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Molecular characterization of induced mutagenesis through gamma radiation using RAPD markers in *Jatropha curcas* L.

R. S. Dhillon[1]*, R. P. Saharan[2], M. Jattan[2], T. Rani[3], R. N. Sheokand[4], V. Dalal[1] and George Von Wuehlisch[5]

[1]Department of Forestry, CCS Haryana Agricultural University, Hisar-125004, India.
[2]Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar-125004, India.
[3]Department of Molecular Biology and Biotechnology, CCS Haryana Agricultural University, Hisar-125004, India.
[4]Computer Section, CCS Haryana Agricultural University, Hisar-125004, India.
[5]Federal Research Institute for Rural Areas, Forestry and Fisheries, Institute for Forest Genetics, Grosshansdorf, Germany.

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Genetic variability in *Jatropha curcas* was induced by different doses (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 kR) of gamma-rays. Gamma radiation induced earliness in flowering and the plants set flowers earlier than that of control, which took longer duration of 327 days for flowering. The improved reproductive and yield parameters such as days taken to first flowering, flowering population, male to female ratio and seed yield per plant were recorded in 25 kR dose and seed germination in 5 and 10 kR treated seeds. Molecular characterization of induced mutants (M_1 generation) with 47 Random amplified polymorphic DNA (RAPD) primers showed 65.27% polymorphism. The variability created by gamma rays ranged from 9 to 28%. The 50 kR mutant was found to be the most diverse from control followed by 25 kR mutant. Thus, this integrated approach can be used for carrying out the mutation-assisted breeding and subsequent selection of desired mutants using molecular markers in *J. curcas*.

Key words: *Jatropha curcas*, random amplified polymorphic DNA (RAPD), gamma-rays, induced mutagenesis.

INTRODUCTION

Bio-diesel is a fast-developing alternative fuel in many developed and developing countries of the world. Less availability of edible oil for human consumption in developing countries do not favour its use for bio-diesel production. Hence, non-edible oil from plants like jatropha (*Jatropha curcas*) and karanj (*Pongamia pinnata*) is favoured for bio-diesel production and the trend is expected to continue. *J. curcas* L. commonly known as physic nut or jatropha, a multipurpose, drought resistant, monoecious perennial plant belonging to family Euphorbiaceae has evoked much interest all over the world as potential biodiesel plant. It has gained special attention in tropical and sub-tropical countries and has spread beyond its centre of origin because of its hardiness, easy propagation, drought endurance, high oil content, rapid growth, adaptation to wide agro-climatic conditions and multiple uses of the plant as a whole. It is a non-food crop that produces fruits containing seeds with a high quality oil representing 35 to 40% of the seed by weight (Achten, 2008; Kumar and Sharma, 2008). It produces high quality oil whose chemical characteristics have allowed it to partially substitute for jet fuels in

*Corresponding author. E-mail: rsdhillon@hau.ernet.in, rsdhillon67@gmail.com.

recent test flights (Lilley, 2008). Although, this plant is proving an excellent feed stock for large scale plantation, the full potential of *J. curcas* has not yet been realized due to several reasons such as technological, economic and lack of quality plant material.

The limitations in available germplasm include lack of knowledge of the genetic base, poor yields and vulnerability to a wide array of insects and diseases. Assessment of genetic diversity using molecular markers disclosed low inter-accessional variability in *J. curcas* germplasm (Basha and Sujatha, 2007; Popluechai et al., 2009; Tanya et al., 2011). Also, inter-specific hybridization in *Jatropha* species for transferring useful traits such as yield, high oil content, maximum number of seeds, more femaleness, and hard stems has limited success due to pollen incompatibility (Kumar et al., 2009; Dhillon et al., 2009). The sound breeding program depends upon the availability of genetic variability for desired trait. Collection, characterization and evaluation of germplasm for oil and yield and agro-morphological trait are in nascent stage. The major activity of genetic improvement is selection and breeding. As *J. curcas* is often a cross-pollinated crop and exploitation of genetic variation may be carried out through mass selection, recurrent selection, mutation breeding, heterosis breeding and inter-specific hybridization (Divakara et al., 2010). Mutation breeding in tree crop is preferred due to demerits of conventional breeding such as time consuming, unpredictable results, long juvenile phase, high heterozygosity and fear of loss of unique genotype. Mutation breeding which is an efficient and much cheaper method than others can play an important role in crop improvement either directly or by supplementing the conventional breeding (Khawale et al., 2007).

