Assessment of Genetic Diversity in Jatropha (*Jatropha Curcus* L.) by using RAPD Markers

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Abstract

In the present study, ten RAPD primers were used to assess molecular polymorphism in twenty 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 %. For the genotypes tested, 6 to 11 bands were obtained, with an average of 7.83 bands per primer. The size of amplified fragments ranged from 200-3000 bp. Analysis of this polymorphism profile, generated using suitable statistical programmes, grouped the twenty genotypes into two major clusters at a similarity coefficient of 0.62. The cluster-I comprised JC-4 and JC- genotypes. Cluster-II comprised 16 genotypes. Cluster- II was divided into two sub clusters at the similarity coefficient 0.65. Sub cluster-I involved three varieties JC-8 and JC-17 and JC-19. The second sub-cluster contained all other genotypes. Genetic similarity matrices of the genotypes ranged from 0.88 to 0.93, indicating a moderate genetic variability among the genotypes. Genotypes JC-3 and JC-2 were genetically most similar with genetic similarity value of 0.88 while the genotypes JC-8 and JC-17 were found out to be genetically most
diverse, at a value of 0.44. Similarity value for all other genotypes varied between these two extreme values. The results obtained indicated that RAPD markers are efficient for identification of Jatropha curcas genotypes and for determination of the genetic relationships among them. Fingerprint data obtained in this study can be further utilized in identification and development of improved Jatropha varieties for further use in breeding programmes.

**Keywords**: Dendrogram, PIC value, Polymorphism, RAPD, UPGMA.

1. **Introduction**

*Jatropha curcas* L., (2n=22), a perennial plant used for bio-fuel production nowadays, belongs to the family *Euphorbiaceae*. It is commonly known as Physic nut, Ratanjot, Jamalghota, Jangli arandi or Kalaaranda. It is originated from Mexico and Central America and now widespread throughout tropical and subtropical regions of the world (Heller, 1996). Portuguese introduced Jatropha as an oil yielding plant in Asia (Burkill, 1966). Jatropha is well adapted to arid and semi arid conditions. Our country has more than 41.93 million hectares of wasteland which has been identified as potential areas for *Jatropha curcas* plantation. It is reported to be cultivated in central and western parts of India. Recently, it has also been introduced in the northern and southern states under massive plantation work to enhance livelihood of rural people and simultaneously to develop wastelands. Ever since it was established that Jatropha methyl esters yield biodiesel of an exceptional quality there has been a surge of interest in Jatropha across the globe for its cultivation (Banerji *et al.*, 1985; Muhlbauer *et al.*, 1998; Manope *et al.*, 2005; Adebowite & Adedire, 2006; Ghosh *et al.*, 2007; Senger *et al.*, 2008, 2010). Several different methods for documenting genetic information are used. These methods include isozyme analysis, restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) (Mulcahy *et al.*, 1995). The RAPD technique employs 8-10 base pair random primers to locate random segments of genomic DNA to reveal polymorphism. It is a simple, reliable and relatively straightforward technique to apply and the number of loci that can be examined is unlimited. RAPD analysis is viewed as having a number of advantages over RFLPs and other techniques (Lynch & Milligan, 1994). The ease and simplicity of the RAPD technique makes it ideal for genetic mapping in plant and animal breeding programs, and DNA fingerprinting, with particular utility in the field of population genetics. In many instances, only a small number of primers are necessary to identify polymorphism within species (Williams *et al.*, 1990). The availability of molecular linkage map in Jatropha offers a great opportunity to apply molecular techniques in Jatropha for various applications such as fingerprinting cultivars, phylogenetic analysis, identification of genes for agronomic ally important traits etc. In view of the importance of Jatropha, it becomes imperative to analyze genetic diversity among its various genotypes using molecular techniques. This would help the Jatropha
breeders to manage their germplasm more effectively and also speed up breeding programs aimed at genetic enhancement of Jatropha. The main objective of the present study was to assess genetic diversity in twenty accessions of Jatropha by using RAPD markers.

2. Material and Methods

2.1 Plant Material
A representative set of 20 accessions of Jatropha (from different geographical regions of India) which were collected and maintained in the Farm Area of Department of Forestry CCS HAU, Hisar and S.V. Patel University of Agri. and Technology, Meerut were used for the present investigation. The whole experimental work was carried out at the Department of Agri. Biotechnology, College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, U. P.

