: Jaccards similarity co-efficients were obtained with seven primers for 12 genotypes of groundnut on the basis of presence or absence of band (Table 3). The similarity index values ranged from 32.6% (TCGS 645 and Tirupati 4) to 92.9% (Narayani and Kalahasti) indicating the presence of wide range of genetic diversity at molecular level among the 12 genotypes. In general, the genotypes viz. Tirupati-4 and Tirupati-3 exhibited least similarity with other cultivars and were more distinct and diverse. The dendrogram revealed varying levels of similarity among the genotypes studied and consisted of six closely knit groups (Fig 5.). Narayani of the first group and Tirupati-4 of the sixth group were the two extremes in the dendrogram between which all the other cultivars were distributed. In the dendrogram, the maximum similarity (92.90%) was observed between the cultivars, Narayani and Kalahasti of group 1a and minimum of 32.60% was found between the genotype, TCGS-645 of group 4 and Tirupati-4 of group 6. Group 1b consisted of one cultivar, TCGS-653 which was genetically closer to both Narayani and Kalahasti.

Group 2a comprised of TCGS-913 and TG-47 which were closely related having 86.5 per cent similarity while group 2b included TCGS-750. Group 3a included two genotypes (Prasuna and Abhaya) having 80 per cent similarity. Group 3b included single genotype, Greeshma (TCGS-888)) which was genetically dissimilar. Group 4, 5 and 6 comprised of one genotype each *viz.,* TCGS-645, Tirupati-3 and Tirupati-4 respectively indicating wide variability among these genotypes.

Among the genotypes analysed, Tirupati 3, Tirupati 4, Prasuna, TCGS 653, TCGS 645 and TG 47 were highly dissimilar with the other genotypes. These lines can be included in hybridization programme to realize higher variability in segregating generations. Highly similar genotypes were Narayani and Kalahasti (92.9%), TG 47 and TCGS 913 (86.5%), TG 47 and TCGS 750 (85%), TG 47 and Narayani (82.5%), TG 47 and Abhaya (82.5%), Narayani and Abhaya (81.4%), Narayani and TCGS 913 (80.5%) and Abhaya and Prasuna (80%), TCGS 653 and Kalahasti (80%). The similarity indices fairly reflect the degree of closeness and diverseness in their pedigrees one exception being the distance between Narayani and Tirupati 4. Though they share the same pedigree *i.e.* they are derived from JL-24 x Ah316/S, Narayani of group 1 and Tirupati-4 of group 4 were clustered at the two extremes. They exhibited only 52.4 per cent of similarity indicating that the diversity might have arisen because of differential contribution from the two parents and also might be due to limited coverage of the genome by the primers used. It is also possible that the primers might have amplified the most dissimilar portion of the genome in these two varieties. The cultivar, Narayani was genetically similar (92.90%) to Kalahasti and TCGS-913 was similar (86.50%) to TG-47 though they have different pedigrees.

It is evident from the present study that RAPD markers could be employed for assessment of molecular genetic divergence and relatedness among groundnut genotypes as they are easy to acquire and use and do not need sequence data. Earlier, Dwivedi *et al.* (2001) and Nalini Mallikarjuna *et al.* (2005) also reported a high level of genetic variation in groundnut using RAPD markers. In a similar study involving 12 released cultivars, Radhakrishnan *et al.* (2004) reported wide genetic diversity among released cultivars *i.e.* within cultivated groundnut in contrast to earlier reports and concluded that with RAPD technique, sufficient polymorphism can be detected in cultivated groundnut which would help in genetic mapping. Vyas *et al.* (2014) used RAPD markers to analyze diversity among 15 genotypes of groundnut and reported similarity of 69% between UG 100 and GG 7 and of 94% between UG 109 and UG 110.

