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ORIGINAL RESEARCH

Assessment of Genetic Diversity in Groundnut (*Arachis hypogaea* L.) Genotypes using PCR based RAPD markers

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ABSTRACT
Cultivated groundnut or peanut is one of the important legume crops of tropical and semiarid tropical countries, where it provides a major source of edible oil and vegetable protein. Knowledge of genetic diversity within and among varieties in a crop is prerequisite to its improvement. The objective of this study is to estimate the genetic diversity and to evaluate the relationship between eight groundnut genotypes using Random Amplified Polymorphic DNA (RAPD). Eight groundnut genotypes were included in this study and were subjected to RAPD analysis. Total 10 primers were used to detect polymorphism but only 8 primers showed amplification. Selected primers generated 52 amplification products, out of which 34 were polymorphic (65.38%). The number of fragments amplified per primers ranged from 2 to 15 and their sizes ranged from ~ 500bp to ~2000bp. Maximum number of amplification products were observed with OPD-02 primer (100% polymorphism). UPGMA cluster analysis among the genotypes revealed that the genotypes Narayani and Dharani are genetically similar with 86.8% whereas Kadiri-6 and Dharani were distant. Genotype specific amplification profiles observed with specific primers would help in the identification of the genotypes resistant to biotic, abiotic stress and agronomically important characters. The alleles represented in 8 groundnut genotypes with 5 RAPD markers and their uniqueness has to be verified over a wide range of genotypes.

KEY WORDS: *RAPD, UPGMA, Polymorphism, Genotypes*

Introduction

Groundnut also known as peanut is an important oil, food, and feed legume crop grown in over 100 countries. Over 60% of global groundnut production is crushed for extraction of oil for edible and industrial uses, while 40% is consumed in food uses and others. It covered 24 million ha area worldwide with a total production of 38 million tons in 2010. The projected demand of groundnut in Asia alone by 2020 is expected to be 1.6 times more than the level of production in 2000. If the projected demands should be met, the productivity and production of the crop has to increase at a much higher growth rate than the present one. The lack of adequate and quality food to the ever-increasing world population is putting more and more pressure on the agricultural industry to deliver higher crop yields with better quality. The targeted traits for improvement are quality, flavor traits, freedom from mycotoxins in the produce, yield and yield contributing parameters.

Knowledge of the genetic diversity is a fundamental aspect in the improvement of a crop species. In cultivated groundnut although the molecular markers are becoming progressively important as useful tools in assessing the genetic diversity in crop breeding programs, their application in groundnut improvement is lagging behind because of the limited

knowledge of the groundnut genome. Extensive variation for morphological and physiological characteristics exits in both wild and cultivated groundnut but abundant DNA polymorphism has been observed only in wild diploid *Arachis* species as compared to cultivated species. The genetic variability observed is much less than the immense phenotypic variability which makes it one of the main reasons for little progress in genetic enhancement of the crop. The utility of PCR based marker variations as phylogenetic markers for investigating evolutionary relationships among plants has been clearly established. These techniques are not affected by environmental factors.

Molecular markers offer plant geneticists and breeders a set of genetic tools that are abundant, non-deleterious and reliable. Markers systems have been successfully used over the last several decades to construct genetic maps, access genetic diversity and locate genes of interest in a number of agriculturally important crops for the desired traits. Random Amplified Polymorphic DNA (RAPD) is a powerful technique for screening different germplasm for assessing their genetic diversities. Therefore the present study was carried out to assess genetic diversity among eight groundnut genotypes using RAPD marker in support of breeder's crop improvement programs.

Materials and Methods

Plant Material

Eight *Arachis hypogaea L*. species seeds procured from Regional Agricultural Research Station, ANGRAU, Tirupati and were used for molecular study. The groundnut genotypes were Greeshma, Narayani, Kadiri 6, TAG 24, TPT-2, Kadiri 9, Dharani and Abhaya. Plants were grown hydrophonically and the DNA was extracted from each genotype separately.