2.2 Genomic DNA isolation
Total genomic DNA was isolated from young leaves of all varieties of Jatropha following CTAB extraction method (Doyle and Doyle, 1987) with some modifications. Five gram of the leaf tissue was hand homogenized to fine powder in liquid nitrogen using sterilized mortar and pestle. The powder was mixed with 15 ml of pre-warmed CTAB extraction buffer (2% CTAB; 1.4 M NaCl; 100 mM Tris-HCl, pH 8.0; 20 mM EDTA, pH 8.0; 0.2% - mercaptoethanol) in sterilized 50 ml polypropylene tubes. The samples were incubated in water bath at 65°C for 1-2 hrs. After incubation, the samples were cooled for 5 minutes at room temperature followed by addition of 15 ml of chloroform: octanol (24:1) solution and centrifuged for about 10 min at 8000 rpm. After centrifugation the upper aqueous phase was transferred to a pre-sterilized centrifuge tube and again extracted with 10 ml of chloroform: octanol (24:1) solution. The upper aqueous phase was collected and DNA was precipitated with equal volume of ice cool isopropanol. DNA was then air-dried at room temperature and subsequently dissolved in appropriate volume of TE buffer.

2.3 Quantification of DNA by UV-spectrophotometer
After isolation of DNA, its quantity was determined at a wavelength of 260 and 280 nm using UV visible spectrophotometer. The ratio between absorbance at 260 and 280 nm (260/280) was used to estimate DNA purity. The DNA was diluted to a working concentration 20 ng/μl and stored at -20°C for further RAPD analysis. DNA concentration = OD_{260} × 50 μg/ml × dilution factor.

2.4 Primers
A total 10 RAPD primers (Genei, Bangalore) were used for genetic diversity analysis. The length of each primer was 10 bp and their different properties including name, sequences, polymorphism, PIC values are given in table-1.
2.5 RAPD-PCR reaction
Amplification reaction was performed according to the method described by Saker et al. (2005) with slight modification, which contains a template containing 40 ng of template DNA, 200 µM dNTPs, 1× Taq PCR buffer, 2.5 mM of MgCl2, 1.2 U of Taq polymerase, and 1 µM each of the RAPD primers. The amplification was carried out in PTC-100 programmable thermal cycler (MJ research and Biometra personal) with PCR profiles: pre denaturation at 94°C for 3 min, 45 cycles at 94°C for 45 sec, 36°C for 30 sec, and 72°C for 2 min, with final extension at 72°C for 10 min, finally amplified product was hold at 4°C. The 15 µl of amplified products were resolved in 1.5 % agarose gel with ethidium bromide at 55 volts for 1-2 hours and visualized under UV-Transilluminator. PCR amplified products were visualized under UV light and photographed using VSD Image Master of Pharmacia, Biotech.

2.6 Data analysis
Individual RAPD patterns were compared with samples. The reproducible well-marked amplified fragments were scored for each genotype represented graph data. The pair wise comparison of banding patterns was evaluated by Nei’s (1978) genetic distance using the Free-Tree-Free ware programme (Pavlicek et al., 1999). The Phylogenetic tree was constructed with help of tree view software.

3. Results
The present investigation was undertaken to assess genetic diversity in physic nut (Jatropha curcas L.) by using RAPD markers. To detect polymorphism, ten random decamer primers having 60 percent or more G +C content were used for RAPD analysis of different genotypes. Four primers did not exhibit any amplification for any of the genotypes. Out of 10 random primers, only six primers (OPH-03, OPH-04, OPH-09, OPH-19, OPA-02 and OPC-19) showed amplification in all the 20 genotype. The DNA amplification and polymorphism generated among various genotypes of J. curcas using random primers are presented in Table-1. RAPD profile among 20 genotypes of J. curcas using primers OPA-02, OPH-04, OPH-03 & OPC-19 are shown in Fig-1: (A), (B), (C), (D). The DNA amplification pattern obtained after amplification of the 20 genotypes was scored on the basis of presence or absence of amplified products on agarose gels. For each primer, the presence of band in genotypes was denoted by one (1) while its absence was represented by zero (0). DNA amplification pattern for all genotypes were obtained in a similar manner for all the primers showing amplification.
Assessment of Genetic Diversity in Jatropha (Jatropha Curcus L.)

