Indian J. Plant Physiol., Vol. 15, No. 3, (N.S.) pp. 283-287 (July-Sept., 2010)

SHORT COMMUNICATION

USE OF RAPD MARKER FOR IDENTIFICATION OF DNA POLYMORPHISM IN GAMMA RAYS TREATED *JATROPHA CURCAS* L.

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Received on 19th Jan., 2010, Revised on 8th Sept., 2010

The aim of this study is to examine the discriminatory power of random amplified polymorphic DNA (RAPD) marker in *Jatropha curcas*, and to determine the effect of various dose exposures (0, 5, 10, 15, 20 and 25 Kr) of gamma rays on *J. curcas*, at molecular level. All the ten random primers used produced reproducible polymorphic bands. PCR products of mutant genome revealed a total of 40 bands, out of which 27 were polymorphic. Polymorphism information content (PIC) values were ranged from 0.00 to 0.40 and the highest PIC value of 0.40 was observed in primer OPU-13 followed by primers OPAL-11 and OPT-18 (0.30) while no PIC value were reported in primers OPH-18 and OPM-13. Jaccard's coefficient of similarity varied from 0.476 to 0.723, indicative of high level of genetic variation among the mutants studied. UPGMA cluster analysis indicated three distinct clusters, one comprising control while the second included four mutants *viz.,* 10, 15, 25 and 20 Kr. The mutant 5 Kr remained distinct and formed third cluster indicating its higher genetic diversity from the rest of the mutants and control. The primer OPU-13 produced maximum number of bands (8) showed highest discriminatory power and PIC (0.40) by showing maximum number of polymorphic bands (5) when compared to other primers used. The study reveals that RAPD molecular markers can be used to assess polymorphism among the mutants and can be a useful tool to supplement the distinctness, uniformity and stability analysis for plant varietal identification and protection.

Key words: **Gamma radiation, *Jatropha curcas,* PCR- RAPD marker**

Jatropha curcas, a member of Euphorbiaceae, native species of tropical America can grow well under such adverse climatic conditions because of its low moisture demands, fertility requirements and tolerance to high temperatures. The genus *Jatropha* is morphologically diverse encompassing more than 200 species, which are distributed chiefly in dry tropical regions of America, and have been later introduced into Africa and Asia and are now cultivated worldwide. Seeds of *Jatropha* contain 46-58% of oil on kernel weight and 30-40% on seed weight. The rate of spontaneous mutations in the nature is too low for plant breeding. Therefore, physical and chemical mutagens can be used for mutation induction in cultivated plants. It is possible to increase the genetic variability by inducing mutations in plants with ionized radiations *in vivo* and *in vitro* studies of mutation breeding. The mutant varieties are analyzed at the molecular level and differences in variability can be determined. Different methods are available to investigate the effect of mutagens on plants: Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeat (SSR) and Randomly Amplified Polymorphic DNA (RAPD).

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Among these, RAPD is an inexpensive and a rapid method not requiring any information regarding the genome of the plant and has been widely used to ascertain the genetic diversity in several plants (Deshwall *et al.* 2005). RAPD markers have been used to assess genetic diversity at molecular level in *Jatropha curcas* by several workers (Basha and Sujatha 2007, Ganesh Ram *et al.* 2008, Ranade *et al.* 2008). Although the most common use of the RAPD marker analysis is related to genetic mapping, taxonomic and phylogenetic studies, the method has also been used to detect DNA alterations (Ong *et al.* 1998). Currently crop improvement work (mutation) in this species is very limited. Hence, the present study on induced mutation in *J. curcas* was undertaken to identify the DNA polymorphism induced by gamma rays.

A physical mutagen (Gamma rays) employed in the present study was given to seeds of *J. curcas* with different doses *viz.,* 0, 5, 10, 15, 20 and 25 Kr from the Gamma chamber – 900 installed at Tamil Nadu Agricultural University, Coimbatore. The fresh leaf material was harvested from the plants treated with gamma rays. Genomic DNA was extracted by adopting the CTAB (Cetyl trimethyl ammonium bromide) method outlined by Doyle and Doyle (1990) with minor modifications. PCR amplification was performed twice for each primer to ensure their reproducibility in a total volume of 25 µl containing 20 mM Tris/HCl (pH: 8.4), 50 mM KCl, 200 µM of each dNTP's, 2 mM $MgCl_2$, 0.8 µM primer, 100 ng of template DNA and 0.5U Taq DNA polymerase in Eppendorf Master Cycler personal which was programmed to include pre-denaturation at 94°C for 1 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min and extension at 72° C for 1 min. Data of RAPD marker analysis were scored as discrete variables, using '1' to indicate presence and '0' to indicate absence of bands for each primer. The polymorphism information content (PIC) was calculated by the formula: PIC= 2 pi (1-pi) (Bhat 2002) where, pi is the frequency of occurrence of polymorphic bands in different primers. Jaccard's coefficient similarity among mutants was calculated according to Jaccard (1908). A dendrogram based on these similarity coefficients was constructed by using Unweighted Pair Group Method of Arithmetic means (UPGMA) in the software, NTSYSpc-2.0.

