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PHENOTYPIC AND GENOTYPIC DIVERSITY ASSESSMENT OF *JATROPHA CURCAS* BY USING RAPD MARKERS TO IMPROVE BIODIESEL PRODUCTION

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ABSTRACT

Jatropha curcas L. is a multipurpose shrub has significant economic importance for its seed oil, which can be converted into Biodiesel. In the present study, the genetic relationships of three *Jatropha curcas* seeds collected from Labland Biotech Private Limited, Mysore (LB), Gandhi Krishi Vigyan Kendra, Bangalore (GKVK) as well as from rural area of Jharkhand (JH), were assessed by Random amplified polymorphic DNA(RAPD) analysis. Amplification of genomic DNA using ten RAPD primers yielded 81 bands, of which 73 bands were polymorphic. The amplification products ranged from 7-11 bands for different primers. Among the chosen ten primers four of them, OPC-2, OPC-9, OPH-1 and OPQ-15, were found to be polymorphic. RAPD analysis confirmed the genetic variability between the accessions in relation to the variation in morphology and seed oil content, LB variety could be considered superior as compare to other two accessions.

Keywords: *Jatropha curcas*, Genetic diversity, Biodiesel, RAPD, Fruit yield

1. INTRODUCTION

Jatropha curcas L. is a potential plant for making biodiesel to cater the growing energy needs [1, 2]. The Jatropha biodiesel has been proven to be superior compared to biodiesel made from other vegetable oils in terms of engine performance and emission [2, 3]. Understanding the genetic diversity is crucial for improving the desired quality any plant species. Morphological markers are routinely used for estimating genetic diversity but are not successful due to strong influence of environmental factors.

Hence the use of molecular markers has complemented the classical strategies and enabled the characterization of genotypes in plant kingdom [4]. These markers are independent of the influences of environmental growth conditions, physiological age of the plant and type of tissue being analysed [5]. In Jatropha, isozyme markers were used to determine the genetic relatedness of the genus of *Jatropha* and *Ricinus* [6].

Random amplified polymorphic DNA (RAPD) is one of the common genetic markers that can be used for population genetic analysis, pedigree analysis and taxonomic discrimination [7, 8, 9]. As compared to other molecular techniques, RAPD analysis has proved to be useful in genetic diversity studies and has principal advantage of being simple, obviates to work with radioisotopes and faster in obtaining results [10].Several studies have demonstrated that the RAPD-PCR method is a powerful tool in the assessment of discriminating differences at intra and inter-population level in a wide range of organisms including plants [11-14]. This technique has earlier been used in assessing genetic variations in wide array of agricultural and forest tree crops.

The main objective of the present study was to assess genetic diversity in three accessions of Jatropha plant by using RAPD markers and to corelate with the seed oil content.

2. MATERIALS AND METHODOLOGY

2.1 Chemicals and explant

RAPD primers, Taq polymerase enzymes, dNTPs mix, Buffer, Agarose, gel loading dye, cell lysis buffer, TE buffer, 3M Sodium acetate, distilled ethanol and Ethidium bromide (EtBr) were purchased from Genei Bangalore. The seeds of three different accessions of *Jatropha* were purchased from Labland Biotech Mysore, GKVK Bangalore and Jharkhand.

2.2 Seed characterization

Seeds of the 3 accessions were collected and dried properly. The physical characteristics like seed length, width and weight were measured by scale method.

2.3 Oil Extraction

Seeds were dried in sunlight and about 500gm was weighed and powdered. The powder was placed in a three neck-flask and hexane was added slowly in the ratio of 1:2 (w/v) and then heated at 40ºC up to 90mins. Contents were subjected to filtration using Whatman no.1 filter paper. The sediment was subjected to same procedure for 3 times. The filtrate collected from each cycle was pooled and hexane was recovered through distillation process. The amount of oil extracted was calculated as percentage of oil present in seed [15] and the extracted oil was used for further experiments.

2.4 Physicochemical characterization of oil

Physicochemical parameters of the oil such as acid value, Iodine value [16], Saponification value [17], Viscosity and density [18] were determined.