The first group comprising of two sub-clusters (1a and 1b) had a minimum similarity of 77.3% and maximum of 92.9%. The cultivars Narayani and Kalahasti of sub-cluster 1a were closely related having 92.9% similarity. These two cultivars possess red seed coat colour, while group 1b comprised of TCGS-653 which shared a similarity of 77.3% with Narayani. Group 2 included 2 sub-clusters within which the maximum similarity (86.5%) was observed

between TCGS-913 and TG-47 of sub group 2a and minimum of 78.6% between TCGS-913 and TCGS-750 of sub group 2b.These genotypes are short statured with narrow leaflets and dark green foliage with high SCMR. Group 3 also included 2 sub clusters with a maximum similarity of 80% between Prasuna and Abhaya of sub-group 3a. These two genotypes possess 3-seeded pods in higher frequency.

Detection of genotype-specific alleles: Amplification profiles of 12 genotypes with seven primers helped in the identification of genotype-specific alleles. The cultivar, Tirupati-4 showed a unique allele of 300 bp with primer, OPA-3 The uniqueness of bands with specific genotype has to be verified further involving large number of genotypes. This allele is absent in other cultivars. Thus, the amplification profile of Tirupati-4 with OPA-3 primer is genotype-specific and can be used for identification of the genotypes. Similarly, the genotype, Greeshma (TCGS-888) also showed a specific allele of 200 bp with OPA-3 primer (Figure 1). In this genotype, a thick specific allele of 400 bp was amplified with OPA-19 primer. The primers, OPA-19 and OPA-3 could be used to differentiate TCGS-888 from others. In the genotypes, Tirupati-3 (400 bp), TG-47 (75-100 bp) and TCGS-913 (75-100 bp), OPA-19 primer amplified unique alleles. In amplification profiles of the genotypes, Tirupati-3 and Prasuna with OPA-19 primer an allele of 500-600 bp was absent which was present in all the other genotypes. Thus ,OPA-19 is also useful to differentiate Tirupati-3 and Prasuna from others (Figure 2). All the genotypes, except Tirupati-4 exhibited a common allele of 75 bp with OPJ-4 primer. The profile with OPJ-4 could be used to distinguish Tirupati-4 from other genotypes. The genotype, Abhaya exhibited two specific small sized alleles with OPJ-4 primer (Figure 4). With primer OPJ-6, the genotypes, Kalahasti (7000 bp) and TCGS-750 (500 bp) produced unique alleles which can be used as markers to identify these cultivars (Figure3). Thus, genotype-specific amplification profiles observed with specific primers would help in the identification of the genotypes. However, unique alleles reported here are in only 12 genotypes with seven markers and their uniqueness has to be verified over a wide range of genotypes.

The study has clearly indicated the scope of using RAPD markers for varietal differentiation and diversity assessment at molecular level. The genotypes, Tirupati 4, Tirupati 3, Prasuna, TCGS 653 and TCGS 645 were found to be more diverse and can be used in hybridization programme to generate wider variability for yield and its attributes. Genotype-specific alleles identified with OPA 19, OPA 3, OPJ 4 and OPJ 6 would be useful in identification of groundnut genotypes viz. Tirupati 4, Tirupati 3, Prasuna, TCGS 913, Kalahasti and TCGS 750.

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Table 1. List of groundnut genotypes used for genetic diversity