Table 1: Different properties of RAPD primers showing polymorphism among 20 accessions of jatropha.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Primer code</th>
<th>Primer sequence (5’→3’)</th>
<th>Range of mol. Size (bp)</th>
<th>Total bands</th>
<th>Polymorphic bands</th>
<th>Mono morphic bands</th>
<th>Polymorphism %</th>
<th>PIC value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OPH-03</td>
<td>AGACGTCCAC</td>
<td>220-1200</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>100.00</td>
<td>0.32</td>
</tr>
<tr>
<td>2</td>
<td>OPH-04</td>
<td>GGAAGTCGCC</td>
<td>200-800</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>100.00</td>
<td>0.46</td>
</tr>
<tr>
<td>3</td>
<td>OPH-09</td>
<td>TGTAGCTGGG</td>
<td>250-950</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>85.71</td>
<td>0.34</td>
</tr>
<tr>
<td>4</td>
<td>OPH-19</td>
<td>CTGACCAGCC</td>
<td>430-1700</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>66.66</td>
<td>0.42</td>
</tr>
<tr>
<td>5</td>
<td>OPA-02</td>
<td>GAGGATCCCT</td>
<td>350-3000</td>
<td>11</td>
<td>11</td>
<td>0</td>
<td>100.00</td>
<td>0.37</td>
</tr>
<tr>
<td>6</td>
<td>OPC-19</td>
<td>GTTGCCAGCC</td>
<td>300-1500</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>100.00</td>
<td>0.57</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>47</td>
<td>44</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td>7.83</td>
<td>7.33</td>
<td>0.50</td>
<td>93.61</td>
<td>0.41</td>
</tr>
</tbody>
</table>

A total of 47 sharp and reproducible bands were obtained from 6 primers. Out of which 44 bands were polymorphic while 3 bands were monomorphic resulting in 93.61% polymorphism among the genotypes. Primer OPH-19 produced the minimum level of polymorphism i.e. 66.66%. Out of 6 primers 4 primers produced 100% polymorphism. Hence the per cent polymorphism ranged from 66.66%-100 % for the 20 genotypes of Jatropha. For RAPD markers the PIC values ranged from 0.32 to 0.57. Out The mean PIC per primer (Diversity index) for polymorphic bands was 0.41. The highest PIC value (0.57) was observed with primer OPC-19 and the lowest PIC value (0.32) was observed with primer OPH-03. For the genotypes tested, 6 to 11 bands were obtained for various primers with an average of 7.83 bands per primer. The highest number of bands i.e.11, were generated by primer OPA-02 followed by OPH-03(9 bands), OPC-19(8 bands) and OPH-09(7 bands). Primers OPH-04 and OPH-19 generated only six bands. The banding pattern of all other primers was more or less around the average i.e. 7.83.

3.1 Genetic relationship and cluster analysis of 20 Jatropha accessions

The RAPD data thus obtained were used to construct similarity matrices of the 20 Jatropha accessions using ‘Simqual’ sub-programme of software NTSYS-PC. The allelic diversity data were used for analysis. ‘Cluster Tree Analysis’ sub-programme of the same software, revealed the genetic linkage and proximity among all the genotypes investigated.
3.2 Similarity Matrices
Similarity matrix of 20 Jatropha genotypes revealed the relationship among them (Table-2). The similarity indices between different genotypes ranged from 0.44 to 0.88 with an average of 0.68 across all the genotypes. A maximum similarity value of 0.88 was observed between JC-3 and JC-2 whereas JC-9 & JC-19 and JC-14 & JC-19 were found to be genetically most diverse with similarity value of 0.44. The maximum similarity was obtained between JC-2 and JC-3, they were in the same group with the similarity coefficient 0.93. The cluster tree, in a way, told the same story as told by the similarity matrices of the 20 Jatropha genotypes. The association amongst different genotypes was presented in the form of dendrogram. Varieties JC-2 and JC-3 were the closest at the similarity coefficient 0.93, followed by JC-16 and JC-18. Genotypes JC-14 and JC-19 were farthest or most diverse and dissimilar to all others at a coefficient of 0.44.

3.3 Cluster Analysis
The average linkage between the genotypes was used for constructing a phylogenetic tree. The relationship among the 20 Jatropha genotypes used during present investigation was represented in fig. 1. The hierarchical cluster analysis identified two major clusters at a similarity coefficient of 0.51. First cluster comprised of two genotypes JC-14 and JC-2 whereas the remaining 18 genotypes fall in second cluster. Second clusters at a similarity coefficient of 0.62 divided into two subclusters.

![Fig. 1](image_url)

**Fig. 1:** (1) RAPD amplification pattern of twenty jatropha accessions by using (A) OPA-02 primer (B) OPH-04 primer (C) OPH-03 primer (D) OPC-19 primer and (2) Dendrogram based on UPGMA cluster analysis.
Subcluster-I comprised two varieties, JC-4 and JC-7, they were close to each other with the similarity of 71%. The remaining 16 genotypes fall in subcluster-II. Subcluster-II again divided into two groups at the similarity coefficient of 0.65. Group-I comprised of three genotypes- JC-8, JC-17 and JC-19 but this group again divided into two sub groups at the similarity coefficient of 0.69. Genotype JC-19 was out grouped as first subgroup. Subgroup-II again divided into two sub-subgroups at the similarity coefficient of 0.81. Group- II divided the genotypes into two sub-subgroups at the value of 0.66. JC-5 was out grouped as first sub-sub group while genotypes JC-6, JC-3 and JC-2 fall in second sub-sub group. Sub Sub group II again divided into two parts in which one part comprised of genotypes JC-3 and JC-2 showing the maximum similarity (0.88) at the similarity coefficient 0.93. Sub group II composed of two Sub Sub groups in which Sub Sub group I JC-18, JC-16, JC-15, JC11and JC-10 out of which JC18 and JC-16 showed more similarity. Sub Sub group II comprised of 4 genotypes viz., JC- 12, JC-13, JC-9 and JC-1.