When no gene or genes, for resistance to a particular disease or for tolerance to stress are found in the available gene pool, plant breeders have no obvious alternative but to attempt mutation induction. Treatment with mutagens alters genes or breaks chromosomes. Mutagenic agents, such as radiation and certain chemicals, can be used to induce mutations and generate genetic variation. Mutation breeding work in *J. curcas* carried out in Thailand using fast neutrons and isolated dwarf or early flowering mutants from the M_3 generation, but the potential productivity of these variants under intensive cultivation conditions was not proved (Sakaguchi and Samabhi, 1987). Dwimahyani and Ishak (2004) used induced mutations in *J. curcas* for improvement of agronomic characters with irradiation dose of 10 Gy and identified mutant plants with early maturity, 100 seeds weight (30% over control) and better branch growth. In India, mutation breeding using chemical and physical mutagens has been initiated to create genetic variation for various traits and developed mutants are being characterized using DNA markers (Punia, 2007). Mutation studies undertaken at National Botanical Research Institute (NBRI), Lucknow, India has led to induction of cotyledonary variabilities in *J. curcas* (Pandey and Datta, 1995). The mutants themselves may not be suitable for direct release, but they do provide the necessary alleles for developing superior cultivars with desirable traits.

In this context, induced mutagenesis is an important breeding tool to improve the desirable characters among the existing commercial varieties. Although, the desired variation is often lacking, radiation can be used to induce mutations and thereby generate genetic variation from which desired mutants may be selected. Different methods are available to investigate the effect of mutagens on plants (Chopra, 2005). However, molecular markers allow a direct comparison of the effects of genotypes at the DNA level. The explorations of Random Amplified Polymorphic DNA (RAPD) as genetic markers have improved the effectiveness of recombinant DNA techniques. RAPD analysis hence can be used for the detection of DNA alterations after the influence of mutagenic agents. Irradiation by gamma rays leads to the increasing level of DNA break formation. These different types of DNA damages must be detected by changes in RAPD profiles (Selvi et al., 2007). Therefore, in the present investigation, mutation induction in *J. curcas* was undertaken to identify the DNA polymorphisms induced by gamma rays.

MATERIALS AND METHODS

Plant source, irradiation and morphology study

In the present study, the seeds of MP-022 accession from Sidhi and MP-031 from Shahdol, Madhya Pradesh, India which showed >95% similarity as investigated in our previous study (Dhillon et al., 2012), were used as basic material. Seeds of MP-031 were irradiated with different doses of gamma rays namely, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 kR, whereas the untreated seeds of MP-022 accession were used as control. Prior to finalization of different treatments of gamma rays, medial lethal dose that causes 50% mortality of the seeds (LD50) was standardized. The gamma rays treated as well as untreated seeds were sown at 1 cm depth in root trainers (250 cc m^3) having vermiculite, perlite and coco-pit in the ratio of 3:2:1. These blocks of root trainers were kept in the poly house of Department of Forestry, CCS Haryana Agricultural University, Hisar. Two seeds were sown in each cell of root trainer and for each treatment 300 seeds were used. Customary care was undertaken after seed sowing. Observations on seed germination and survival of germinated seedlings were recorded regularly.

At the age of four months, 18 plants from each treatment were transplanted in experimental field following randomized block design with three replications and with six plants in each replication. Observations were made for growth, reproductive and yield parameters. The data were analyzed using the statistical model suggested by Panse and Sukhatme (1978).

DNA extraction

DNA extraction was carried out from young leaves of putative mutants (gamma-rays treated) and control plants (untreated) following the cetyl trimethyl ammonium bromide (CTAB) extraction method (Murry and Thompson, 1980) with some modifications. DNA

extraction buffer was comprised of 1.5% CTAB, I00 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 2% PVP and 2% β-mercaptoethanol. The homogenized mixture (CTAB buffer and ground tissue) was incubated at 60°C for 90 min.

