Genetic Diversity of Indian Groundnut Cultivars

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Groundnut was introduced to India in 1800 AD at the then Madras presidency and has been spread to other states by about 1910. Subsequently, through breeding methods around 140 groundnut varieties have been developed for commercial cultivation in India. Groundnut cultivars, cultivated in early 1900, like Gangapuri and Spanish improved were selections from the germplasms. Of the commercially available cultivars, more than 40% are selections from ruling varieties and about 50% have been developed through hybridisation and selection. Analyses of the pedigrees of the commercial cultivars, released so far, indicates that only a few parents like M13, GAUG 10, and Robut 33-1 have been used in the hybridisation programmes. Selections have also been made from very few accessions only for instance, the cultivars ICGS 1, ICGS 11, ICGS 44, and Kadiri 3 were selections from Robut 33-1.

In the genus *Arachis,* considerable variability with regard to morphology and economically important traits like resistance to pests and diseases have been documented (Stalker and Moss, 1987; Mehan *et al.,* 1992) but the morphological variability exhibited in the cultivated species (*Arachis hypogeae* L.) doesn't reflect at the biochemical and molecular level till recent times. Application of molecular approaches like RFLP, RAPD could detect very limited polymorphisms in cultivated groundnut (Kochert *et al.,* 1991, Paik-Ro *et al.,* 1992) due to a very narrow genetic base of the breeding materials used. With the advent of the recent techniques like AFLP, DAF and SSRs, better polymorphisms have been detected in cultivated groundnut (He and Prakash, 1997; Hopkins *et al.,* 1999).

In the present investigation, we attempted to examine the variability, at molecular level by RAPD, of some of the old cultivars and the new cultivars developed out of them through hybridisation programme.

Materials and Methods

Plant materials

Seed materials were obtained from the gene bank of cultivated groundnut at the National Research Centre for Groundnut. Twelve Indian cultivars belonging to the four different habit groups were used in the study (Table 1). The cultivars (old and new ones) were selected keeping in mind the pedigree as well as the geographic locations.

Isolation of DNA

Approximately 2 g of leaf material from the plants grown in dark (etiolated) was used for isolation of genomic DNA. Fresh leaves were ground in liquid nitrogen and the urea-based method for DNA isolation as described by Chen and Dellaporta (1994) was followed. The quantity and quality of DNA were determined using the spectrophotometric method.

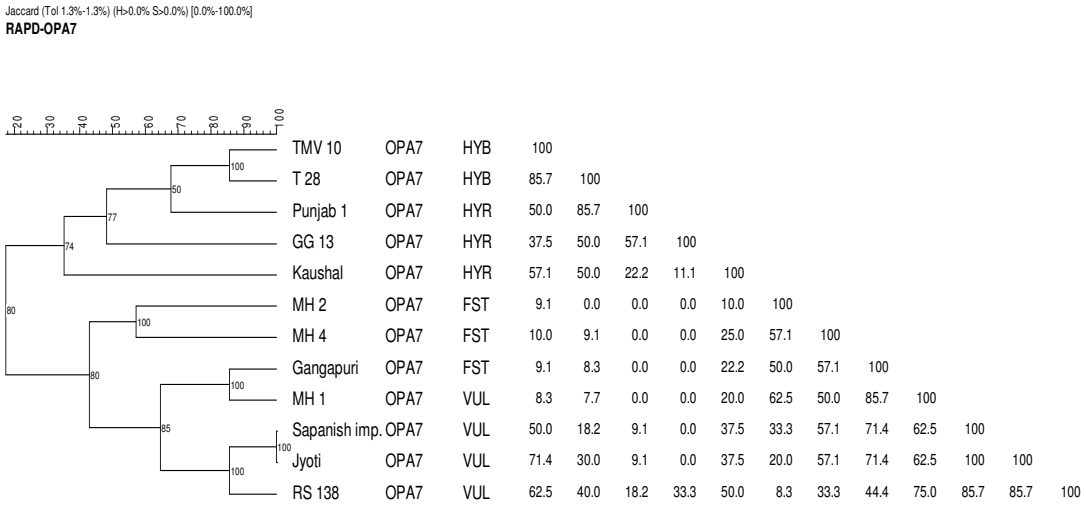
PCR amplifications and analysis of products

Primers: From the preliminary screening of 72 primers, 8 primers (Table 2) capable of amplifying DNA were selected for RAPD (Table 2) analysis. Twenty-five µl of the reaction mixture was prepared for amplification by mixing *Taq polymerase* buffer 10X (Promega) 2.5 µl; DNTPs 2mM (Fermentas):1.6 µl; Primer 25 pM: 1.0 µl; *Taq polymerase* (Biobasic): 3.0 U; Genomic DNA: 50 ng; Distilled water: 20 µl. The mixture was amplified using a Technic (model Genius) thermal cycler with one cycle of 92°C for 3.5 min; 35°C for1 min; 72°C for 2 min; followed by 44 cycles of 92°C for 1 min; 35°C for 1 min; 72°C for 2 min and a final primer extension cycle of 72°C for 15 min. The amplification products were separated on an agarose gel lectrophoresis and the gel was scanned using gel documentation system. The gel analysis was performed using the Gelcompare II software.

