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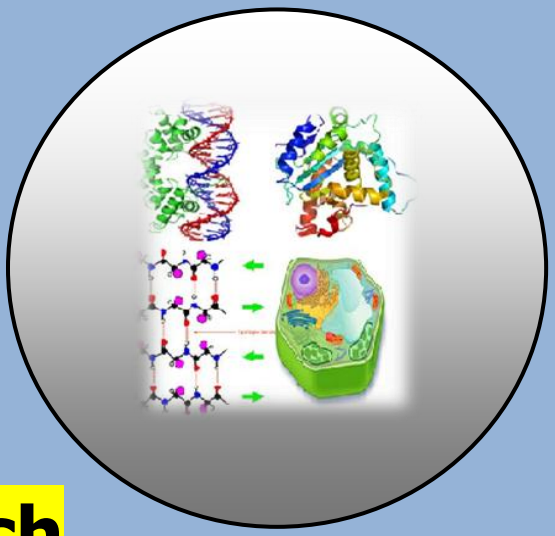

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RESEARCH PAPER
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Genotypic Characterization of *Jatropha curcas* L. germplasm by RAPD Analysis

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ABSTRACT

Jatropha curcas L. is a rapidly emerging biofuel crop attracting a lot of interest, triggering large investments and rapid expansion of cultivation areas. In the present investigation, the genetic relationships of 5 J. curcas accessions were assessed based on randomly amplified polymorphic DNA (RAPD) analysis. Amplification of genomic DNA of the 5 genotypes, using RAPD analysis, yielded 24 fragments that could be scored, of which 11 were polymorphic with average pf 2.2 polymorphic fragments per primer. The number of amplified bands varied from 2 to 10 with a size range of 300-3000bp. Molecular polymorphic was 45.8%. Jatropha curcas L. plant leaf isolates from different geographical locations exhibit almost same level of phylogenetic similarity.
Keywords:- Jatropha curcas L., Randomly Amplified Polymorphic DNA (RAPD) Analysis

INTRODUCTION

Jatropha curcas L. mainly cultivated for extraction of bio diesel and one of the best source of biodiesel and biofuels. In study of various biofuel one hectare of *Jatropha curcas* L. yielded 6-8m.t. of seed. One ton of *Jatropha curcas* L. seed yield 300kg oil product and 700kg cake before Jatropha oil is mixed with diesel this is resulted in production of glycerin and disposal of this glycerin is a problem.

In India Jatropha oil is used for powering firm equipment and diesel generator, southern railway also uses the biofuel Jatropha oil it is also used in making candle and soap. The Jatropha seed fruit shell is used as fuel for burning. The seed cake that used as organic fertilizer or for animal feed.

The meal after extraction of excellent organic manur other than extracting biodiesel from *Jatropha curcas* L. plant leaf and bark are used for various other industrial and pharmaceutical uses. Localized production and availability of quality fuel restoration of degraded land over a period of time.

Approximately 31.37% of oil extracted from *Jatropha curcas* L.seed can be used for any diesel engine without modification. Identification of *Jatropha curcas* L. is a large course annual shrub or small short live tree. Which can grow 6-15 feet tall. It has thin greenish bark which exude copious amount of watery sap when cut.

Jatropha curcas L. or psychic nut has become new source of biodiesel it is higher yielding crop in India Jatropha oil use for powering farm equipment and diesel generator. Southern railway also uses the biofuel Jatropha oil.

Jatropha oil also uses in making candle and soap. Seed fruit shell is used as fuel for buring the seed cake remain after extraction of Jatropha can be used as organic fertilizer for animal feed. *Jatropha curcas* L. is resistant to drought and can be planted in the desert climate and it can thrive in any type of soil including sandy, gravel and saline soil. Jatropha need minimal input or management, it has no inspected pest and it is not browsed by cattle or sheep. Jatropha propagation is easy. It has rapid growth and rapidly forms a thick live hedge. After only one month of planting, it starts yielding from the second year and continues up to 40 year the meal after extraction and excellent organic manure other than extracting biodiesel from *Jatropha curcas* L. plant.

