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Analysis of Genetic Diversity on Mutants *Jatropha curcas* Using RAPD

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Abstract

Physical and chemical mutagen studies on experimental of mutagenesis in *Jatropha curcas* are limited.The study wasaimed to estimate the genetic diversity on *J. curcas* mutants using RAPD. Eighteenmutants resulted from colchicine treatment and one wild type of *J. curcas*were used in this study. Dendrogram derived from UPGMA clustering analysis using simple matching coefficient of RAPD markerindicate thatthe eighteenmutants and one wild type were divided into three major groups consist of three, eight and seven genotypes respectively. The study reveals genetic diversity within mutants of *J. Curcas* based on RAPD fingerprinting techniques.

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Keywords: *J. curcas;* genetic diversity; mutant; colchicine; RAPD

1. Introduction

Jatropha curcas is native to Mexico, Central America and South America where it grows in the forests of coastal regions. It has been widely disseminated and become naturalized in many parts of the tropical and subtropical world [1]. *J. curcas* has shown wide adaptability to a range of soil and climatic conditions.Availability of genetic variability of *J. curcas*can be exploited for selection of superior genotypes [2].

The effectiveness of tree breeding and improvement programs depends upon the nature and magnitude of existing genetic variability. A systematic germplasm collection, characterization and evaluation program is essential to identify superior planting material from the existing natural variations[3].

Physical and chemical mutagen studies on experimental of mutagenesis in *J. curcas* are very limited, mutation breeding studies in this plant carried out by Sakaguchi and Somabhi [4] in Thailand using fast neutrons and isolated dwarf or early flowering mutants from the M3 generation, but the potential productivity of these variants under intensive cultivation conditions was not proved. Pandey and Datta [5] in India have led to induction of cotyledonary variables in *J. curcas*. Irradiation dose of 10 Gy was used to induce mutations in *J. curcas* for improvement of agronomic characters and identified mutant plants with early maturity, 100 seeds weight (30% over control) and better branch growth [6]. Treatments of gamma rays were found to be greater compared to those of EMS treatments. Based on the variation in flowering and yield traits of gamma rays and EMS treated

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plants, superior strain will be screened by PCR-RAPD marker [7]. The study on molecular characterization of induced mutagenesis through gamma irradiation using RAPD marker analysis in *J. curcas* reported that RAPD analysis is a highly suitable method for the detection of DNA polymorphism induced by gamma radiation. Dhakshanamoorthy *et al.* [8] discovered that the RAPD marker generated genetic variation among control and gamma rays treated populations will help to distinguish the plants showing differences in morphological characters. Various studies on the genetic diversity of *J. curcas* have been undertaken involving a variety of populations and molecular marker technologies [9].
The present study was done to estimate the genetic diversity on mutants *J. curcas* using RAPD.

2. Material and method

2.1. Plant material

Eighteen mutants as the result of colchicine treatment and one wild type of *J. curcas*were used in the present study for analysis of genetic diversity. Details of the plant material are given in Table 1.

Table 1.Character of eighteen mutans and wild type of *J. curcas*.

Code of Mutants	Number of leaves	Shoot color	Stalk color	Number of primery branches	Plant height (cm)	Stem diameter (cm)
0.1%+6x	72	red	l-green	7	79.0	7.92
0.2%+6x	124	red	l-green	16	92.2	8.00
0.3%+6x	95	g-red	l-green	18	81.0	7.00
0.4%+6x	223	r-green	l-green	19	78.4	6.25
0.5%+6x	125	g-red	d-green	9	88.0	9.00
0.6%+6x	134	r-green	l-green	8	91.2	7.48
0.1%+8x	194	green	l-green	13	82.3	7.49
0.2%+8x	130	g-red	l-green	13	92.0	6.74
0.3%+8x	120	r-green	l-green	8	89.0	7.40
0.4%+8x	162	red	l-green	9	92.0	7.48
0.5%+8x	114	g-red	d-green	12	81.7	6.63
0.6%+8x	65	green	l-green	6	76.3	8.00
0.1%+10x	103	r-green	d-green	17	98.0	5.00
0.2%+10x	151	r-green	l-green	19	73.0	5.40
0.3%+10x	108	g-red	l-green	9	83.2	6.94
0.4%+10x	213	green	l-green	11	79.0	7.12
0.5%+10x	76	r-green	l-green	8	67.4	6.67
0.6%+10x	119	green	d-green	8	88.0	7.33
IP1P (wt)	55	r-green	l-green	11	78.0	7.00