Results and Discussion

A total of eight primers were used for detecting polymorphisms in selected twelve released cultivars. Analyses of DNA polymorphisms indicated that the primers used for the amplification of DNA could produce a total of 586 bands in twelve cultivars tested (Table 3). The maximum number of bands (96 bands) was obtained with the primer OPA1 (5' CAGGCCTTC3') whereas OPA3 (5'AGGTCAGCCAC3') produced the least number of bands (45 bands) across the twelve cultivars. The polymorphism ranged

between 7 and 9.22 with the maximum in GG13 and the minimum in TMV10 (Table 3). OPA 20 produced the maximum of 10 bands in GG13 whereas OPA 1 failed to produce any bands in TMV10. OPA 2 uniformly produced 6 bands in all the genotypes. The following are the similarity matrices for the different primers with the 12 genotypes.



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The primers OPA 20 and OPA 2 could group the HYR and HYB cultivars together where as OPA 1 grouped all the Spanish cultivars together. The primers OPA 4 and OPA 6 could not differentiate between most of the genotypes based on their banding patterns (Table 3).

Of the different primers studied for their ability to distinguish cultivars, OPA6 could differentiate GG 13 from MH1, Jyoti, and Spanish Improved. It could also differentiate between MH 2 and Kaushal, as well as Gangapuri and Punjab 1 (Table 3). OPA 7 was identified as the best among the primers studied. This primer has grouped majority of the cultivars according to their habit groups. It could differentiate GG 13 from MH2, MH 4, Gangapuri, MH1, Spanish improved and Jyoti. Similarly, it could differentiate MH2 from T28, Punjab 1, and GG13. Thus, the present RAPD analyses indicated that genetic diversity exists in the released cultivars within and across habit groups of the genus *Arachis*. This was contrary to the earlier reports of the existence of very low degree of DNA polymorphisms in the released cultivars of groundnut determined on the basis of RFLP and fewer primers. Thus, despite the earlier conclusions that the cultivated groundnut has a very narrow genetic base, we report that with RAPD and suitable primer combinations, polymorphims in cultivated groundnut could be attained.

Reference

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Table 1. The names and pedigree of the groundnut cultivars studied

Cultivar	Habit type	Pedigree	Year of Release
1. T 28	HYB	Selection from Bombay collection	1960
2. TMV 10	HYB	Natural mutant from Argentina	1970
3. GG 13	HYR	GAU 10 x TMV 10	1994
4. Kaushal	HYR	Selection from T 28	1984
5. Punjab 1	HYR	Selection	1953
6. MH 1	VUL	Selection from Faizpur 1-5	1975
7. Spanish Improved	VUL	Selection from Spanish peanut	1905
8. Jyoti	VUL	Selection from Exotic 7	1971
9. RS 138	VUL	Selection from Brazilian culture	1989
10. MH 2	FST	Selection from Gujarat dwarf	1978
11. MH 4	FST	Not traceable	1990
12. Gangapuri	FST	Not traceable	--

Table 2. The list of primers used and their nucleotide sequences

Primer designation	Sequence
OPA1	5'CAGGCCTTC3'
OPA2	5'TGCCGAGCTG3'
OPA3	5'AGTCAGCCAC3'
OPA4	5'AATCGGGCTG3'
OPA5	5'AGGGGTCTTG3'
OPA6	5'GGTCCCTGAC3'
OPA7	5'GAAACGGGTG3'
OPA20	5'GTTGCGATCC3'

Table 3 DNA polymorphism with RAPD primers

Genotype	OPA20	OPA1	OPA2	OPA3	OPA4	OPA5	OPA6	OPA7	Total	%polymphsm
T 28	7	8	6	4	5	8	5	6	49	8.36
TMV 10	8	0	6	4	5	8	4	6	41	7.00
GG 13	10	9	6	4	5	8	5	7	54	9.22
Kaushal	9	9	6	3	5	5	8	7	52	8.87
Punjab 1	8	9	6	4	5	8	6	6	52	8.87
MH 1	8	9	6	4	5	8	6	5	51	8.70
Spanish Improved	7	9	6	3	5	8	4	6	48	8.19
Jyoti	7	9	6	4	4	8	3	6	47	8.02
RS 138	9	9	6	4	5	7	4	7	51	8.70
MH 2	6	7	6	4	5	8	4	5	45	7.68
MH 4	6	9	6	3	5	8	5	5	47	8.02
Gangapuri	6	9	6	4	5	8	5	6	49	8.36
Total	**91**	**96**	**72**	**45**	**59**	**92**	**59**	**72**	**586**	