4. Discussion

RAPD analysis has proved to be useful in genetic diversity studies and it has detected sufficient number of polymorphic DNA markers (Winter & Kahl, 1995). These markers are an important tool in a molecular biology to analyze a gene pool at a molecular level. In order to impart meaning to the results obtained and to fully comprehend their implications, it is imperative to statistically analyze the results. Random amplified polymorphic DNA (RAPD) is one of the common genetic marker that can be used for population genetic analysis, pedigree analysis and taxonomic discrimination (Rodriguez et al., 1999; Muneer et al., 2008; Nair & Keshavachandran, 2006). Several authors 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 (Bautista et al., 2001; Wu et al., 2006; Archak & Karihaloo, 2002; Malviya & Yadav, 2010). In the present study, a total of 47 sharp and reproducible bands were obtained from 6 primers. Out of which 44 bands were polymorphic while 3 bands were monomorphic resulting in 93.61 % polymorphism among the genotypes. Primer OPH-19 produced the minimum level of polymorphism (66.66%) while OPH-03, OPH-04, OPC-19 & OPA-02 showed highest level of polymorphism (100%). Out of 6 primers 4 primers produced 100% polymorphism. Hence the per cent polymorphism ranged from 66.66-100% for the 20 accessions of Jatropha. For RAPD markers the PIC values ranged from 0.32 to 0.57. Out the mean PIC per primer (diversity index) for polymorphic bands was 0.41. The highest PIC value (0.57) was observed with primer OPC-19 and the lowest PIC value (0.32) was observed with primer OPH-03. For the genotypes tested 6 to 11 bands were obtained for various primers with an average of 7.83 bands per primer. The present investigation revealed that Jatropha accessions used for diversity assessment through RAPD, showed a moderate level of genetic variations. The average linkage between the genotypes was used for constructing a phylogenetic tree. Different levels of
polymorphism were also observed in other studies conducted on several accessions of jatropha (Sujatha et al., 2003; Ganesh et al., 2007; Basha et al., 2007; Makker et al., 2008; Sudheer et al., 2008) by using many RAPD markers. The levels of variability for RAPD in Jatropha were comparable to other medicinal and oil bearing plants studied such as Cassava (Asante & Offei, 2003) and Phyllanthus amarus (Jain et al., 1997). The results observed in the present study were almost similar to the above studies.

The cluster tree, in a way, told the same story as told by the similarity matrices of the 20 Jatropha genotypes. The association amongst different genotypes was presented in the form of dendrogram. Varieties JC-2 and JC-3 were the closest at the similarity coefficient 0.93, followed by JC-16 and JC-18. Genotypes JC-14 and JC-19 were farthest or most diverse and dissimilar to all others at a coefficient of 0.44. Previous studies using alloenzymes, RAPD and microsatellites, indicated a low level of variation in Jatropha (Staubmann et al., 1999; Ginwali et al., 2004). The genotypes which were lying nearer to each other in dendrogram were more similar to one another than those lying apart. Varieties JC-2 and JC-3 were the closest, followed by JC-16 and JC-18. Genotypes JC-14 and JC-19 were farthest or most diverse and dissimilar to all others. The results revealed that RAPD is an efficient technique to characterized the Jatropha genotypes and classify different genotypes based on the RAPD markers generated. It was also indicated that RAPD analysis has determined the genetic relationships and estimated the genetic diversity among the genotypes of Jatropha. The results of the present study can be used as a stepping stone for evolving well defined approach based on evaluation and characterization of genetic variation in Jatropha which can be further used for the improvement of Jatropha for various traits through different breeding methods.

5. Conclusion

The present study revealed that the levels of genetic differentiation between cultivars of Jatropha curcas increased with geographical distance. The polymorphism detected among the 20 accessions will be helpful in selecting genetically diverse cultivars in future breeding programmes. However, there were some precincts in the present study that only twenty cultivars and ten primers were used in RAPD analysis and hence reduce the chance to obtain a reliable knowledge precisely about the genetic structure of each cultivar of jatropha. Further studies involving large number of accessions and primers need to be conducted to get more precise information.

References


