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RESEARCH ARTICLE

Genetic Diversity of Indian Jatropha Species as Revealed by Morphological and ISSR Markers

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Abstract

The selection of Jatropha based on morphological information and molecular markers is essential as it is more reliable and consistent. Hence, twelve Jatropha accessions from different geographical areas of India were screened for genetic diversity using 19 morphological traits and 21 ISSR primers. The analysis of morphological traits grouped the accessions into five clusters. The cluster I consisted of *J. curcas* (*CJC 18*), *J. curcas* (*CJC 20*), *J. curcas* (*CJC 22*), *J. curcas* (*CJC21*), and *J. curcas* (*CJC 25*), and contained the maximum number of accessions; clusters II and IV contained the minimum number of accessions. Among all the characters, the highest range was exhibited by plant height and the least value by the number of branches. The twenty-one ISSR primers generated 156 polymorphic alleles. The average number of ISSR alleles generated was 7.47 per primer. The ISSR primer UBC 884 was highly informative with the maximum of 12 alleles. The 12 genotypes were grouped into eight clusters. The cluster I contained the maximum number of accessions, namely *J. curcas* (*CJC 18*), *J. curcas* (*CJC 20*), *J. curcas* (*CJC 22*), *J. curcas* (*CJC21*), and *J. curcas* (*CJC 25*). The clusters II, III, IV, V, VI, VII, and VIII (*J. tanjorensis*, *J. gossypiifolia*, *J. glandulifera*, *J. podagrica*, *J. ramanadensis* *J. villosa*, and *J. integerrima*) contained the minimum number of accessions. Maximum diversity between *J. villosa* and *J. integerrima* was noticed and the least diversity between *J. curcas* (*CJC21*) and *J. curcas* (*CJC 25*) seen because the ISSR markers differentiated the Jatropha accession into a wide genetic diversity as compared to the morphological data. The species-specific diagnostic markers identified in the study such as 1000 bp alleles for *J. glandulifera* by the primer UBC 826 is suitable for discriminating species of Jatropha, and thus can be used for identifying a Jatropha species from any mixed population comprising other members of the Jatropha complex.

Key words: Jatropha spp., cluster analysis, genetic diversity, ISSR primers, morphological variation

Introduction

Jatropha curcas L. (Family Euphorbiaceae), also known as Sabudam, purging nut is, a multipurpose plant with several attributes and considerable potential and has evoked interest all over the tropics as a potential biofuel crop (Martin and Mayeux 1985; Takeda 1982). Jatropha is a perennial shrub to small evergreen trees of up to 6 meters in height, adapted to all kinds of soils and does not demand any special nutritive regime (Patil and Singh 1991). *J. curcas* is a native of Mexico and Central American regions and was later introduced into many parts of the tropics and subtropics where it is grown as a hedge crop and for traditional use (Heller 1996). Among the potential oil bearing tree species, *J. curcas* has assumed importance due to its short gestation period, drought endurance, high oil content, and easy adaptation on marginal and semi-marginal lands.

In the recent past, the oil crisis and depleting fossil fuel reserves has rekindled interest in promotion of tree-borne oil species in several African, Asian, and Latin American countries. Global biofuel production has tripled from 4.8 billion gallons in 2000 to about 16.0 billion in 2007, but still accounts for less than 3% of the global transportation fuel supply (Paul 2007).

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Jatropha seeds contain 46-58% of oil on kernel weight and 30-40% on seed weight (Subramanian et al. 2005). It shows promise for use as an oil crop for biodiesel (Foidl and Elder 1997; Henning 1998). The oil is renewable resource and a safe source of energy and a viable alternative to diesel, kerosene, LPG, furnace oil, coal and fuel wood (Chandhari and Joshi 1999). Jatropha species are essentially cross pollinated, which result in a high degree of variation and offers the breeder ample scope to undertake screening and selection of seed sources for the desired traits (Ginwal et al. 2005).

An understanding of the extent of genetic diversity is critical for the success of a breeding program. The selection based on genetic information using morphological and molecular markers is essential as it is more reliable and consistent. In Euphorbiaceae, molecular markers such as, RAPD, RFLP and SSRs have been employed for determining the extent of genetic diversity in elite rubber (*Hevea brasiliensis*) clones (Besse et al. 1994). In Jatropha, RAPD markers were previously employed to confirm hybridity of inter-specific hybrids (Sujatha and Prabakaran 2003) and to determine the similarity index between Indian and Mexican genotypes (Sujatha et al. 2005).

DNA marker-based fingerprinting can distinguish species rapidly using small amounts of DNA and therefore can assist to deduce reliable information on their phylogenetic relationships. DNA markers are not typically influenced by environmental conditions and therefore can be used to help describe patterns of genetic variation among Jatropha species/varieties and to identify duplicated accessions within germplasm collections. Ganesh et al. 2007, analyzed diversity of 12 Jatropha genotypes using RAPD markers. However, the RAPD markers showed less reproducibility when compared to ISSR markers.

Based on ISSR profiling, the present study was formulated to understand the morphological and molecular diversity among the local genotypes of Jatropha.

Materials and Methods

Plant material

Twelve accessions of Jatropha representing various growth habitats were selected for this study (Table 1). The Jatropha accession seedlings were planted at the Centre of Excellence in Biofuels, Tamil Nadu Agricultural University, Coimbatore, TN, India during December 2007. All the recommended agronomic packages of practices were adopted during the entire crop period and the observations on various morphological characters were recorded. Leaf samples were collected from all the accessions to study the molecular diversity at the DNA level.

