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RAPD analysis of induced mutants of groundnut (*Arachis hypogaea* L.)

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Abstract. Radiation-induced mutants of groundnut cv. Spanish Improved showing distinct morphological differences and the parent were screened for RAPD variability. The analysis revealed characteristic band differences among the 12 mutants and the parent. The polymorphic RAPD bands were dominant in the F_1 and segregated in a Mendelian fashion in the F_2. The RAPD technique brought out greater genome variability than RFLP.

Keywords. RAPD; variability; groundnut mutants.

1. Introduction

Groundnut (*Arachis hypogaea* L., $2n = 4x = 40$) is the second most important oilseed crop in India, grown over 8 million hectares which accounts for two-thirds of the world's area under this crop. Groundnut originated in South America and was introduced in India by Portuguese traders in the middle of the sixteenth century. In USA and other countries it is mainly grown for the nuts which are consumed after roasting. Despite its commercial importance, genetic and cytogenetic studies on this crop are limited, in part, because of the small size of its chromosomes, which makes cytogenetic observations difficult. Rapid advances in the use of molecular markers based on variation in the DNA sequence have led to development of saturated linkage maps making marker-assisted selection feasible in plant breeding programmes. Many agronomically useful genes have been tagged in different crops (Mohan *et al.* 1997), including legumes (Young *et al.* 1996). Application of molecular markers in plant breeding programme has established the need for information on variation in DNA sequences even in those crops in which little classical-genetic and cytogenetic information is available.

Variability using molecular markers has been examined in groundnut among American cultivars, and unadapted germplasm lines from South American centres of origin, Africa and China (Halward *et al.* 1991, 1992; Kochert *et al.* 1991). In all cases a very low level of variability was found in the cultivated groundnut. Abundant RFLP was however detected among wild diploid species (Paik-Ro *et al.* 1992). Hence the first RFLP linkage map of groundnut was constructed using interspecific hybridization between two related diploid wild species *Arachis stenosperma* and *A. cardenasii* (Halward *et al.* 1993). Using this framework map introgression analysis was done (Garcia *et al.* 1995) of the *A. cardenasii* genome into the tetraploid

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A. hypogaea. Garcia *et al.* (1996) later reported the first molecular marker linked with nematode resistance in groundnut.

Development of groundnut cultivars in India has largely utilized the primary gene pool of introduced material under the name Spanish. A large number of true-breeding mutants were isolated in cv. Spanish Improved (SI) following exposure of seeds to X-rays at our centre (Patil 1966). These mutants are morphologically distinct in plant height, growth habit, branching pattern, and pod and kernel size. Four groundnut cultivars, SI, 12 induced mutants of SI, and *A. monticola* (the proposed tetraploid progenitor of cultivated groundnut) were earlier examined for RFLP using 20 low-copy and three multicopy probes at six restriction enzyme sites (*Eco*RI, *Bam*HI, *Hin*dIII, *Eco*RV, *Taq*I and *Hae*III). As in previous studies (Halward *et al.* 1991, 1992; Kochert *et al.* 1991), the probe–enzyme combination tried did not show polymorphism (unpublished). We therefore initiated the studies reported in this paper with the object of exploring the utility of RAPD markers in molecular analysis of radiation-induced mutants.

2. Materials and methods

2.1 *Plant material*

Groundnut mutants obtained by X-ray irradiation of seeds of cv. Spanish Improved were used in this study (Patil 1966). Comparison of their morphological features is given in table 1.

2.2 *Plant DNA isolation*

The plant DNA used for analysis was isolated from green leaves of field-grown plants by the miniprep method of Dellaporta *et al.* (1983). The quantity and quality

Table 1. The mutants used for RAPD analysis.

No.	Name	Plant habit	Flowering pattern	Distinguishing feature	Mean height in cm
1	Spanish Improved	Spanish bunch	Sequential	Large, oblong leaflet, HKW* 58 g	$75 \pm 2{\cdot}6$
2	Short-1	„	„	20% less in height	$60 \pm 0{\cdot}7$
3	Short-3	„	„	33% less in height	$50 \pm 2{\cdot}8$
4	Short-4	„	„	10% less in height	$68 \pm 1{\cdot}7$
5	Tall	„	„	50% more in height	$125 \pm 4{\cdot}3$
6	Small leaf	„	„	50% reduced leaflet size, light yellow flowers, light green leaves	
7	Virescent	„	„	Chlorophyll deficient	$40 \pm 2{\cdot}3$
8	Spreading	Virginia runner	„	—	
9	Tertiary branching	Spanish bunch	„	—	$72 \pm 5{\cdot}1$
10	Fused top leaflet	„	„	5% of leaves have top pairs of leaflet fused	
11	Minute pod	„	„	HKW 18 g vs. 58 g	
12	Small pod	„	„	HKW 30 g vs. 58 g	$65 \pm 3{\cdot}3$
13	Large pod	Virginia bunch	Alternate	HKW 90 g vs. 58 g	$66 \pm 3{\cdot}5$

*HKW - Hundred Kernel Weight

of the DNA preparations were determined by measuring absorbance at 260 nm and 280 nm.