After incubation, chloroform:isoamylalcohol (24:1) extraction was carried out twice and DNA was precipitated with chilled isopropanol. DNA was washed with wash 1 (76% ethanol with 0.2 M sodium acetate) for 20 min and then subsequently washed with wash 2 (76% ethanol with 10 mM ammonium acetate) for 2 min. Afterwards, the DNA was left for air drying. Dried DNA was dissolved in appropriate TE buffer. RNase A (50 µg/ml) treatment was given to remove RNA at 37°C for 2 to 3 h. After incubation, DNA was extracted with chloroform:isoamylalcohol and precipitated with ice-cold absolute alcohol. The quality and quantity of DNA were tested by submerged horizontal agarose gel (0.8%) electrophoresis (Sambrook et al., 1989) using λ DNA (25 and 50 ng) as standards.

RAPD analysis

A total of 47 RAPD primers with high GC content (synthesized from Life Technologies, India) were used for molecular characterization of gamma-rays induced mutants in *J. curcas* (Table 1). PCR amplifications were carried out in 10 µl reaction mixture containing 12.5 ng template DNA, 1× PCR buffer, 2.5 mM $MgCl_2$, 0.2 µM primer (Life Technologies), 200 µM dNTP mix (MBI Fermentas) and 1.0 U of Taq DNA polymerase (Life Technologies, India). PCR amplifications were performed using MJ Research, Inc. PTC-150 minicycler with initial denaturation at 94°C for 4 min, followed by 40 cycles of amplification with denaturation at 94°C for 1 min, annealing at 35°C for 1 min, extension at 72°C for 2 min and final extension at 72°C for 10 min. The amplified products were electrophoresed on 1.5% agarose gel containing ethidium bromide (0.5 µg/ml) using 0.5 × TBE buffer at 80 V for 4 h. A 500 bp DNA ladder was used as a molecular weight standard. The DNA fragments were visualized under UV light and photographed using the Vilber Lourmat gel documentation system.

Data analysis

The data were scored for presence (1) or absence (0) of bands and set in a binary matrix. Jaccard's coefficient of similarity was measured and a dendrogram based on similarity coefficients was generated by using unweighted pair group method with arithmetic mean (UPGMA). Principal coordinate analysis was done using the package NTSYS-PC (Rohlf, 1990).

RESULTS AND DISCUSSION

Seed germination, survival and yield attributing parameters

Table 2 shows that seed germination in the untreated seeds started after five days of sowing; however, germination was delayed in irradiated seeds which commenced after nine days of sowing. Significant variation was observed in seed germination and survival percent of both irradiated and untreated seeds. In case of percent seed germination, the data shows that 5 and 10 kR doses have a stimulatory effect on seed germination (84.9 and 73.1%, respectively); whereas, when the dose was increased to 20 kR and above, the seed germination percentage was reduced up to 16.6% (50 kR). The percent survival was highest (98%) in control plants followed by plants obtained from the seeds treated with 10 kR dose of gamma rays (92.3%). It was observed that the percent survival decreased radically with increase in the irradiation level of gamma rays. The higher exposures to irradiation are usually inhibitor on seed germination of both agricultural crops and tree species (Akhaury et al., 1993; Thapa et al., 1999); whereas, lower exposures stimulated percent seed germination (Taylor, 1968; Chauhan, 1978).

The reasons of these stimulations are acceleration in cell division rates (Zaka, 2004) as well as activation of auxins (Gunckel and Sparrow, 1991). Percent reduction/stimulation in seed germination might have been due to the effect of mutagens on meristematic tissues of the seed. The decrease in germination and survival at higher doses of the mutagens may be attributed to disturbances at cellular level (caused either at physiological or physical level) including chromosomal damages. Kumar and Mishra (2004) reported that in okra (*Abelmoschus esculentus*), germination percentage decreased with increase in gamma rays dose. Reduced germination and survival percentage with increasing doses of gamma radiation was also reported in *Pinus kesiya* and *Pinus wallichiana* (Thapa, 2004) and *J. curcas* (Songsri et al., 2011). Similar pattern was observed for plant morphological (plant height and basal diameter) characters. Stunted plant growth was recorded under 45 and 50 kR treatments. The higher doses of gamma irradiation lead to reduction in plant growth parameters in the present study, which may be partly due to the fact that the cells which have relatively more chromosomal damage at high irradiation exposures are at a disadvantage due to diplontic section, which cannot complete well with the normal cells and are thus prevented from making any further contribution. Days to first flowering ranged from 256 to 327 days (Table 2). A maximum decrease in days to first flowering (256 days) was recorded in 25 kR followed by 50 kR dose (273 days). Gamma radiation induced earliness in flowering and the plants set flowers earlier than that of control, which took longer duration of 327 days for flowering. Total number of flowers per inflorescence had a high degree of variability as 20 kR dose had 292 flowers (male to female flower ratio 23:1) which were more than two times as compared to the 45 kR dose of gamma irradiation which had only 136 flowers (male to female flower ratio 31:1). However, the maximum femaleness (19:1) was observed in the plants obtained from 25 kR treatment.