Mutants of *J. curcas* were analyzed using 10 random primers (Table 1) and all of them produced

Table 1. List of primers, number of amplified products, polymorphic bands, polymorphism percentage and polymorphism information content (PIC).

S. No.	Primers	Sequence (5' - 3')	Total amplified	Total polymorphic bands	Percent polymorphism	PIC values
1.	OPH18	GAATCGGCCA	2	1	50	0.00
2.	OPH12	GGGACGTTGG	5	3	60	0.33
3.	OPM13	GGTGGTCAAG	2	1	50	0.00
4.	OPM14	AGGGTCGTTC	3	2	66	0.25
5.	OPAL11	GTCACGTCCT	5	4	80	0.37
6.	OPT18	CATGCCAGAC	6	4	66	0.37
7.	OPA4	AATCGGGCTG	4	3	75	0.33
8.	OPAK14	CTGTCATGCC	2	2	100	0.25
9.	OPM15	GACCTACCAC	3	2	66	0.25
10.	OPU13	GGCTGGTTCC	8	5	62	0.40
		Total:	40	27	6752	2.55
		Mean:	3.7	2.7	67	0.25

polymorphic banding patterns. A total of 40 bands were scored, of which 27 (67.5%) were polymorphic (Table 1). The number of bands generated per primer varied from 2 to 8 and a minimum of 2 bands were generated by the primers OPH18, OPM13 and OPAK14, while the maximum of 8 and 6 bands were scored with OPU13 (Fig. 1) and OPT18 (Fig. 2) respectively. The primer OPU 13 revealed the highest polymorphism information content (PIC) value of 0.40 followed by the primers OPAL 11 and OPT 18 (0.37). As the PIC provides a measure that is influenced by both the number and frequency of alleles, the maximum PIC for a RAPD marker is 0.5 since two alleles per locus are assumed in RAPD analysis (Henry 1997). Jaccard's coefficient similarity varied from 0.476 to 0.944. The highest Jaccard's coefficient similarity (0.723) was observed in 25 Kr mutant while the lowest similarity (0.476) observed in 5Kr mutant when compared to that of control. The Jaccard's coefficient similarity matrix showed that 5 Kr mutant was more distinct to control than other mutants, whereas 25 Kr, 10 Kr, 15 Kr and 20 Kr mutants were quite close to each other with control (Table 2). The dendrogram showed three distinct clusters, one comprising control while second cluster included mutants *viz.,* 10, 15, 25 and 20 Kr, third included 5 Kr mutant only (Fig. 3). In the present study, the solid mutant (5 Kr) was identified based on Jaccard's coefficients similarity and dissimilarity (dendrogram) obtained by the RAPD profile. The present investigation coincides with the previous study conducted in grapevine by Khawale *et al.* (2007), who obtained solid mutants by induced mutation, based on RAPD analysis. DNA polymorphism due to different doses effect of gamma rays could be distinguished based on the RAPD profiles. The main changes in the RAPD profiles of the present investigation were the appearance or disappearance of different bands with variation in their intensity. This might be due to the structural rearrangements in DNA caused by different types of DNA damages (breaks, transpositions, deletions, etc). Radiation is one of the best known physical mutants as it dissociates the atoms of water molecules and causes the generation of hydroxyl radicals that are the most reactive. They react with most of the biomolecules including DNA and scavenge electrons from them causing genetic alterations on the DNA molecules. Several workers have found that RAPD markers, which can quickly detect a large number of genetic polymorphisms, lead to the creation of genetic maps in a number of woody fruit crops and detection of mutation in sunflower (Erdem and Oldacay 2004), grapes (Khawale *et al*. 2007) and amla (Senthamizh Selvi *et al.* 2007), including changes due to DNA damage. The results obtained suggest that gamma radiation as a