S.No.	Cultivar	Pedigree	Salient characteristic features	Reference
1	Tirupati-3	Isogenic selection from TMV-10	Virginia bunch type, dark green foliage, 6-7 primaries, 3-4 secondaries, narrow leaflets, dormant, shelling out-turn 70-73%, pods 2-seeded, medium bold, without constriction, slight reticulation. Testa colour red. Kalahasti malady resistant. LLS tolerant, duration 120-135 days. High oil content (52%).	Vasanthi *et al.* (1998)
2.	Tirupati-4	JL-24 x Ah316/s	Erect growth habit with 4-5 primaries, foliage green with large leaflets, suitable for both *Kharif* and *rabi* seasons, tolerant to mid-season moisture stress, shelling out-turn 72-76%, oil 49%, non-dormant, pods 2 seeded medium bold with moderate to deep constriction moderate reticulation and slight beak. 100 pod weight 100-150 g, 100-kernel weight 43-47 g. Duration 105 days.	Reddy *et al.* (2000)
3.	Narayani	JL-24 x Ah 316/s	Erect growth habit with 4-5 primaries, leaflets long and large, elliptical and green, stem with light greenish purple pigmentation. Suitable for both the seasons, tolerant to mid-season moisture stress. Synchronous maturity of pods, light-red testa, shelling out-turn 75-76%, oil content 49%, pods, 2-seeded, medium bold with moderate constriction, moderate reticulation and slight beak, 100-pod weight 90-100 g, 100-kernel weight 40-45 g, duration-100 days.	Vasanthi *et al.* (2003)
4.	Kalahasti	TCG-1709 x TCG-1518	Decumbent growth habit, short stature, leaflets short, broad and oval shaped, dark green with 6-8 primaries, resistant to Kalahasti malady and recommended for *rabi* cultivation throughout Andhra Pradesh specifically to endemic areas of Kalahasti malady suitable for high rainfall areas, shelling out-turn 74-76%, oil content 52%, pods 2 seeded, medium bold with shallow constriction, slight reticulation and moderate beak, SMK 85-90%, 100-pod weight 95-105 g, 100-kernel weight, 50 g and red testa. Duration 105-110 days.	Vasanthi *et al.* (2003)
5.	Prasuna	TCG-1717 x TCG-1518	4 to 5 primaries, dark green foliage, medium sized, elliptical leaflets, medium bold pods with slight constriction, reticulation and moderate beak. 105-110 days duration, testa rose, moderately tolerant to Kalahasti malady, moderately tolerant to LLS and sucking insects. Oil content 50%.	Vasanthi *et al.* (2011)
6.	Abhaya	K-134 x TAG-24	Short and compact type, pods mostly 3-seeded, reticulated with slight beak. Leaflets narrow, elliptical and dark green, pods slender and medium bold. Testa colour rose. Duration 105-110 days, high WUE, drought and LLS tolerant, tolerant to sucking insects and spodoptera. High oil content (52%).	Vasanthi *et al.* (2006)
7.	TCGS-653	JL-24 x ICGV-86031	Erect, 4-5 primaries, leaflets narrow, elliptical, dark green, pods 2-seeded, medium bold, smooth with slight constriction and beak. 110 days duration. Rose testa, oil-49%.	Vasanthi and Padmavathamma (2000)

8.	TCGS-888	TIR-46 x JUG-37	Short, compact plant type, 5-6 primaries, narrow leaflets, oval and dark green, duration 95 days. High WUE and drought tolerant. Thin pod shell. Smooth pods with slight to moderate constriction, slight beak, flesh testa.	Vasanthi *et al* (2012)
9.	TCGS-913	Tirupati-4 x TIR-45	Short, compact plant type, 5-6 primaries, leaflets narrow, medium sized, oval and dark green. 95 days duration, high WUE, LLS susceptible, pods 2-seeded.	Vasanthi *et al* (2012)
10.	TCGS-750	Tirupati-3 x ICGV-86031	Short duration virginia bunch, leaflets round, dark green, medium sized, leaves and stems hairy. 5-6 primaries, pods 2-seeded ,slight constriction, smooth, slight beak. Tolerant to sucking insects. 110-120 days duration.	John *et al* (2004)
11	TG 47	TAG-24 x TG-19	Short, statured, compact type, narrow, dark green and elliptic leaflets, pods mostly 3-seeded, bold with moderate constriction and beak. Tolerant to LLS, 115-120 days duration. Oil content 45%. Pods with slight reticulation.	Vasanthi *et al* (2012)
12	TCGS 645	JL-24 x ICGV-86398	Virginia bunch, leaflets broad and round, dark green with ashy coat. Pods mostly 2-seeded, medium bold with slight reticulation and beak, tolerant to sucking insects and spodoptera.	Unpublished

Table 2. List of random primers along with their nucleotide sequence selected for PCR amplification in groundnut

Sl. No.	Primer code	Nucleotide sequence (5' – 3')	Total number of bands	Polymorphic bands
1	OPA – 3	AGTCAGCCAC	10	9
2	OPA – 19	CAAACGTCGG	7	6
3	OPH-7	CTGCATCGTG	10	8
4	OPH-20	GGGAGACATC	8	5
5	OPJ-1	CCCGGCATAA	7	6
6	OPJ-4	CCGAACACGG	5	5
7	OPJ-6	TCGTTCCGCA	11	9