2.3 *RAPD analysis*

Random decamers either synthesized at BARC or obtained from Operon Technology, USA, were used for the RAPD experiments. Each primer was used in the amplification reactions with DNA from the 12 mutants and SI. The nucleotides used were from Boehringer–Mannheim, the *Taq* polymerase was from Bangalore Genei, India. Reaction mixtures for PCR (50 µl) contained 10 mM Tris-Cl, pH 8·3, 50 mM KCl, 1·5 mM $MgCl_2$, 0·001% gelatin, 200 µM each of dATP, dCTP, dGTP and dTTP, 0·2 µM primer, 200 ng of plant DNA and 2 units of *Taq* DNA polymerase. The amplification reactions were carried out in a MJ Research thermal cycler. Initially denaturation was carried out at 94°C for 5 min, followed by annealing at 35°C, and then the enzyme was added. Fifty cycles of the following program were used for amplification: 72°C for 2 min, 92°C for 1 min and 35°C for 1 min. The products of amplification were analysed by electrophoresis in Tris-acetate buffer using 1·5% agarose gels followed by staining with ethidium bromide and viewing under UV transilluminator.

3. Results

A total of 42 decamers and one octamer were used to analyse the mutants in comparison with the parent SI in this study. Twelve primers that produced relatively simple banding pattern were selected for use in further experiments. A total of 1182 fragments were amplified, of which 65 bands were polymorphic (5·5%) giving a ratio of 1·51 polymorphic bands per primer, Illustrative examples of typical RAPD pattern are shown in figure 1 (a & b). Number of polymorphic bands identified varied with the primer used, e.g. primer OPJ06 yielded high polymorphism among the mutants examined (figure 1a) while primer OPC02 showed differences in only a few of the mutants (figure 1b). The band profiles obtained with the 12 primers used are summarized in table 2. Minimum and maximum numbers of bands obtained with different primers were in the ranges 2–8 and 7–14 respectively. Mutants were compared with the parent SI and the numbers of bands that were different for each of the plant-height and pod-size mutants are shown in table 3. Among the plant-height mutants Short3 showed the maximum difference of 20 bands, while among the pod-size mutants the large-pod mutant was different in 15 bands.

A cross between large-pod mutant and SI was made and the F_1 and F_2 plants were analysed by RAPD using primer OPJ17 which earlier had shown absence of one band (showing slowest mobility) in the large-pod mutant (figure 2, lanes 1 and 2). Dominance of the amplified bands was seen in the F_1 (figure 2, lane 3). Segregation was followed in the F_2 progeny. Parental and recombinant segregants are seen in figure 2 (lane 4 onwards).

4. Discussion

Few studies analysing induced mutations at molecular level have been carried out in *Arabidopsis*, lettuce, maize and tomato (Weck *et al.* 1996). In *Arabidopsis*, mutations at