The days taken for fruiting to maturity ranged from 57 to 65. The least time taken was 57 days by 25 and 40 kR doses and the highest time of 65 days was taken by control plants. Seed yield per plant (g) varied from 53.9 to 213.7 g. The highest seed yield per plant was obtained from the plants obtained from the 25 kR treatment and the lowest seed yield per plant was recorded at 50 kR

Table 1. List of primers and DNA fingerprint profile of γ-rays induced mutants in *J. curcas.*

Primer	Sequence(5'-3')	% GC content	*TNB	**PP	Band size range	PIC value
UP-1	CAGGCCCTTC	70	13	46	250-1900	0.23
UP-2	TGCCGAGCTG	70	10	80	550-2500	0.49
UP-3	AGTCAGCCAC	60	14	71.4	475-1750	0.24
UP-5	AGGGGTCTTG	60	14	57.1	200-1975	0.14
UP-6	GGTCCCTGAC	70	3	33.3	650-950	0.09
UP-7	GAAACGGGTG	60	13	38.5	300-2500	0.19
UP-13	CAGCACCCAC	70	14	35.7	530-1750	0.14
UP-14	TCTGTGCTGG	60	13	53.8	520-2350	0.21
UP-15	TTCCGAACCC	60	5	60	600-2500	0.11
UP-16	AGCCAGCGAA	60	14	57.1	380-2200	0.26
UP-17	GACCGCTTGT	60	5	40	400-1600	0.09
UP-18	AGGTGACCGT	60	13	38.5	270-2100	0.14
UP-19	CAAACGTCGG	60	6	50	650-1800	0.23
UP-20	GTTGCGATCC	60	10	80	400-2000	0.26
UP-24	CCGAACACGG	70	16	56.2	230-2100	0.16
UP-26	TCGTTCCGCA	60	7	57.1	250-2000	0.29
UP-27	CCTCTCGACA	60	8	62.5	380-2400	0.22
UP-29	TGAGCCTCAC	60	13	69.2	250-1950	0.36
UP-30	AAGCCCGAGG	70	9	77.7	280-1200	0.64
UP-32	GTCCCGTGGT	70	6	50	550-2250	0.18
UP-35	TGTAGCAGGG	60	9	66.7	300-1400	0.36
UP-36	CTGCTTAGGG	60	8	75	550-2000	0.28
UP-39	GGACACCACT	60	9	66.7	100-820	0.32
UP-40	AAGCGGCCTC	70	7	28.6	475-2000	0.16
UP-41	CCCAAGGTCC	70	7	57.1	550-2000	0.05
UP-44	GTGACATGCC	60	11	63.6	250-2200	0.21
UP-45	TCAGGGAGGT	60	13	92.3	430-2200	0.43
UP-46	AAGACCCCTC	60	16	75	200-1500	0.38
UP-47	AGATGCAGCC	60	18	83.3	350-2300	0.33
UP-49	CTTCACCCGA	60	9	77.7	550-1700	0.39
UP-51	GAGTCTCAGG	60	11	63.6	250-1950	0.08
UP-54	TGCGGCTGAG	70	11	36.4	200-1900	0.15
UP-55	ACGCACAACC	60	11	72.7	450-1300	0.28
UP-56	GGTGACTGTG	60	8	75	500-2400	0.25
UP-58	GGACTGCAGA	60	14	71.4	300-1850	0.33
UP-60	AACGGTGACC	60	11	63.6	390-1900	0.30
UP-63	CTGTTGCTAC	50	9	88.9	300-1300	0.36
UP-65	CCCAGTCACT	60	8	37.5	600-1900	0.06
UP-67	CAGCACTGAC	60	11	72.7	150-1400	0.25
UP-82	GGGAACGTGT	60	11	63.6	200-2000	0.30
UP-85	GTCGCCGTCA	70	15	80	350-1800	0.27
UP-86	TCTGGTGAGG	60	10	90	475-1750	0.41
UP-87	GGTCTACACC	60	6	83.3	780-2000	0.38
UP-88	CACCGTATCC	60	11	72.7	300-1500	0.32
UP-96	GGGACGATGG	70	13	69.2	475-1475	0.22
OPN-12	CACAGACACC	60	11	54.5	700-2000	0.30
OPO-03	CTGTTGCTAC	50	17	100	300-2500	0.42