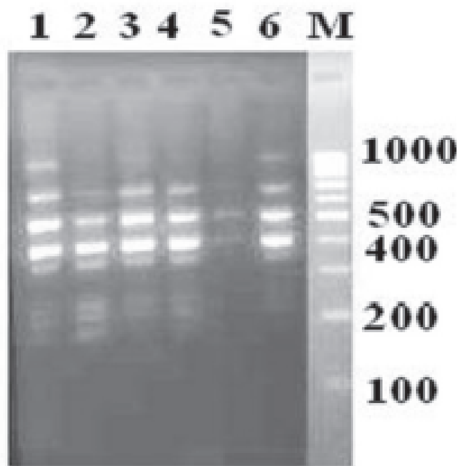

Fig. 1. Gel electrophoresis (2%) showing PCR profiles of amplified DNA from control and mutants using primer OPU-13 (lane-1: control, lane-2: 5 Kr, lane-3: 10 Kr, lane-4: 15 Kr, lane-5: 20 Kr, and lane-6: 25 Kr) M: 100 bp Marker.

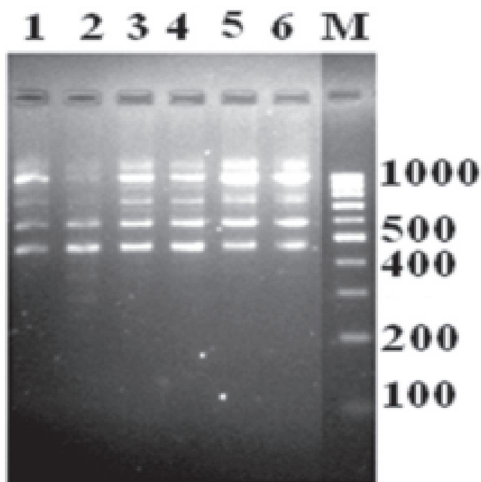

Fig. 2. Gel electrophoresis (2%) showing PCR profiles of amplified DNA from control and mutants using primer OPT-18 (lane-1: control, lane-2: 5 Kr, lane-3: 10 Kr, lane-4: 15 Kr, lane-5: 20 Kr, and lane-6: 25 Kr) M: 100 bp Marker.

Table 2. Jaccard's similarity matrix of gamma rays induced mutants in *Jatropha curcas.*

	Control	5 Kr	10 Kr	15 Kr	20 Kr	25 Kr
Control	1.000					
5 Kr	0.476	1.000				
10 Kr	0.714	0.632	1.000			
15 Kr	0.667	0.662	0.944	1.000		
20 K r	0.571	0.647	0.833	0.882	1.000	
25 Kr	0.800	0.550	0.895	0.842	0.737	1.000

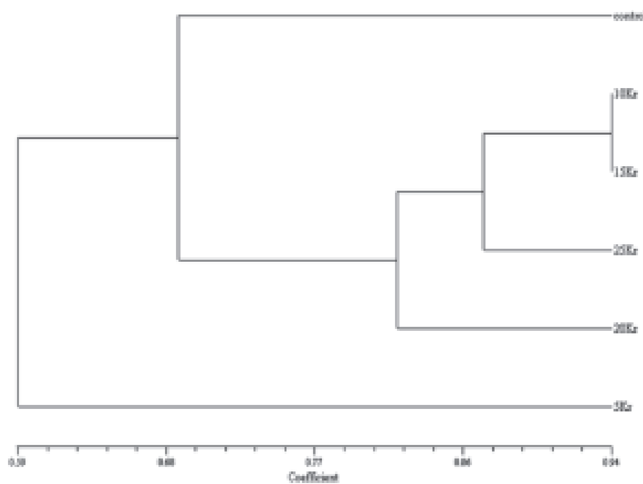

Fig. 3. Dendrogram constructed from similarity coefficients, showing the clustering of five mutants (control, 5 Kr, 10 Kr, 15Kr, 20 Kr and 25 Kr).

physical mutagen could effectively be used for mutation selection in plant breeding, and newly evolved 5 Kr mutant and other mutants can easily be differentiated from their parents through RAPD marker analysis. Hence, there is a need to adopt and incorporate induced mutations and marker assisted selection for early selection and recognition of the desired types in tree species, which in turn, result in quick production of stable mutant genotypes in mutation breeding.

ACKNOWLEDGEMENTS

Authors are thankful to the University Grant Commission (UGC), New Delhi, India for providing fund for the present study. They also thank the authorities of Annamalai University and Dr. R. Panneerselvam, Professor and Head, Department of Botany, Annamalai University, for having provided laboratory facilities and encouragements.

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