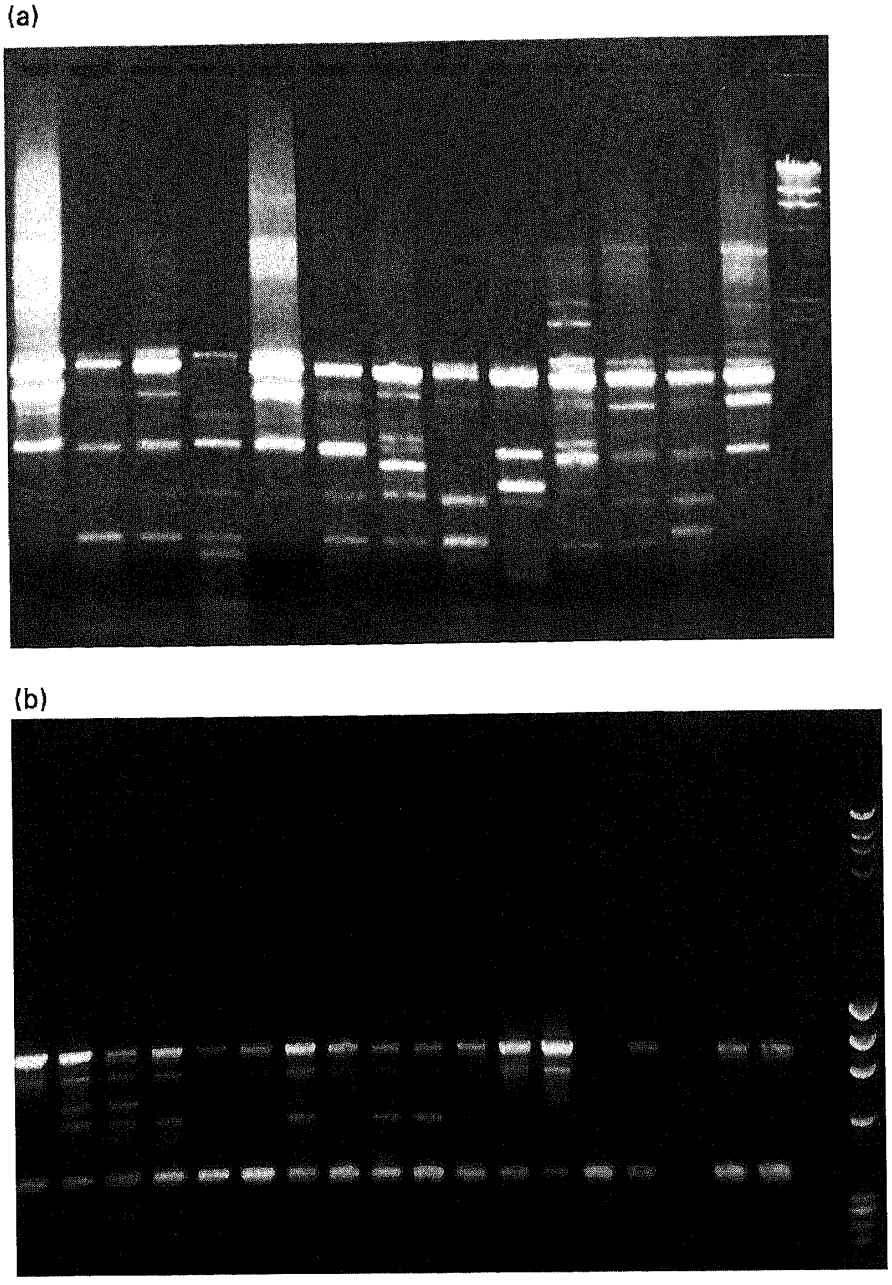

Figure 1. Amplification of DNA from SI and the mutants using (a) primer OPJ 06 and (b) primer OPC 02. DNA sources in the lanes are in the sequence in table 1. The last lane is λ DNA digested with *Hin*dIII + ϕx174 DNA digested with *Hae*III.

abscisic acid insensitive *abi*3–6 (Nambara *et al.* 1994); auxin, ethylene and abscisic acid resistance *axr*2–1 (Timpte *et al.* 1994); chlorate resistance *CHL*3 and *NAI*2 (Wilkinson and Crawford 1991), gibberellin responsive dwarfs *ga*1 (Sun *et al.* 1992); and trichome differentiation gene *gl*1–1 (Oppenheimer *et al.* 1991) involved deletions ranging from 17 bp to 19 cM. A chalcone flavanone isomerase (*CHI*) mutation obtained following fast neutron irradiation revealed a 1·4-kb inversion within the *CHI* gene and a 272-bp insertion adjacent to another inversion from the same chromosome (Shirley *et al.* 1992). A dihydroflavanol-4-reductase (*DFR*) allele, induced by

Table 2. Analysis of RAPD patterns generated using 12 primers comparing the mutants and SI.

No.	Primer #	Total no. of bands	No. of bands monomorphic	No. of bands polymorhic
1	Oligo 1*	121	10	1
2	Oligo 6*	77	2	4
3	OPA 01	84	2	5
4	OPA 12	139	6	6
5	OPA 16	119	4	6
6	OPA 17	96	5	6
7	OPA 18	99	5	4
8	OPC 02	77	5	2
9	OPC 04	83	3	4
10	OPC 13	107	2	10
11	OPJ 06	100	0	14
12	OPJ 17	80	5	3

* Sequence of Oligo 1: CGCGGCCA; sequence of Oligo 6: TGCTCACTGA.

Table 3. Comparison of the plant-height and pod-size mutants with parent SI, showing numbers of bands that were different.