DNA Extraction

Leaves of 15 days old seedlings were used for DNA extraction. Plant DNA extraction was done by procedure given by Murray and Thompson(1980) with slight modifications. Groundnut plant leaves were weighed (1g) and ground in pestle and mortar and the mixture was transferred into centrifuge tubes. Preheated (65°C) 2% CTAB extraction buffer was added to make slurry. The tubes were then incubated at 65°C for an hour and stirred occasionally with the help of the sterile glass rod. Centrifuged at 14,000 rpm for 10 min. Equal volume of chloroform:Isoamylalcohol (24:1) was added to each tube and mixed gently. Samples were centrifuged at 10,000 rpm for 10 min at room temperature. Then upper aqueous phase was precipitated with 0.6 volume of ice cold isopropanol and 0.1 volume of 3M sodium acetate (pH 5.2) and spinned at 15,000 rpm for 15min at room temperature and kept for overnight incubation. Supernatant discarded and 70% ethanol was added to pellet and centrifuged at 10,000 rpm for 10min. Ethanol was pipetted out carefully and kept for drying at room temperature. Nucleic acid obtained was dissolved in sterile distilled water and stored at -20°C in small aliquots and the DNA was quantified using UV-Vis Spectrophotometer.

Random Amplification Of Polymorphic DNA (RAPD)

Ten primers (Sigma Aldrich, USA) were used for initial screening of repeatable amplification with eight groundnut genotypes (Table. 1).

Table 1: List of the RAPD primers for PCR amplification in groundnut

S.No	Name of the Primer	Primer Sequence ($5^1 – 3^1$)
1	OPA-03	AGTCAGCCAC
2	OPA-05	AGGGGTCTTG
3	OPA-10	GTGATCGCAG
4	OPA-15	TTCCGAACCC
5	OPA-19	CAAACGTCGG
6	OPD-02	GGACCCAACC
7	OPD-06	ACCTGAACGG
8	OPD-11	AGCGCCATTG
9	OPD-17	TTTCCCACGG
10	OPD-20	ACCCGGTCAC

PCR was carried out in 25µl reaction volumes containing template DNA 25ng, Taq DNA polymerase (Fermentas)-0.5 units; $MgCl_2$-5mM; dNTP (Fermentas) 200µM each; primer (Sigma Aldrich, USA.)-1µm, Buffer (Fermentas).The amplication was performed in a Biorad Thermo cycler with the initial denaturing at 94°C for 2min follower by 35 cycles of denaturing at 92°C for 1min; annealing at 37°C for 1min, extension at 72°C for 2min with final extension of 72°C for

5min and hold the reaction at 4°C. Primer screening was carried out using 10 primers from OPA and OPD series (Sigma Adrich, USA) for molecular variation analyzing. The primer that gave reproducible and recordable amplification was used in the analyzing of variability of the genotypes.

Agarose Gel Electrophoresis

To the 25µl amplification product obtained after the PCR reaction, 4µl loading dye (Bromophenol blue) was added and loaded into individual wells of 1% agarose in 1X TBE buffer. Electrophoresis (Genei) was carried out at 60V for 3 hours and there after the gel was stained with ethidium bromide (1µg/ml). The observation was made on a transilluminator under UV light. 1kb ladder (Fermantas) was also loaded in one lane as a marker.

Cluster Data Analysis

Each amplification product was considered as RAPD marker and recorded across all sample. Data was entered using a Similarity matrix generated by SPSS software (Version-20) in which all observed bands or characters was listed. The RAPD pattern of each isolate 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 similarity co-efficient for each pair-wise comparision. The co-efficients were calculated following Jaccard (1908) and the similarity co-efficient were subjected to Unweighted Pair-Group Method on Arithmetic Average (UPGMA) cluster analysis and sub-sequent Dendrogram preparation was done.

Results and Discussion

Five selected primers gave total of 52 amplification products, out of which 34 were polymorphic (Table. 2). Among the primers of OPA and OPD series, 5 primers produced scorable and reproducible amplifications in all the isolates. Maximum polymorphism was showed in PCR reaction with OPD-02. These primers showed 100% polymorphism as all these obtained were polymorphic in size ranging from 1 to 2 kb. They were closely followed by OPA-03 with 60% polymorphism. The banding pattern of OPA-05 and OPA-10 was found to be identical with many primers and in case of primer OPD-02 total number of bands were 15 out of which 15 were polymorphic. The size of amplified products varied from 500 to 2 kb (Fig 1-5). Jaccard's similarity co-efficient between the isolates were calculated (Table. 3). Banding profiles obtained with 5 random primers for 8 genotypes of *Arachis hypogaea L.* were analyzed on the basis of presence and absence of the bands.