2.5 Fatty acid composition analysis

Fatty acid composition of the oil was determined by using gas chromatography (Agilent 6890 series) equipped with flame ionization detector and capillary column (30m×0.25mm×0.25mm). The fatty acids were determined using their methyl esters and were injected into gas-chromatography for analysis [15]. The identification of peaks was achieved by retention times by means of comparing them with authentic standards analysed under the same conditions.

2.6 Genomic DNA Isolation

Total genomic DNA was isolated from young leaves of all three varieties of Jatropha using GeNei Plant DNA Extraction kit (for PCR amplification) KT22Cat #2102200051730 with slight modifications. About 350 gram of leaf tissue was washed with ethanol and distilled water and then homogenized using sterilized motor and pestle, kept in dry ice. Then 1ml of precooled solution A, 350µl of PVP and 350µl of SDS were added and incubated at 65ºc for 1 hour in dry bath through intermittent thawing. Then chloroform and isoamyl alcohol (24:1) were added in equal volume of sample then centrifuged at 12000 rpm for 20 min. To the supernatant twice the volume of cold ethanol and 1/10th of 3M sodium acetate were added and kept for overnight incubation at -20ºC. After incubation, centrifugation was carried out at 12000 rpm for 30 min, the pellet was washed with 70% ethanol twice then air dried. 50µl of solution B (TE buffer) was added to the pellet and kept at 65ºC for 10 min for complete DNA solubilization and was stored at 4ºC.

2.7 Primers

A total of 10 RAPD primers were used for genetic diversity analysis. The length of each primer was 20 bp and details are given in Table 4.

2.8 RAPD- PCR reaction

Amplification reaction was performed according to the method described by Saker method [19] with slight modification. Reaction mixture contained 40 ng of template DNA, 2µl of dNTPs mix (2.5Mm each), 1 x Taq PCR buffer, 1.5 U of Taq DNA polymerase, and 1 µM each of RAPD primers. The amplification was carried out in programmable thermal cycler with PCR profiles:Pre denaturation at 94ºC for 5 min,45 cycles at 94ºC for 45 sec, 36ºC for 1 min and 72ºC for 2min,with final extension at 72ºC for 10 min, finally amplified product was hold at 4ºC.

2.9Agarose gel electrophoresis:

The 15 µl of amplified PCR products were resolved in 1.8% Agarose gel with ethidium bromide at 100 volts for 1 hour and visualized under UV transilluminator (GeNei^{TM}, Bangalore).

3. RESULTS AND DISCUSSION

3.1 Seed characteristics

Table 1 show the different seed characteristics such as seed length, width and weight of three different accession of Jatropha plant. In the present study the percentage of oil was found 33% in G1 seeds followed by G2 (31%) and G3 (30%). Among three accessions, Labland seed shows the better seed characteristics with compare to other two accessions.

Similar study carried out by [20] shows that Maximum percentage of oil from kernels was recorded in Jatropha KM accession followed by RSAD, FWB and MB accessions and the highest weight of seedcake was obtained from KM accession. Similarly Ghosh *et al* [21] reported that the seed oil content varied considerably from 27.68% (JCN01) to 37.49% (JCN14) and had high heritability, but it had low PCV and GCV and moderate GA among those different accessions. Kumar *et al* [22] assessed the variability in seed traits and oil content of 24 accessions of *J. curcas* collected from different agroclimatic zones of Haryana state, India and reported significant differences in seed size, weight and oil content between accessions.

3.2 Physicochemical properties of the oil

The various physicochemical characterizations includes acid value, iodine value, saponification value, viscosity and density of Jatropha seed oil were shown in Table 2. There is no significant difference between the three accessions of Jatropha seeds. Acid value and iodine value of oil will give an estimation of free fatty acids and the degree of unsaturation respectively. Jatropha oil has a high degree of unsaturation due to the amount of linoleic acid present in the oil [23, 24]. The acid value has significant effects on the transesterification reaction of oil with alcohol using catalyst. High acid value causes soap formation during alcoholysis process and lead to difficulties in separation of biodiesel from its by-product; thus, it reduces the biodiesel yield and production cost [25].