*TNB- Total number of bands, **PP- percentage polymorphism.

Table 2. Effect of gamma radiation on percent germination, growth and reproductive parameters of *J. curcas.*

Treatment (^{60}Co)	Germination (%)	Survival (%)	Days taken to first flowering	Flowering plants/ population (%)	Plant morphological characters at reproductive age		Number of flowers/ inflorescence	Male to female flower ratio	Days taken from flowering to fruit maturity	Seed yield/ plant (g)
					Plant height (cm)	Basal diameter (cm)				
Control	67.3 ± 4.49	98.0 ± 1.97	327 ± 16.04	1.7 ± 0.94	127.8 ± 9.78	4.53 ± 1.18	189 ± 23.86	36:1	65 ± 2.43	96.2 ± 6.45
5 kR	84.9 ± 5.03	84.9 ± 4.21	294 ± 29.43	8.3 ± 3.12	114 ± 14.36	5.34 ± 0.86	263 ± 31.42	39:1	60 ± 1.81	163.8 ± 13.87
10 kR	73.1 ± 5.79	92.3 ± 3.37	304 ± 11.68	13.8 ± 1.49	122 ± 5.50	4.84 ± 0.53	251 ± 17.97	33:1	59 ± 0.75	183.3 ± 5.03
15 kR	69.5 ± 2.98	80.8 ± 5.04	281 ± 18.37	32.4 ± 4.76	104 ± 19.42	4.39 ± 0.97	176 ± 24.20	32:1	63 ± 1.50	155.5 ± 9.27
20 kR	50.9 ± 4.23	76.1 ± 2.53	290 ± 8.91	65.4 ± 1.88	97 ± 19.03	4.44 ± 1.05	292 ±22.53	23:1	58 ± 1.08	176.1 ± 7.91
25 kR	44.3 ± 2.52	61.3 ± 6.12	256 ± 19.47	69.7 ± 6.03	94.5 ± 11.27	4.29 ± 1.49	194 ± 16.68	19:1	57 ± 1.67	213.7 ± 12.38
30 kR	36.2 ± 6.40	43.9 ± 4.45	299 ± 28.03	61.5 ± 2.97	100.08 ± .95	4.60 ± 0.70	263 ± 26.34	23:1	59 ± 1.32	189.6 ± 3.66
35 kR	27.4 ± 5.33	33.4 ± 5.10	285 ± 25.32	57.6 ± 2.53	94.3 ± 13.74	4.45 ± 0.37	249 ± 30.72	31:1	59 ± 2.69	147.8 ± 15.62
40 kR	20.5 ± 3.58	28.1 ± 2.09	302 ± 10.93	54.3 ± 5.35	91.4 ± 10.58	5.10 ± 0.66	188 ± 29.16	26:1	57 ± 4.36	101.6 ± 5.84
45 kR	20.1 ± 6.14	23.6 ± 5.71	294 ± 15.89	36.8 ± 2.09	63.8 ± 8.43	4.95 ± 0.28	136 ± 17.53	31:1	61 ± 2.28	98.3 ± 17. 41
50 kR	16.6 ± 5.51	20.1 ± 3.93	273 ± 26.76	8.0 ± 3.63	62.13 ± 5.51	5.13 ± 0.73	162 ± 23.49	22:1	58 ± 5.82	53.9 ± 10.28
CD (P≤0.05)	5.81	7.39	11.96	3.07	4.22	0.91	13.69	-	3.35	16.79

followed by control.