Note: g- = greenness, r- = redness, l- = light, d- = dark, wt = wild type as a control;
0.1-0.6% = colchicine concentration, 6x-10x = frequency of solution that was dropped on shoot of *J. curcas* seedling

2.2. DNA isolation

The fresh young leaf material was harvested from the twelve-month-old plants of mutants and wildtype. Genomic DNA was extracted by adopting the method outlined by Sambrook *et al.* 1989, with minor modifications. About 0.5 g of leaf tissue was grinded it in liquid nitrogen and put in a 2ml Eppendorf tube. A 800 µl of extraction buffer (SDS=sodium dudocyl sulphate) was added and incubated at freezer for 5 min. The mixture was centrifuged at 12000 rpm/4^0C/5 min, the supernatant was thrown, and 500 µl of lysis buffer was added at precipitate, vortex and incubated at 65 ^{o}C for 30 min. The mixture was added with 500 µl chloroform : isoamylalcohol (24:1) and centrifuged at 12000 rpm/4^0C/5 min. The supernatant was collected in a new tube, addedwith 500 µlchloroform : isoamylalcohol (24:1) and centrifuged at 12000 rpm/4^0C/5 min. The supernatant was collected in a new tube, addedwith 800 µl ethanol absolute and 80 µl NaOAc 3M, shake gently and centrifuged at 12000 rpm/4^0C/5 min. Supernatant was thrown and precipitatewas washed with 500 µl ethanol 70%. The pellet was air-dried and dissolved in 30 µl TE buffer.

2.3. PCR amplification

PCR amplification was performed in a total volume of 25 µl containing 0.5 U Taq DNA polimerase and 10x buffer Taq Polimerase (100 mM Tris-Cl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01 % gelatin), dNTP'S mix (Pharmacia), dH_20 and 30 ηg of template DNA. Amplification was performed in a programmed thermal cycler with pre-denaturation at 94 ^{o}C for 2 min., followed by 40 cycles of denaturation at 94 ^{o}C for 1 min., annealing at 50^{o}C for 1 min. and extension at 72^{o}C for 2 min. The PCR products were analyzed by electrophoresis through 1.5% agarose gels in 1XTBE buffer, performed at 50 V for 1 h. After electrophoresis, the gel was stained in ethidium bromide and then visualized and the images were photographed using Gel Documentation System.

2.4. Datascoring and statistical analysis

Data of RAPD marker analysis were scored as discrete variables, using '1' to indicate the presence and '0' to indicate absence of bands for each primer. The faint and unclear bands were not considered for data scoring. The binary data so generated were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. A dendrogram based on Jaccard similarity coefficients was constructed by using Unweighted Pair Group Method of Arithmetic means (UPGMA) with the SHAN module of NTSYS-PC 2.02 to show phenetic representation of genetic relationships as revealed by the similarity coefficient.

3. Result and discussion

PCR amplification products generated by the representative RAPD primer are presented in Fig. 1-3.The experiment resulted that the eighteen mutants and one wild typeof*J. curcas* exhibited variations in their DNA fingerprint profiles as revealed by RAPD markers. A comparison of the eighteen mutants and one wild type of *J. curcas* analyzed showed a total of169 amplified products. Primers OPA-20, OPH-20 and OPH-15 produce score of bands. The amplified products ranged between 200 and 2000 bp in size. These primers yielded a total of 56, 61, 52 bands with an average of 2.95, 3.21, 2.74 bands per primer and 6, 8, 7 polymorphsm bands respectively in theeighteen mutants and one wild type of *J. Curcas* (Table 2).

In this research, polymerizing comparison indicated differences among the mutants improved by colchicine application to *J. curcas* which examined using RAPD marker. The results demonstrated that RAPD markers are powerful tools for fingerprinting and analyzing variability in *J. curcas*. From a tree improvement perspective, the considerable degree of genetic variance of *J. curcas* found among mutants and wild type as confirmed by RAPD, is encouraging.

RAPD being dominant, detect multiple loci distributed throughout the genome and hence strongly preferred for genetic variation studies in tree species. RAPD is one of the simplest and the most common methods used in the determination of genetic similarity and diversity [3].