Table 2: List of polymorphic bands produced after PCR amplifications

Name of the Primer	Primer Sequence ($5^1 - 3^1$)	Total Number of bands	Polymorphic bands
OPA-03	AGTCAGCCAC	14	8
OPA-05	AGGGGTCTTG	3	1
OPA-10	GTGATCGCAG	8	2
OPA-15	TTCCGAACCC	0	0
OPA-19	CAAACGTCGG	12	8
OPD-02	GGACCCAACC	15	15
OPD-06	ACCTGAACGG	0	0
OPD-11	AGCGCCATTG	4	3
OPD-17	TTTCCCACGG	0	0
OPD-20	ACCCGGTCAC	12	08

Similarity matrix thus produced indicated that Dharani and Kadiri-9 were genetically distinct as they showed only 40.9% similarity followed by Kadiri-9 and Narayani with 41.3%. While the varieties Narayani and Dharani were found to be genetically similar with 86.8% similarity followed by 78.4% similarity between TAG-24 and Greeshma, 77.8% between Kadiri-6 and Abhaya, 70.8% between Kadiri-9 and Greeshma.

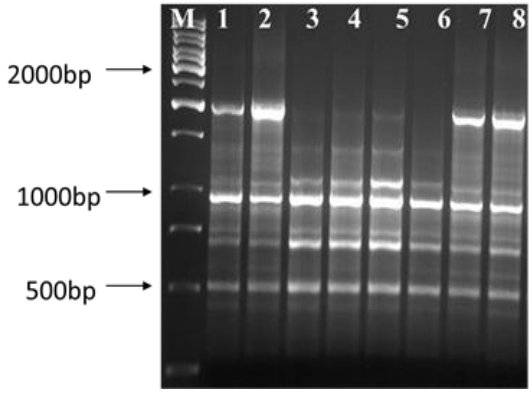

Figure 1: RAPD profiles of 8 groundnut genotypes with primer (Lane M-Marker, 1-Greeshma, 2-Narayani, 3-Kadiri-6, 4-TAG-24, 5-TPT-2, 6-Kadiri-9, 7-Dharani, 8-Abhaya)

The similarity co-efficients subjected to SPSS software (version-20) to produce a dendrogram, produced 2 major clusters (Fig. 6) First cluster having Narayani and Dharani with first sub-cluster having TPT-2 and Kadiri-6, second sub-