Table 3. Similarity matrix of 12 groundnut cultivars using Jaccards coefficient of similarity

Cultivars	Tirupati-3	Tirupati-4	Narayani	Kalahasti	Prasuna	Abhaya	TCGS-653	TCGS-888	TCGS-913	TCGS-750	TG-47	TCGS-645
Tirupati-3	1											
Tirupati-4	0.410	1										
Narayani	0.500	0.424	1									
Kalahasti	0.500	0.489	0.929	1								
Prasuna	0.537	0.487	0.756	0.705	1							
Abhaya	0.468	0.488	0.814	0.761	0.800	1						
TCGS-653	0.533	0.488	0.773	0.800	0.674	0.733	1					
TCGS-888	0.526	0.432	0.675	0.628	0.694	0.634	0.718	1				
TCGS-913	0.477	0.429	0.805	0.750	0.700	0.762	0.721	0.750	1			
TCGS-750	0.522	0.444	0.756	0.783	0.738	0.795	0.756	0.659	0.786	1		
TG-47	0.488	0.475	0.825	0.767	0.763	0.825	0.780	0.771	0.865	0.850	1	
TCGS-645	0.500	0.326	0.596	0.592	0.438	0.563	0.596	0.488	0.578	0.583	0.591	1

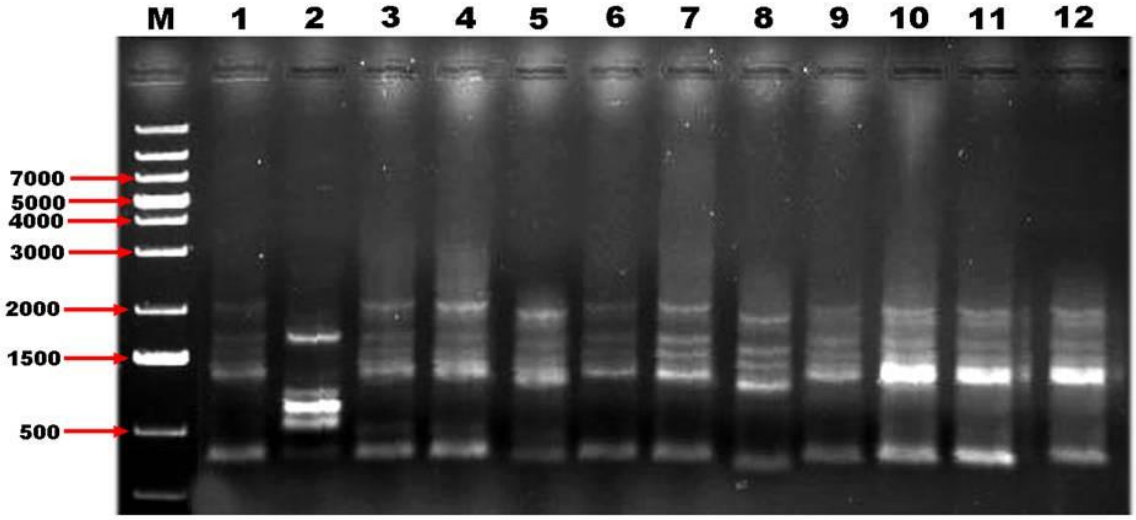


Fig 1. RAPD gel profile of 12 groundnut cultivars with OPA-3 primer

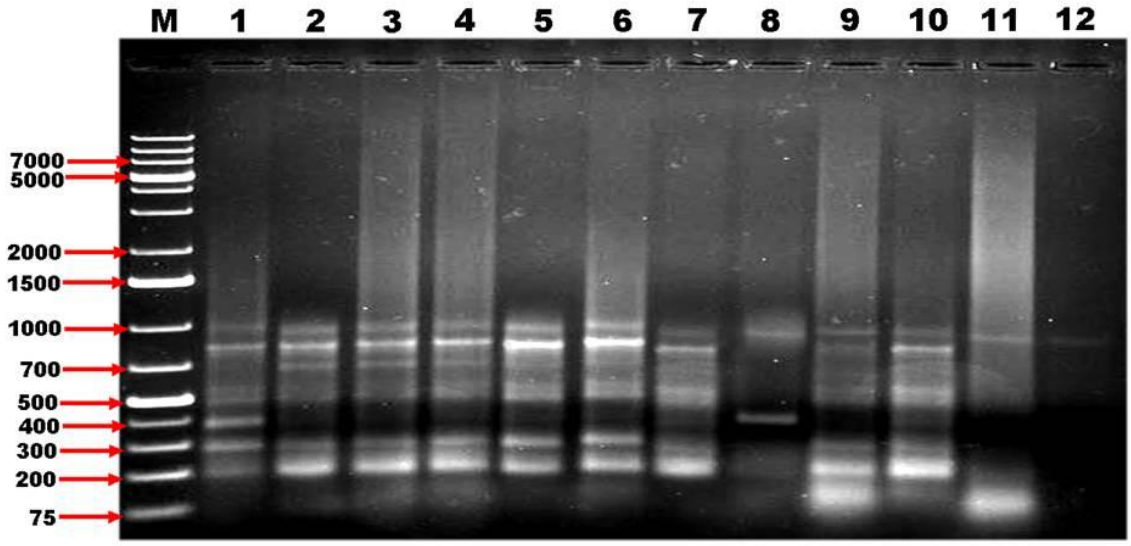


Fig 2. RAPD gel profile of 12 groundnut cultivars with OPA-19 primer

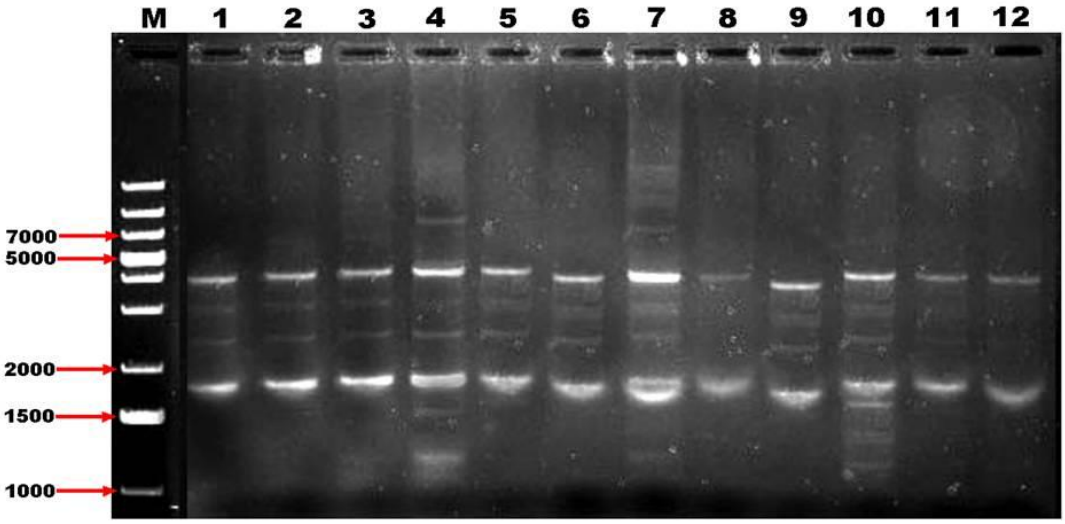
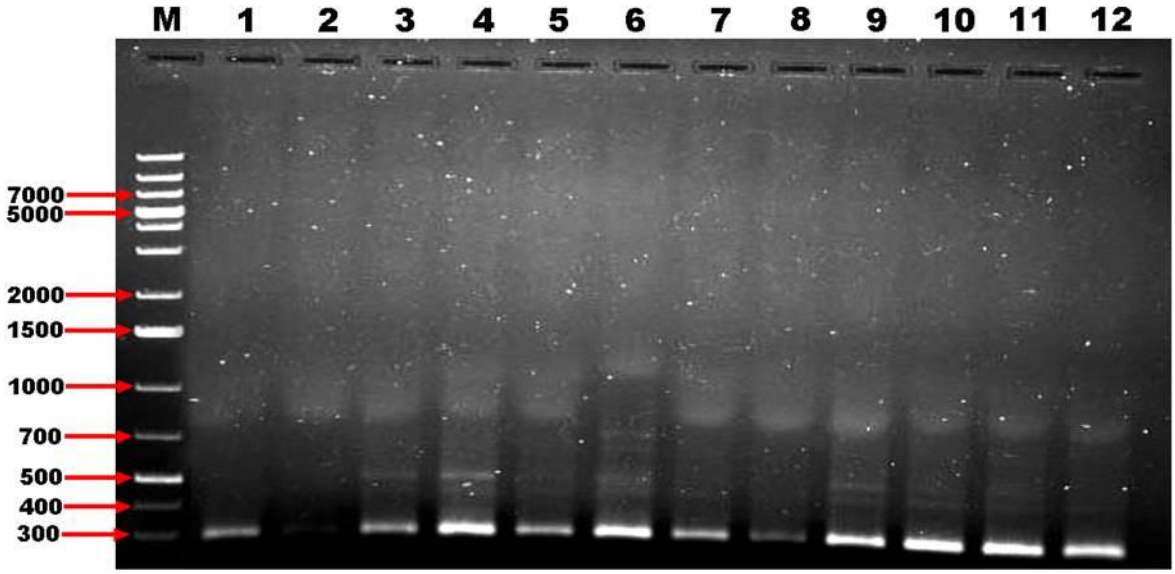