Approximately 31-70% of oil is extracted from *Jatropha curcas* L. seed. It can be used for any diesel engine without modification the oil is used as illuminant without being refined and it burns with clear smoke free flame. Oil has very high saponification. The latex of Jatropha contains and alkaloid known as “jatrophn” which is believed to be having anticancerous property. It is also externally applied for skin disease and rheumatism and for sores on domestic livestock. In addition twig of plant used for cleaning teeth while the juice of leaf is used as an external application for piles. Finally the roots are reported to be used as an anti dotes for snake bite.

The bark of *Jatropha curcas* L. yield a dark blue dye which is used for coloring cloth. Jatropha oil cake rich in nitrogen phosphorous potassium and can be used as organic manure Jatropha leaf used as food for tussar silkworm Divakara et. al (2010), Ginwal et. al (2004), Gopinath and Sudhakaran (2009), Kaushik et. al (2007), Kumar and Sharma (2008) and Rao et. al (2008).

Presence of genetic diversity is crucial for improvement of any plant species. An understanding of the magnitude and pattern of genetic diversity in crop/forestry plant has important application in breeding program and for conservation of genetic resources. The markers are routinely used for estimating genetic diversity but are not successful due to strong influence of environment. Hence rapid use of molecular marker has complimented the classical strategies and helped in the characterization of genotype in plant kingdom. These markers are independent of influence of environmental growth conditions. Physical age of plant and the type of tissues being analyzed in Jatropha isozyme markers were used to determine the genetic relatedness of the member of the genus *Jatropha curcas* L.. RAPD marker were employed to confirm hybridity of interspacific hybrids beteen *Jatropha curcas* L. and ISSR marker were used to determine inter and intra population variability in *Jatropha curcas* L. as compare to the other molecular technique random amplified polymorphic DNA (RAPD) has principle advantage of eliminating the need to work with radioisotope and faster in obtaining result. This technique has earlier being used in assessing genetic variation in wide array of agricultural and forest tree crops.

Among the various molecular markers employed to assess diversity, PCR based markers such as RAPD is popular and its application does not need any prior sequence information. Arif M. Zaidi et. al (2009), Caetano-Anolles and Gresshoff (1997), Basha and Sujatha (2007), Basha et. al (2009), Ikegami et. al (2009), Raina et. al (2001), Willams et. al (1990).

MATERIAL AND METHODS

We report here the application and reliability of RAPD marker to investigate the extent and distribution of genetic diversity in *Jatropha curcas* L. from different regions of Uttar Pradesh, India (Kanpur, Lucknow, Sitapur, Lakhimpur, Bareilly) in year 2014. Total genomic DNA was extracted from young leaves following the standard CTAB method (Doyle and Doyle 1990).

In the genome study greatly enhace the speed and efficiency of crop improvement. Molecular marker closely linked to traits of economic importance has been developed in several crops. These have allowed the section of desirable trait in a genotype. DNA finger printing technique like restriction fragment length polymorphism (RFLP), amplified fragment lenght polymorphism (AFLP), random amplified polymorphism DNA (RAPD), microsatellite marker like SSR s, sequence characterized amplified region (SCAR), sequence tagged sites (STS), and rDNA- it's have been used for generation of molecular marker and there efficient use in breeding. For the PCR based marker assisted selection, suitable modified extraction method for genomic DNA in term of quality and quantity is essential for a particular crop.

Normally the objective of PCR is to generate defined fragment of DNA from highly specific primers. In case of RAPD (pronounced of rapid). Short oligonucleotide primer is arbitrarily selected to amplify a set of DNA fragments randomly distributed throughout the genome. This technique random amplified polymorphic DNA is also known as arbitrarily primed PCR (AP-PCR).

Five plants of *Jatropha curcas* L. from different ecogeographical regions of India were used for the assessment of genetic diversity. The accession were selected randomly from 30 leaf sources of *Jatropha curcas* L. which were collected and maintained at CSA university Kanpur, bio-tech park Lucknow, Sitapur, Lakhimpur and Bareilly districts of Uttar Pradesh, India.

Amplication reaction was carried out in PTC-100 programmable thermal cycler (MJ research and biometra personal) with an initial denaturation at 94^{o}C for 30 seconds and extension at 72 ^{o}C for 2 minute with a final extension at 72 ^{o}C at 10 minute.