Similar findings were also reported earlier in *Vitis vinifera* (Charbaji and Nabulsi, 1999), *Pisum sativum* (Zaka et al., 2004), *Triticum durum* (Melki and Marouani, 2009) and *J. curcas* (Dhakshanamoorthy et al., 2011). The variation observed in reproductive characters such as days to first flowering, flowering population, number of flowers per inflorescence, male to female flower ratio and days taken for flowering to fruit maturity in the present investigation can be useful for plant cultivation with high seed yield as the primary objective.

The findings on plant morphological and reproductive parameters showed that gamma rays treated plants can change the flowering and its maturity in either a positive or negative direction which result in sufficient variability in the treated population that can be utilized for selection of early or late flowering plants for further improvement of this versatile crop.

Days to flowering and maturity in the case of irradiated populations were consistently shifted towards earliness. It is valuable in obtaining varieties associated with escape from pests, drought and other stress injuries that occur in late growing winter season.

DNA polymorphism

A total of 47 RAPD primers were used which resulted in the generation of a total of 501 reproducible RAPD bands. Out of these, 174 were monomorphic and 327 were polymorphic. The overall polymorphism percentage was 65.27%. The number of bands (DNA fragments) per primer ranged from 3 (UP-6) to 18 (UP-47), the average number of bands per primer being 10.66. The size of amplified products ranged from 100 to 2500 bp (Table 1). The selection of primers with high GC content (mostly 60 to 70%) was useful in terms of reproducibility of results and production of higher frequencies of RAPD because of increase in total frequency of amplified fragments (Fritsch et al., 1993). The PIC values ranged from 0.05 (UP-41) to 0.64 (UP-30) (Table 1).

The highest PIC (0.64) was exhibited by primer UP-30 followed by UP-2 (0.49), UP-45 (0.43), OPO-03 (0.42) and UP-86 (0.41) primers. These primers revealed more informativeness and can be used for further studies. The banding profile of mutants and control with different RAPD primers is depicted in Figure 1. The main changes observed in RAPD profiles were both in the presence or absence of different bands with variations of their intensities as well.

As evident from Figure 1, some of the RAPD bands appeared as new bands in specific γ-ray induced mutants while some of the RAPD bands which were present in the control disappeared in some specific mutants. These effects of γ-rays may be correlated with structural re-arrangements