Inekwe *et al* [26] reported that saponification value of about 194 mg/g and Oladele and Oshodi [27] recorded as 193.33 mg/g, which is nearer to the present study. High saponification value indicated that oils are normal triglycerides and very useful in production of liquid soap and shampoo industries [18]. High saponification value indicates the presence of high percentage of fatty acids in the oil and therefore implies the possible tendency

to soap formation and difficulties in separation of products if utilized for biodiesel production. This would also suggest that using the oils for biodiesel production would lead to very low yields in the methyl esters.

Viscosity is an important property in the selection of oil for any engine. Viscosity and densities are other important parameters for diesel fuel injection system, high density of oil can lead to incomplete combustion and particulate matter emission [28, 29]. Olasheu *et al*. [30] showed that Jatropha oil is suitable as alternative to conventional lubricating in auto engines and observed that most of the values complied with standard specified by ASTM, Jatropha oils are of good quality and could be recommended for suitable industrial usage. In another study carried out by [31] also conferred the same that fuel properties of Jatropha methyl ester and its blends with petroleum diesel. They also found viscosity values for all fuels falls within specifications of American Standard Test Methods (ASTM), with a maximum variation of 21% observed between BO and B100.

3.3 Fatty acid composition of oil

Fatty acid profile of three accessions was shown in Table 3. The major fatty acids in Jatropha seed oil were palmitic acid 15.11%, Oleic acid 39.41% and linoleic acid 38.67 % and are rich in unsaturated fatty acids. Akbar *et al*. [18] finds the fatty acid Composition of *Jatropha Curcas* oil seed from Malaysia as palmitic acid 14.2 %, oleic acid was 44.7% and linoleic acid 32.8 %.Studies carried outby [26] showed the fatty acid content of oils from the three sampling locations of India and Nigeria had the composition of palmitic acid ranged from 13.16 -14.69%, linoleic acid ranged from 79.08- 81.87%, oleic acid ranged from 5.87-6.06% and stearic acid was 5.23%.

In another study reported by [23] that fatty acid composition of *Jatropha curcas*, which were collected from the outskirts region of Bardoli (Gujarat) was palmitic acid 16.69%, stearic acid 7.67%, oleic acid 40.39%, linoleic acid 33.09% and linolenic acid 0.28%. Results of the present study show the fatty acid composition of Karnataka and Jharkhand accessions were similar to other Indian Investigators [23, 18].

3.4 RAPD analysis

Among 10 primers, only four primers (OPC-2, OPC-9, OPH-1 and OPQ-15) showed genetic difference in all the three genotypes of Jatropha plant.Fig.1 shows the RAPD amplification pattern of Jatropha accessions using ten primers. A total of 81 sharp and reproducible bands were obtained from 10 primers, out of which 73 bands were polymorphic while 8 bands were monomorphic, resulting in

88.93 % polymorphism among the genotypes. Out of 10 primers 6 primers produced 100% polymorphism whereas primer OPQ-15 produced the minimum level of polymorphism about 50%. The average polymorphic fragments were 7.3 per primer.

Kaul *et al.* [32] were using 47 RAPD primers for amplification of DNA of the 29 genotypes of Jatropha plants which yielded 552 fragments, among 334 were polymorphic with an average of 7.1 polymorphic fragments per primer. Similarly, Kumar *et al*. [22] were used 10 RAPD primers were used to assess molecular polymorphism of 20 *Jatropha curcas* genotypes. A total of 47 amplified products were obtained out of which 44 were polymorphic and 3 were monomorphic. Average polymorphism across twenty genotypes was found to be 93.61%.

Studies carried out by Pamidimarri *et al*. [33] were successfully identified the polymorphic markers that are specific to non-toxicand toxic variety using RAPD and AFLP techniques respectively. Totally 371 RAPD were analysed and 56 (15.09%) RAPD markers were found specific to either of the varieties. Genetic similarity between non-toxic and toxic verity was found to be 0.92 by RAPD technique.