The PCR products were separated on 1.0% agarose gel in 1XTAE buffer using ethidium bromide staining dye. The size of amplified fragment was determined by using size standard gene ruler 3kb. DNA loader machine or transilluminator and DNA fragment were visualized under UV light and photographed using VSD image master (Pharmacia biotech). To test the reproducibility of RAPD marker the reaction was repeated at least twice.

Agarose gel electrophoresis was employed to quickly determine the yield and purity of DNA isolated or check product of PCR reaction check progression of a restriction enzyme digestion and to size fractionate DNA molecules which then could be eluted from the Agarose gel is a natural product purified from red seaweed (Rhodophyta).

It i a polysaccharide of alternate 1, 4- linked □- D- galactopyranose and 1, 4-linked 3, 6-anhydro - □- L – galactopyranose residue and arranged into double helix (Araki 1958, Arnott et al 1974). It dissolves in water on boiling and forms a gel when cooled down to about 40^{0}c.

Agarose gel is a first choice for nucleic acid analysis and protein separation as early as 1974 since the gel is easily prepared in a laboratory Nontoxic Optically clear (preferred for densitometry sanning and photography) Chemically inert Having large pores for wide range of molecules to pass through available with least or minimum electro endosmosis (EEO) possessing good gel strength accessible for manual staining and destaning procedures and dried and preserved easily.

Agarose gels have a larger pore size and they can be used to separate macromolecules such as nucleic acids, large proteins complexes. Agarose gels are hydrocolloids held together by hydrogen and hydrophobic bonds.

So they are somewhat brittle and break when they are bent. Hence, agrose gels should always be handled carefully with some form of support for the entire gel such as gel tray or wide spatula. The pore size and sieving characteristics of agarose gel to a certain extent is determined by its concentration. The higher the concentration of agarose the lower the pore size. Agarose gel is generally used in the concentration range of 0.4-4.0 % (w/v).

The separation of DNA on the gel i carried out under an electric applied to the gel matrix. DNA molecules migrate towards the anode due to negatively charged phosphates along the backbone of the DNA. Fragments of linear DNA migrate through agarose gels with a mobility that is inversely proportional to the log 10 of their molecular weight. Thus the larger molecules Travel at a slower speed than the smaller ones. Circular forms of DNA migrate in agarose distinctly from linear DNAs of the same mass. Typically uncut plasmids will appear to migrate more rapidly than the same plasmid when linearised. Additionally most preparations of uncut plasmid contain at least two topologically different forms of DNA, corresponding to super coiled forms and nicked circles. Several parameters like agarose concentration, voltage applied and molecular size of DNA affect the migration of DNA. RAPD-PCR was performed using RAPD primer in the present analysis took five random primer for five sample. The primers which are used for this analysis are given below-

OPE-21(5'-TCG GGC TGA G-3'), OPE-22(5'ACG GCG TAT G-3'),

OPE-23(5'AAC GGT GAC C-3'), OPE-24(5-GGT CGT GTT G-3'),

OPE-25(5'CTC TGT TCG G-3'), Supplied by operon technologies.

(1) Clean the gel casting apparatus and seal both ends of the gel casting tray with an adhesive tape.

(2) Place the combs of electrophoresis set such that it is approximately 2cm away from the top edge.

(3) Prepare 1% agarose gel by dissolving appropriate amount of agarose in 1X TAE buffer. Boil till agarose dissolves completely and a clear solution results.

(4) Pour the agarose gel solution in the tank when the temperature reaches approximately 550 C. Do not generate air bubbles. The thickness of the gel should be around 0.5 to 0.9cm. Keep the gel undisturbed at room temperature for the agarose to solidify.

(5) Remove the comb carefully, ensuring the wells remains intact and peel of the tape from the gel casting tray.

(6) Place the gel in horizontal electrophoresis apparatus containing 1X TAE electrophoresis buffer in the reservoirs. The buffer level should be such that is just covers the agarose gel.

(7) Take 10 □ l of DNA sample mixed with 3 □ l of gel loading dye and load into the gel.