The polymorphic bands in this research was showed by arrows in Fig. 1-3.Polymorphism of amplified fragments is caused by: (i) base substitutions or deletion in the priming sites, (ii) insertions that render priming sites too distant to support amplification or (iii) insertions or deletions that change the size of the amplified fragment [10].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 alternation and mutation. The polymorphism in genomic DNA was detected by RAPD profiles through the randomly primed PCR reactions. In this sense, the obvious disappearance of normal bands and appearance of new bands generated from the plants exposed to different treatment of colchicine in comparison to the untreated control. In our study, the number of lost bands was found higher than that of extra bands. RAPD markers, which can quickly detect a large number of genetic polymorphism, have led to creation of genetic maps in a number of woody fruit crops [11] and sunflower [12] including changes due to DNA damage and RAPD markers have been used to detect mutations and DNA damage [13].

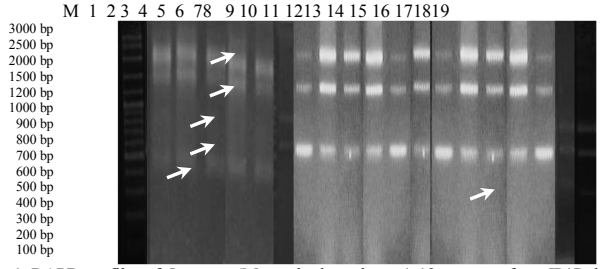

Fig. 1. RAPD profiles of *J. curcas* (M: markerlane; lanes 1-18: mutants from IP1P; lane 19: wild type) generated using OPA-20 primer (arrows showing the polymorphic bands).

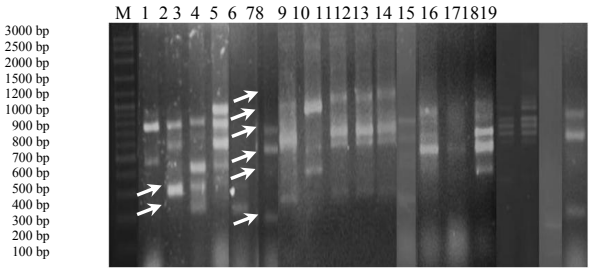

Fig. 2. RAPD profiles of *J. curcas* (M: markerlane; lanes 1-18: mutants from IP1P; lane 19: wild type) generated using OPH-20 primer (arrows showing the polymorphic bands).

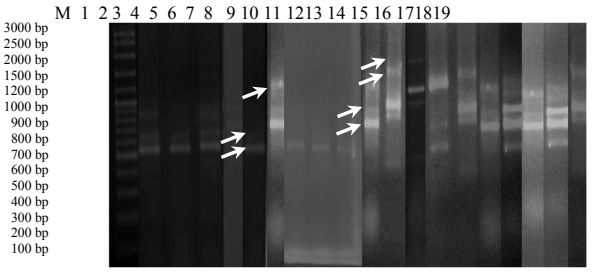

Fig. 3. RAPD profiles of *J. curcas* (M: markerlane; lanes 1-18: mutants from IP1P; lane 19: wild type) generated using OPH-15 primer (arrows showing the polymorphic bands).

Molecular characterization using RAPD marker analysis of *J. curcas* mutants inducedby gamma irradiation was first reported by Dhakshanamoorthy *et al.* [8]. Their data support in term of RAPD analysis is a highly suitable method for the detection of DNA polymorphism induced by gamma radiation. The RAPD marker generated genetic variation among control and gamma rays treated populations will help to distinguish the plants showing differences in morphological characters.

Current advancement in molecular techniques has proved that the absence of any morphological differences does not preclude the possibility of any genetic changes during the mutation. Therefore, it is required to assess the genetic stability by the highly efficient molecular markers [14]. These markers offer numerous advantages over conventional phenotype based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation and development, as they are not confounded by environment, pleiotropic and epistatic effects [15] The use of molecular markers such as RAPD, AFLP, MSAP, RFLP and ISSRs are being rapidly integrated as routine laboratory tools available for quick assessment of the genetic stability of plants [16,17, 18, and 19] The efficiency of these marker systems has been reported by Sudheer *et al.* [20 and 21] for the assessment of Jatropha diversity.

Table 2.Polymorphism of 3 operon random primers used for fingerprinting 18 mutans and 1 wild type of*J. curcas*.