Fig 3. RAPD gel profile of 12 groundnut cultivars with OPJ-6 primer



1. Tirupati-3
2. Tirupati-4
3. Narayani
4. Kalahasti
5. Prasuna
6. Abhaya
7. TCGS-653
8. TCGS-888
9. TCGS-913
10. TCGS-750
11. TG-47
12. TCGS-645

Fig 4. RAPD gel profile of 12 groundnut cultivars with OPJ-4 primer

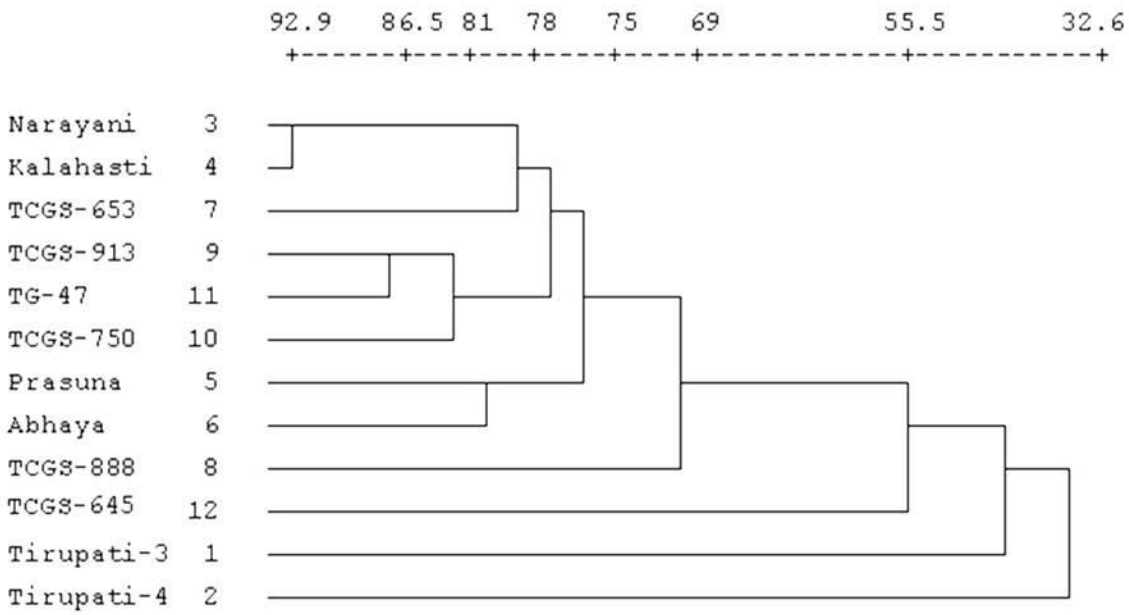


Fig 5. Dendrogram generated through UPGMA analysis