Genomic DNA loaded in the well appeared as distinct bands and DNA sample loaded in the gel appeared as thick band far the well under UV illuminator respectively. The size of the amplication products was determined by comparison to □ □ DNA digested with EcoR1 and HindIII. Genomic DNA loaded on the gel appeared to be broken and hence there was smearing of the smear on the gel along with intact DNA. Agarose gels perform separation according to size, the molecular weight of a DNA fragment may be determined from its electrophoretic mobility by running a sample of *Jatropha curcas* L.. DNA that has been cleaved with a restriction enzyme such as EcoRI. Since the base sequence of □ □ DNA and the cleavage site for EcoRI are known. This generates fragments of Occur a fluorescent molecule which inter calates within the DNA bases extending the length of linear and nicked DNA molecules and making them more rigid. When Et. Br. is added UV radiation at 254 nm it is absorbed by the DNA and transmitted to the bound dye. The energy is re-emitted at 590nm in the red-orange region of the spectrum. EtBr is a powerful mutagen and hence the gel should be handled carefully with the gloves. The DNA bands can be visualized under UV and data can be recorded by gel documentation system.

RESULT AND DISCUSSTION

The RAPD techniques have been reliable to obtain useful system for evaluation and genotypic characterization among 5 plants of *Jatropha curcas*. by analysing the genetic structure of *Jatropha curcas* DNA. RAPD analysis optimization thus adds the reliability to the techniques. This approach may greately facilitate genetic & ecological study of plants Phylogenetic analysis demonstrated that isolated *Jatropha curcas* plant species from different areas of India are related to each other. The genomic relatedness of these species is supported by its similar band pattern thus, indicating the close relationship.

Table 1. Pair group of 5 samples (RAPD) analysis *Jatropha curcas.*

Band	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1	1	0	0	0	0
2	1	0	0	0	0
3	0	1	1	1	0
4	1	0	0	0	0
5	1	0	0	0	0
6	1	0	1	1	0
7	1	0	1	1	1
8	1	0	0	0	1
9	1	0	1	1	0
10	1	0	1	1	0
11	1	1	0	0	1

Table 2. Detail of DNA marker systems in *J. curcas* L.

Marker System	no. of primers used	Total bands scored	Polymorphc bands	total no. of polymorphism	% polymorphism	Av. polymorphism (bands/primer)
RAPD	5	24		11	45.8	2.2

Analysis of this RAPD with primer OPE-21 the bands are visualized in which the band seen in sample-1 was not common in all, only one band is common in all the samples, another band is common in only two sample and one band is similar only with one sample. RAPD analysis when carried out with primer OPE-22, there was no any polymorphic band present in sample 1,3 & 5. Sample-2 contain polymorphic band in comparison with the entire four sample having molecular weight of 300bp, 400bp, 500bp and 600bp. Sample number 04 contained only one polymorphic band at molecular weight 600bp. Then after RAPD analysis with primer OPE-23 the result shows that no polymorphic variation is obtained in all the five sample, only sample number 01 contain one polymorphic band at 800bp. Rest bands were visualized in all the samples at 1000bp. Analysis of sample number 04 with primer OPE-24, the result showed that sample number 05 contained two polymorphic bands at 2000bp and 3000bp in comparison to other samples but sample number 04 contain only two polymorphic band at 800bp and 700bp, and sample number 04, 05 and 06 contained band at 400bp. Rest whatever bands are seen, they are common in all the samples. Sample number 02 contained one under express band at 500bp in comparison to other sample. At last RAPD analysis with primer OPE-25 showed that in sample number 02, only one polymorphic band is present at 1000bp, where as sample number 03, and sample number 04 contained two bands at 800bp and 700bp. In sample number 05 one band is under expressed at 600bp. Rest whatever bands are seen, are common in the entire sample. Amplified products for RAPD analysis were scored based on presence (taken as-1) absence (taken As-0) of band for each primer was scored by visual observation where only clear and un ambiguous band were scored the size (in nucleotide base pair) of the amplified band was determined based on its migration relative to molecular size of marker Table-1, (DNA ladder from Bangalore genie Pvt. Ltd. India. The data entry was done in to a binary data matrix at discreet variable jacord coefficient of similarity was measured and a dendrogram based on similarity coefficient was generated by using un weighted pair group method with arithmetic mean RAPD analysis.