Primer	Size of bands scored (bp)	Number of bands scored	Average bands per individu	Number of polymorphic bands
OPA 20	2000–300	56	2.95	6
OPH 20	1200–200	61	3.21	8
OPH 15	2000–500	52	2.74	7
Total		169	2.97	

The genetic distance between eighteen mutants and one wild type of *J. curcas* is illustrated by the UPGMA dendrogram (Fig. 4).The eighteen mutants and one wild type were divided into three major groups or clusters consisting of three, eight, and seven genotypes respectively by RAPD marker. First cluster comprising control, mutant 0.6%+10x and 0.4%+6x (64% similarity). Second cluster included0.5%+10x, 0.3%+10x, 0.4%+10x, 0.1%+10x, 0.2%+8x and 0.1%+8x (66.3% similarity). Third cluster included0.5%+8x, 0.4%+8x, 0.3%+8x, 0.6%+6x0.5%+6x, 0.2%+6x, 0.3%+6x and 0.1%+6x (64.4% similarity). The dendrogram (Fig. 4) also represent that there are high diversity of 16 mutants and 1 wild typeof *J.curcas* (52% similarity). The highest genetic distance from wild type were mutants 0.5%+8x and 0.4%+8x. While the closest correlation between the mutant and control wild type was mutant 0.6%+10x (84%similarity).

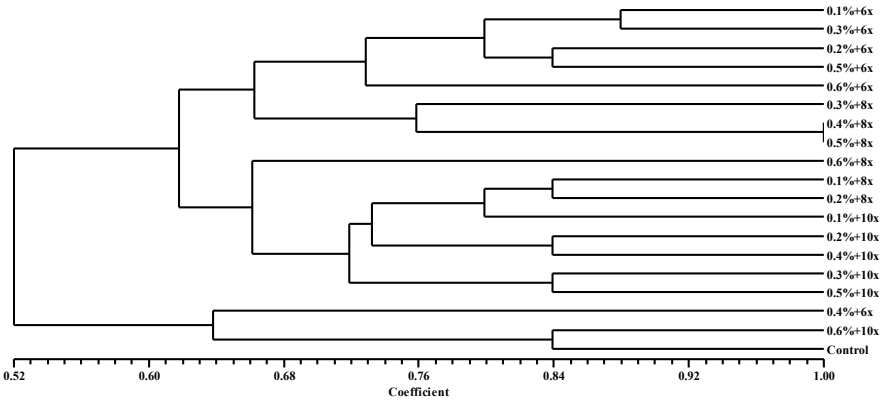

Fig. 4. Dendrogram derived from UPGMA clustering analysis using simple matching coefficientof RAPD marker.

The elucidation of the variability among the candidate tree and the identification of genotype specific markers are important resources for devising strategies for breeding and efficient management in tree germplasm. Introgression of desired traits like oil biosynthesis genes needs to be further conducted to enhance the germplasm for elite genotype development in candidate plus trees of *Pongamia pinnata* [3].The observed variations were not confined to any individual, but were generalized mutations. Other study reported that RAPD and AFLP analyses for assessment of the genetic stability of micropropagated shoots from the three generations and three genotypes is the first illustration of the utility of these marker systems in the discrimination of somatic mutants from parental linesin *J. curcas* [14].

In this sense, the phenetic approach differs from the genetic approach in that RAPD profiles are not considered as genotypes. In the present research, when markers obtained the end of RAPD analyses done at the plants showing improved agronomic traits and control were examined, it was seen that there was a potential polymorphism between mutants and controls. In the dendrograms done according to RAPD markers, the genetically distance to control and mutants replying to colchicine in various concentration and frequency of solution droped doses in particular, 0.5%+8x and 0.4%+8x were rather different with control wild type (Fig. 4).

Dhakshanamoorthy *et al.* [8] reported that in a dendrogram constructed based on genetic similarity coefficients, the mutants were grouped into three main clusters; (a) control, 10,15 and 20 Kr dose mutants clustered together, (b) 25 Kr dose grouped alone, (c) 5 Kr dose also grouped alone. 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. According to Kalpana *et al.* [22] that dendrogram generated by UPGMA clustering method clustered the genotypes of mulberry into three and four groups by RAPD and ISSR fingerprinting respectively. Bansal *et al.* [23] also reported that cluster analysis of the genotypes of *Lapedium sativum* based on UPGMA divided the 18 genotypes into two main clusters, with first cluster having only HCS-20 genotype of *L.sativum* and other having rest of all 17 genotypes. The dendrogram based on similarity matrix revealed 23–66% genetic relatedness among 18 genotypes. Several researches indicated that genetic diversity within genotipes included mutants could be revealedby cluster analysis of the genotypes based on UPGMA used data of RAPD fingerprinting techniques.

4. Conclusion

The study reveals genetic diversity within mutants of *J. Curcas* based on RAPD fingerprinting techniques. The highest genetic distance from wild type were mutants 0.5%+8x and 0.4%+8x. While the closest correlation between the mutant and control wild type was mutant 0.6%+10x.The mutants of *J. curcas* resulted in this study could be used in conventional breeding to improve better genotype.

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