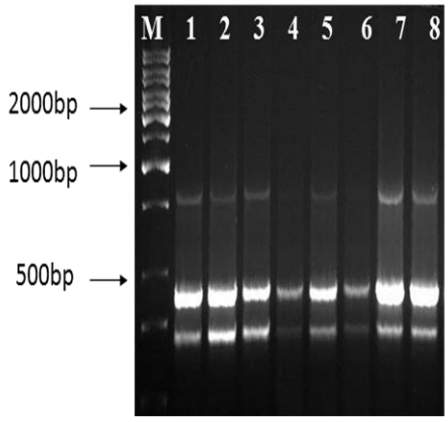

Figure 2: RAPD profiles of 8 groundnut genotypes with primer OPA-05

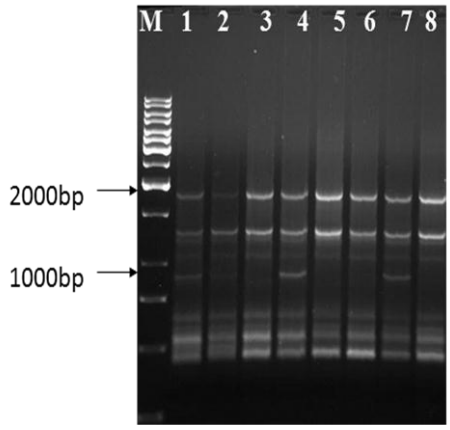

Figure 3: RAPD profiles of 8 groundnut genotypes with primer OPD-10

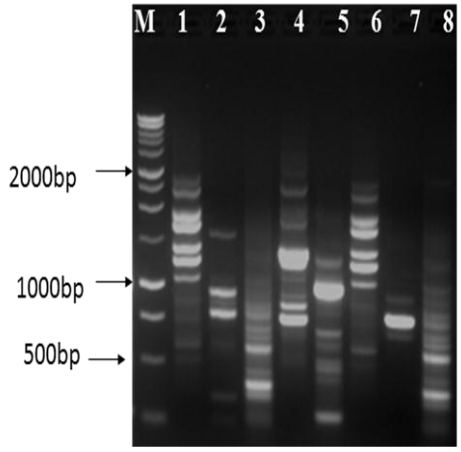

Figure 4: RAPD profiles of 8 groundnut genotypes with primer OPD-02

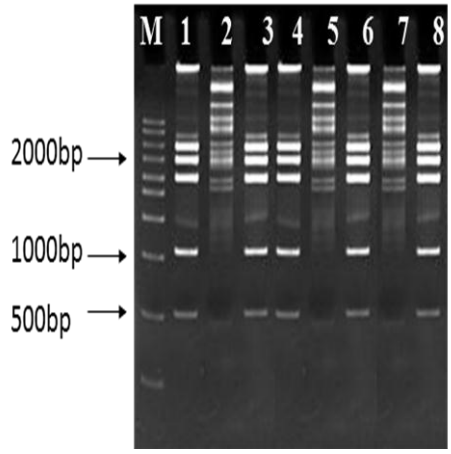

Figure 5: RAPD profiles of 8 groundnut genotypes with primer OPD-19

(Lane M-Marker, 1-Greeshma, 2-Narayani, 3-Kadiri-6, 4-TAG-24, 5-TPT-2, 6-Kadiri-9, 7-Dharani, 8-Abhaya)

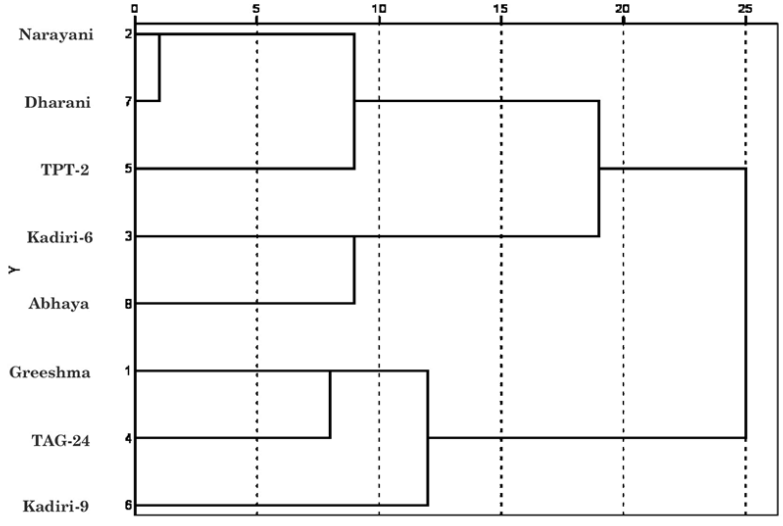

Figure 6: Dendrogram generated for 8 groundnut varieties using UPGMA cluster analysis based on Jaccard's similarity coefficient

Table.3: Similarity matrix obtained from RAPD data of 8 groundnut genotypes

Similarity Matrix								
Groundnut	**Matrix File Input**							
Varieties	Greeshma	Narayani	Kadiri-6	TAG-24	TPT-2	Kadiri-9	Dharani	Abhaya
Greeshma	1.000							
Narayani	0.532	1.000						
Kadiri-6	0.548	0.489	1.000					
TAG-24	0.784	0.478	0.605	1.000				
TPT-2	0.523	0.683	0.590	0.500	1.000			
Kadiri-9	0.703	0.413	0.611	0.735	0.500	1.000		
Dharani	0.533	0.868	0.524	0.512	0.784	0.409	1.000	
Abhaya	0.605	0.651	0.778	0.585	0.610	0.550	0.700	1.000

cluster having Kadiri-6 and Abhaya. Second cluster having Greeshma and TAG-24 with sub-cluster having Greeshma and Kadiri-9. Genotypes TAG-24 and TPT-2; Dharani and Kadiri-6 are distantly related and from separate branches as they were not grouped with these clusters. Among all the genotypes there is close relationship between two genotypes Narayani and Dharani.

The characterization of *Arachis hypogaea* L. varieties by RAPD has proved useful in separating all the isolates from each other. It has also provided us with primer markers that can be used to separate and distinguish each isolate. This possibility of distinguishing different isolates by a rather simpler technique of genomic fingerprinting based on PCR-RAPD could be of great importance for use in patent protection of plant strains of biotechnological use, where additional more easily detectable markers are not available.

Conclusion

The study has clearly indicated the scope of using RAPD markers for varietal differentiation and diversity assessment at molecular level. Genotype-specific amplification profiles observed with specific primers would help in the identification of the genotypes resistant to biotic, abiotic stress and agronomically important characters. The alleles represented in 8 groundnut varieties with 5 RAPD markers and their uniqueness has to be verified over a wide range of genotypes.

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