Primer→	Oligo # 1	OPA 12	OPA 16	OPA 17	OPA 18	OPC 02	Total
Height Mutant ↓							
Short-1	0	2	1	1	0	2	6
Short-3	3	2	9	1	3	2	20
Short-4	2	1	4	1	2	1	11
Tall	3	3	—	2	3	2	13
Primer→	OPA 12	OPA 16	OPA 17	OPA 18	OPC 02	OPJ 17	Total
Pod size Mutants ↓							
Minute pod	2	2	2	2	1	3	12
Small pod	2	3	3	2	1	1	12
Large pod	2	5	3	2	2	1	15

X-irradiation, contained two 72-bp and 7·4-kb deletions flanking a 2·8-cM inversion. However, mutations in two genes, namely chalcone flavanone isomerase (*CHI*) and dihydroflavanol-4-reductase (*DFR*), have revealed complicated alterations (Shirley *et al.* 1992). Nine independent deletion mutations, induced following fast neutron irradiation, in downy mildew resistance genes (*Dm*) in lettuce were genetically analysed and their linear order along the chromosome was established (Anderson *et al.* 1996); one mutant showed a deletion of at least 6 Mb. Somaclonal variants of rice examined with 34 random primers revealed band differences, with six primers giving a total of 67 scorable polymorphic bands across five plants and 65 R_2 lines (Sangduan *et al.* 1995).

Mutations other than point mutations are caused by deletions, duplications, inversions or translocations of segments of different lengths. As stated above, most of the

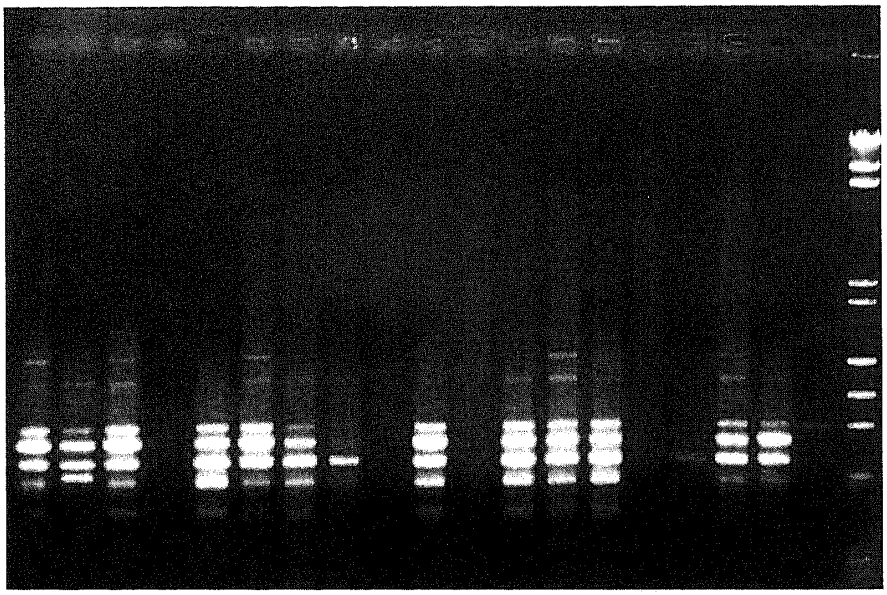

Figure 2. Amplification of DNA from SI and large-pod mutant using primer OPJ 17. Lane 1, SI; lane 2, large-pod mutant; lane 3, F_1; lanes 4 onwards, the F_2 progeny.

X-ray or fast-neutron-induced mutations analysed have mainly revealed deletions, and others inversions and deletions. The nature of alteration was pinpointed in the studies mentioned earlier because mutations in known and well characterized genes were available for comparison in those crops along with the classical phenotypic maps. In groundnut genetic and cytogenetic studies have been hampered owing to small chromosome size and hence a classical genetic map based on morphological traits has not been developed. The mutants examined in the present study represent gross morphological alterations which may be due to changes at a single locus or at more than one loci. The characters virescent and small leaf have revealed monogenic inheritance (Patil 1969, 1984). Characters like pod size and plant height show polygenic inheritance. In the present study the large-pod mutant differed from the parent in more bands than the other pod-size mutants, indicating larger extent of alteration in its DNA. Similarly in case of plant-height mutants the short-3 mutant showed greater sequence alteration. Using additional primers and following the segregation of the traits along with the polymorphic RAPD markers, it may be possible to find some close associations.

The general low level of polymorphism in cultivated groundnut has been attributed to the comparatively recent and single event of polyploidization (Young *et al.* 1996). Later, due to the ploidy barrier between the tetraploid groundnut and the diploid wild species, further gene flow was prevented, resulting in a highly conserved genome. The early cultivators selected the morphological variants suitable for agronomy, traits like larger seed size and branching pattern, which are controlled by only a small number of genes. The existing variation in morphological traits observed among the varieties of domesticated groundnut and different landraces are perhaps all due to differences in those small numbers of genes selected in the beginning.

This study shows that in absence of RFLP in groundnut, as reported in earlier studies (Haldward *et al.* 1991, 1992; Kochert *et al.* 1991; Bhagwat 1996), RAPD analysis

can provide the required molecular markers. Microsatellite or simple-sequence repeats which detect simple sequence length polymorphism (SSLP) may reveal substantial variation (Panaud *et al.* 1996).

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