In another research, Subramanyam *et al*. [34] reported that genetic diversity and pedigree analysis of *Jatropha curcas* L using RAPD markers. A total number of 10 accessions from different zones of India, screened with 43 random decamer primers to evaluate polymorphism. Selected 10 primers generated 125 bands, 76 of which were found to be polymorphic. Each primer produced on an average 12.5 band per primer of which 7.6 were polymorphic. Similarly, Kumar *et al*., (2013) also reported that 36 genotypes of *J. curcas* collected from different districts of Assam and Meghalaya provinces of Northeast India showed variation in seed-oil content. Single primer amplification reaction (SPAR) methods were used to determine diversity at DNA level. Upon analysis of the data generated, both two SPAR methods revealed genetic variation among genotypes.

Therefore, results of the present study show RAPD analysis of different accessions of *Jatropha curcas* from Karnataka and Jharkhand were well corelates with the previous researches reported by other investigators. RAPD analysis study helped to confirm the genetic variability between the accessions in relation to the variation in morphology and seed oil content. The RAPD study also helps to identify the potential accessions.

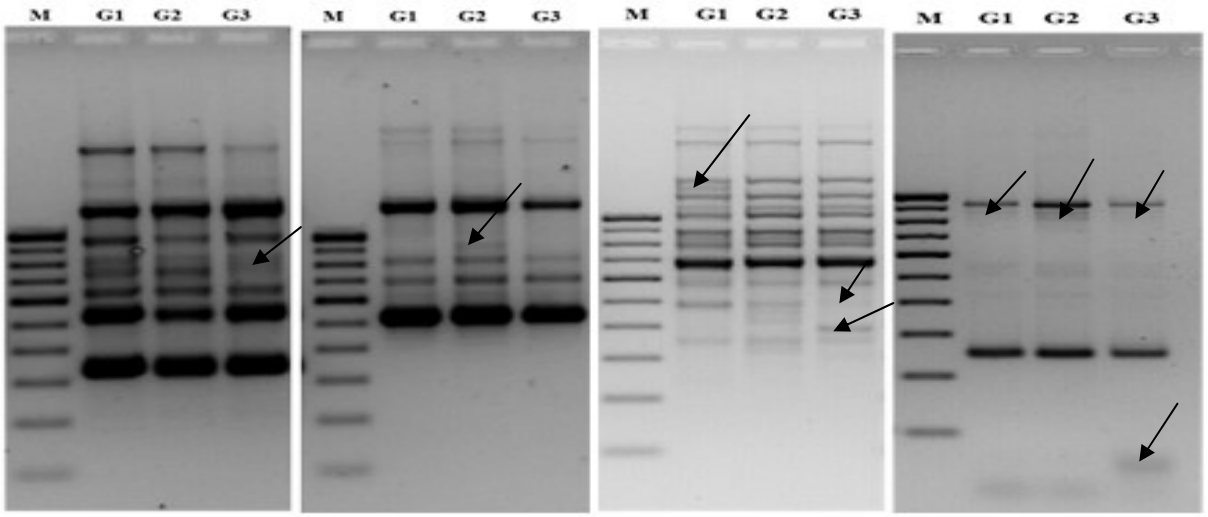

Fig.1a) Primer 2 **Primer 3** **Primer 6** **Primer 10**

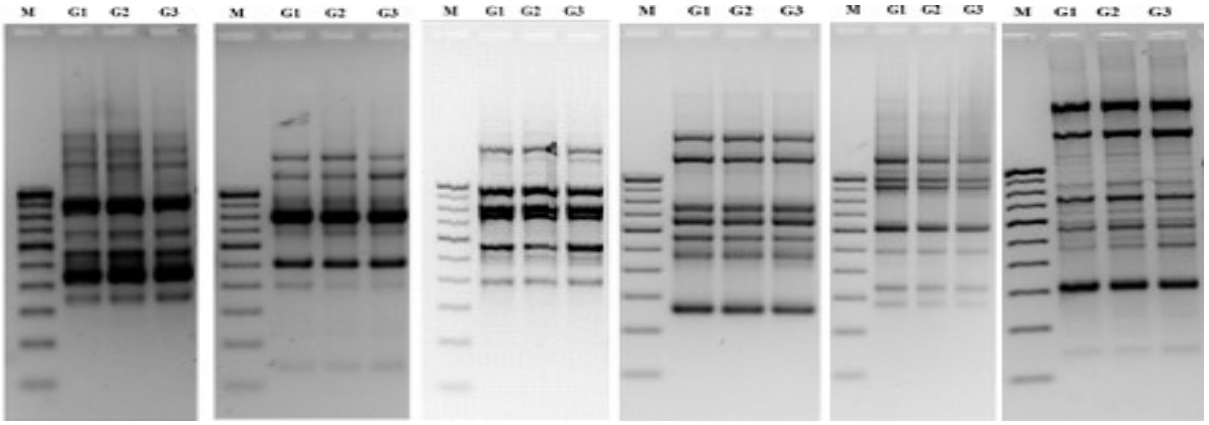

Fig.1b) Primer 1 **Primer 4** **Primer 5** **Primer 7** **primer 8** **primer 9**

Fig 1(a): RAPD amplification pattern of three Jatropha accessions by using random primers 2, 3, 6 and 10 (b) 1, 4, 5, 7, 8 and 9 (M: DNA ladder, G1: LB seeds, G2: GKVK seeds and G3: JH seeds)

Table 1: Seed characteristics of three accessions of *Jatropha curcas* seed

Accessions	Seed length (cm)	Seed width (cm)	Wt. of 100 seeds (gm)	Wt. of 1000 seeds (gm)	Oil (%)
LB (GI)	1.7	1.1	70.41	70,410	33
GL (G2)	1.6	1.0	62.30	62,300	31