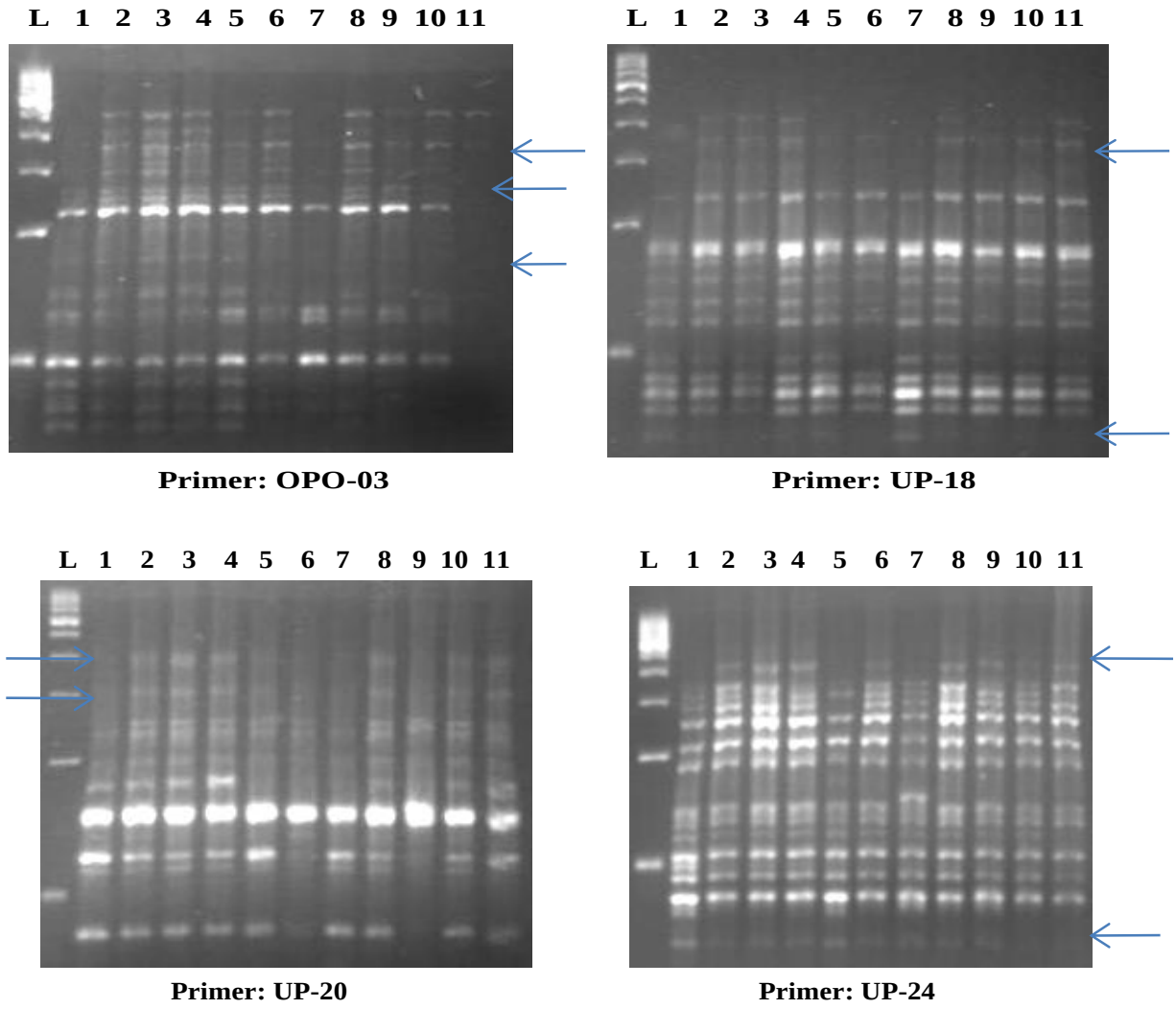

Figure 1. RAPD pattern obtained in γ-rays induced mutants in *J. curcas* with different primers. L, 500 bp DNA ladder; lanes 1 to 11, control, 5 kR, 10 kR, 15 kR, 20 kR, 25 kR, 30 kR, 35 kR, 40 kR, 45 kR and 50kR, respectively.

in DNA (breaks, transpositions, deletions, etc.) caused by different types of DNA damages (Selvi et al., 2007).

Bhagwat et al. (1997) screened radiation induced mutants of groundnut (*Arachis hypogaea*) for RAPD variability and observed 57% polymorphism. Similarly, RAPD method was successfully employed to study genetic variability in radio mutants from *Chrysanthemum* (Ruminska et al., 2004; Kumar et al., 2006) and amla (Selvi et al., 2007).

In these studies, genetic variation rate of mutants was markedly increased as evaluated by RAPD markers. Dhakshanamoorthy et al. (2011) used 23 RAPD primers to evaluate the gamma rays induced mutation in *J. curcas* and reported 55.16% polymorphism. Based on Jaccard's coefficient of similarity values, the maximum similarity (0.906) was found between 10 and 15 kR mutants (Table 3). Minimum similarity (0.634) was found between 40 and 50 kR mutants. Control plant had minimum similarity with 50 kR mutant (0.657) followed by 25 kR (0.687), thereby showing that as the dose of mutagen (gamma rays) increases, variability at the DNA level increases proportionately. However, the trend of genetic variability was not exactly proportional to the doses of gamma rays when similarity coefficient values were examined. The reason may be that genetic variation of radio mutants is proportional to the dosage of mutagen within a certain range (Teng et al., 2008). Average similarity was found to be 0.771 and range of diversity/variation was 9 to 28%. Two main clusters were delineated from the dendrogram (Figure 2). Cluster 1 comprised of control, 20, 25, 30 and 40 kR mutants.

Cluster 2 comprised of 5, 10, 15, 35, 45 and 50 kR mutants. Similar findings were reported by Dhakshanamoorthy et al. (2011) in gamma rays induced mutants in *J. curcas* using 23 RAPD primers. The proximity matrix based on RAPD analysis of EMS-induced mutants showed that

Table 3. Similarity matrix of γ-rays induced mutants in *J. curcas* using database generated by RAPD markers.