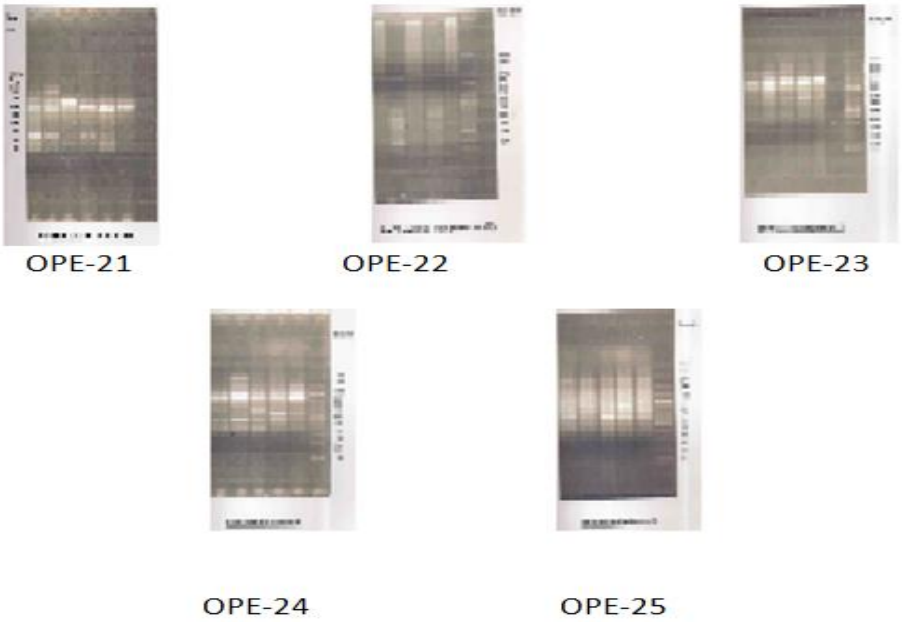

Figure 1. RAPD profile of *J. curcas* genotypes with different primers.

From genomic finger prints of the *Jatropha curcas* plant isolates generated by RAPD PCR. The bands were generated using primers OPE-21 (lane-1), OPE-22(lane-2), OPE-23(lane-3), OPE-24(lane-4) and OPE-25(lane-5). Lane 7 was used as negative control (Fig.1). Random Amplified Polymorphic (RAPD) is very simple but powerful and effective tool in genetic analysis of plants, animals or microorganisms was described by Williams et.al. (1990). Diversity of plant has been revealed by many studies and almost all of the data reported previously indicated that there is high level of genetic diversity. As assessment of the genetic diversity and genetic relationship among plant could provide valuable information about plant genotype that are well adopted to a certain environment. In the present study, the genetic diversity among plants in relation to the environmental and geographic origin has been studied. The result obtained show that samples no 04. When analyzed by RAPD with primer 05 by primer OPE-25, but it is under expressed in sample number 03 and 04 by primer OPE-23 and OPE-24. In sample number 02, the 600bp bands were expressed by OPE-23, OPE-24 bands but with these primers 800 bp and 900bp were also expressed, but these bands are under expressed with other primers and only expressed by primers OPE 23 and OPE 25 (Fig.1). All the bands of sample number 03 were under expressed by primer OPE-21, but 600bp band was expressed by primer OPE 23, OPE 24, OPE 25 of 600bp, 700bp, 2000bp. So by this finding it is easy to conclude that phenotypically these species were similar but genotypicaly they are get separated because of the environmental factor. Finally the analysis of all the data for every sample, it is found that some genes were expressed and many were under expressed. Which were indentified with the help of different primers. It is clear from the result obtained with primer OPE 21 in the entire sample, the most of the genes under expressed. By using primer OPE 22 the genes mostly expressed was 700bp and 3000bp. With primer OPE 23 most of the genes were expressed, the size of which was in between 400bp to 3000bp. The expression from primer OPE-24 showed that mostly the genes are in the range of 400 bp-3000 bp, this expression is similar to primer OPE-25. This analysis of RAPD by primer OPE-25 show mostly smeared gene but the expressed gene was at range of 600 bp-900bp (Fig.1). Here these finding shows that the expressed gene was in the range of 400bp these all are the same gene so all these gene expression show that these plant are phenotypicaly and genotypicaly similar to each other. there is not much variation in there gene expression because all varieties of these plant are of similar species and only few get new expressed or under expressed gene, on the basis of their environmental factor which leads to same stress condition in this plant. RAPD analysis indicating that the plant species are of common origin and are of same species.

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