JH (G3)	1.5	0.9	60.28	60,280	30

Table 2: Physico-chemical properties of the seed-oil of three accessions of *Jatropha* seed

S. No	Parameters	LB (G1)	GL(G2)	JH G3)
1	Acid value (mg/g)	7	9	7
2	Iodine value (mg/g)	106	107	109
3	Saponification value(mg/g)	193	195	196
4	Viscosity (mm^2/sec)	33.2	34.4	33.6
5	Density (kg/m^3)	912.1	912.3	912.4

Table 3: Fatty acid composition analysis of seed-oil of three accessions of *Jatropha curcas*

Fatty acid composition	LB (GI-%)	GL (G2-%)	JH (G3-%)
Myristic acid	0.01	0.11	0.09
Palmitic acid	15.11	17.42	16.01
Stearic acid	3.20	3.38	4.33
Oleic acid	39.41	37.42	42.89
Linoleic acid	38.67	40.80	41.25
Linolenic acid	0.34	0.88	0.85
Arachidonic acid	0.00	-	0.04

Table 4: Different properties of RAPD primers showing polymorphism among three accessions of *Jatropha*

Sl. No.	Primer code	Primer Seq.	Total bands	Polymorphic bands	Monomorphic bands	Polymorphism %
1	OPA-2	TGCCGAGCTG	8	8	0	100.0
2	OPC-2	GTGAGGCGTC	8	6	2	75.00
3	OPC-9	CTCACCGTCC	7	6	1	85.71
4	OPC-18	TGAGTGGGTG	8	8	0	100.0
5	OPC-19	GTTGCCAGCC	7	7	0	100.0
6	OPH-1	GGTCGGAGAA	14	11	3	78.57
7	OPH-3	AGACGTCCAC	7	7	0	100.0
8	OPH-4	GGAAGTCGCC	7	7	0	100.0
9	OPH-9	TGTAGCTGGG	11	11	0	100.0
10	OPQ-15	GGGTAACGTG	4	02	2	50.0
Total			81	73	08	
Average			8.1	7.3	0.8	88.93

4. CONCLUSION

The polymorphism detected among the three accessions will be helpful in selecting genetically diverse cultivars in future breeding programmes. RAPD analysis confirmed the genetic variability between the accessions in relation to the variation in morphology and seed oil content, LB variety could be considered superior as compare to other two accessions. Further studies involving more number of primers may be conducted to get more precise information.

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CONFLICT OF INTEREST

All the authors of this research papers have no conflict of interest.

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