	Control	5 kR	10 kR	15 kR	20 kR	25 kR	30 kR	35 kR	40 kR	45 kR	50 kR
Control	1.00										
5 kR	0.728	1.00									
10 kR	0.698	0.862	1.00								
15 kR	0.700	0.844	0.906	1.00							
20 kR	0.816	0.740	0.738	0.764	1.00						
25 kR	0.686	0.798	0.724	0.758	0.742	1.00					
30 kR	0.852	0.716	0.674	0.684	0.796	0.734	1.00				
35 kR	0.710	0.822	0.852	0.858	0.754	0.760	0.730	1.00			
40 kR	0.710	0.662	0.664	0.690	0.714	0.768	0.746	0.684	1.00		
45 kR	0.744	0.780	0.786	0.796	0.772	0.774	0.760	0.822	0.738	1.00	
50 kR	0.656	0.788	0.818	0.804	0.688	0.682	0.672	0.782	0.634	0.808	1.00

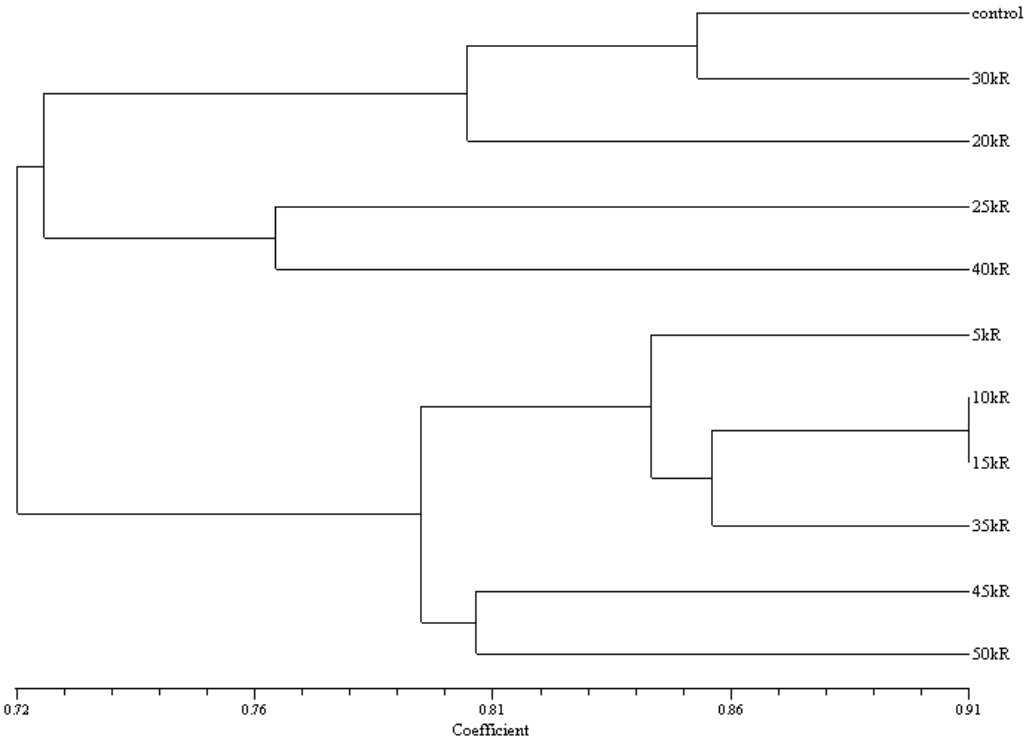

Figure 2. Dendrogram illustrating clustering of γ-rays induced mutants in *J. curcas* based on Jaccard's coefficient of similarity (327 polymorphic bands used).

three mutants were more distinct from the control and other mutants whereas the remaining were quite close to each other and with the control.

In the present study, major differences were observed in RAPD profiles of exposed samples with different doses of γ-rays. The γ-rays exposure changed the patterns of RAPD in comparison with control and hence can be adopted in mutation breeding of *J. curcas.* Further, molecular characterization of mutants might be helpful for the set-up of an efficient mutation induction protocol, for example, identifying proper doses of mutagen by assaying DNA damage. The mutants showing the differences in morphological traits showed DNA polymorphism in PCR profile amplified by RAPD marker. It is concluded that DNA polymorphism detected by RAPD analysis offered a useful molecular marker for the identification of mutants in gamma radiation treated plants.

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