Nineteen different quantitative and qualitative data were recorded as per the NBPGR minimal descriptors on five randomly selected competitive plants in each of the accessions at various phenophases. The quantitative characters like plant height, number of branches, average branch length, stem diameter, leaf petiole length, internode length, leaf length, leaf breadth, number of leaf lobes and qualitative characters like stem color, leaf petiole color, leaf nerve color, leaf shape, flower color, plant growth habit, stem shape, seed coat color, leaf size, and fruit type were recorded in five plants per accession per replication and the mean values were utilized for statistical analysis. The genetic diversity among the accessions was assessed using NTSYS-pc 2.02i version.

Table 1. List of the jatropha accessions used for the diversity analysis.

S No	Scientific name	Place of collection	State	Country
1	*J. curcas* (CJC 18)	Coimbatore	Tamil Nadu	India
2	*J. curcas* (CJC 20)	Coimbatore	Tamil Nadu	India
3	*J. curcas* (CJC 22)	Coimbatore	Tamil Nadu	India
4	*J. curcas* (CJC21)	Coimbatore	Tamil Nadu	India
5	*J. curcas* (CJC 25)	Coimbatore	Tamil Nadu	India
6	*J. integerrima*	Hyderabad	Andhra Pradesh	India
7	*J. ramanadensis*	Ramanathapuram	Tamil Nadu	India
8	*J. villosa*	Ramanathapuram	Tamil Nadu	India
9	*J. glandulifera*	Sivagangai	Tamil Nadu	India
10	*J. gossypiifolia*	Mettupalayam	Tamil Nadu	India
11	*J. podagrica*	Coimbatore	Tamil Nadu	India
12	*J. tanjorensis*	Trichy	Tamil Nadu	India

Genomic DNA was extracted from freshly harvested leaves of each Jatropha species by adopting the procedure outlined by Dellaporta et al. (1983).

ISSR primer screening

Twenty-one ISSR primers from first base (Singapore) were initially screened for their repeatable amplification with five accessions. Primers were selected for further analysis based on their ability to detect distinct polymorphic amplified products across the accessions. To ensure reproducibility, the primers generating weak products were discarded.

PCR amplification

PCR amplification was performed in a total volume of 15 µl containing 1.50 µl of 10X assay buffer, 1.20 µl of 2.5 mM dNTPs, 0.20 µl 0.3 units/µL of Taq polymerase, 2.00 µl of 2.5 mM UBC Primer, 2.00 µl of 40 ng/µl DNA (40 ng/µl). After a denaturation step for 5 min at 94 °C, the amplification reactions were carried out for 40 cycles. Each cycle comprised of 1 min at 94 °C, 2 min at 55 °C, and 2 min at 72 °C. The final elongation step was extended to 5 min. Amplified products were separated on 1.5% agarose gels in TBE buffer and stained with ethidium bromide and photographed under UV light.

Statistical analysis

ISSR markers across the 12 accessions were scored for their presence '1' or absence '0' of bands for each primer. By comparing the banding patterns of genotypes for a specific primer, genotype-specific bands were identified and faint or unclear bands were not considered. The binary data so generated were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. 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. Pairwise similarity matrices were generated by Jaccard's coefficient of similarity (Jaccard 1908) by using the SIMQUAL format of

NTSYS-pc (Rohlf 2002). A dendrogram was constructed by using the unweighted pair group method with arithmetic average (UPGMA) with the SAHN module of NTSYS-pc to show a phenetic representation of genetic relationships as revealed by the similarity coefficient (Sneath and Sokal 1973). The binary data was also subjected to principal component analysis (PCA) using the EIGEN and PROJ modules of NTSYS Pc.

Results and Discussions

The 12 Jatropha accessions showed a wide range of morphological variability. The maximum variability was found in the plant height which was followed by the average branch length.

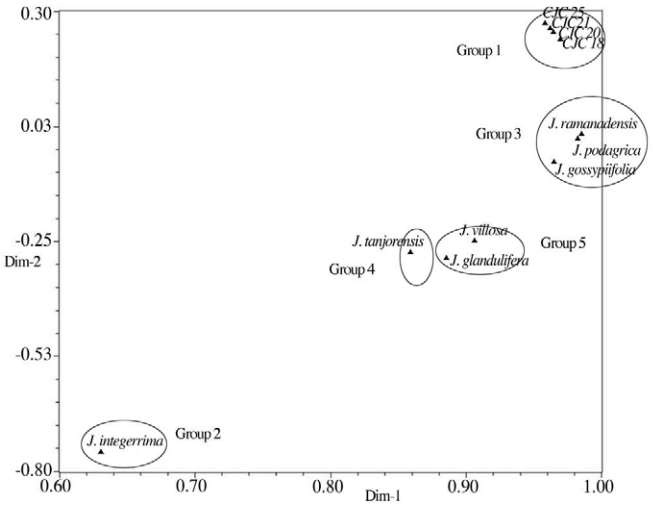

Fig. 1. Genetic relationships of 12 Jatropha accessions based on principal component analysis for morphological data.

Correlation coefficients were worked out between nine quantitative characters. The high positive and significant correlation value were obtained for plant height and number of branches (0.874). From these results it is evident that these traits are associated with yield and are inter-correlated among them. It indicates that the selection in any one of these yield attributing traits will lead to increase in the other traits, there by finally enhancing the yield.

On the basis of factor loadings of the 19 morphological traits that are contributing maximum variability to the first three factors are selected for principal component analysis. The first three factors contributed to 84.8% of the total variance observed. The first factor had high contributing factor loadings from stem diameter, leaf petiole length, leaf length, leaf breadth, plant growth habit, and stem shape and contributed 35.7% of the total variation. The second factor had high contributing loadings from leaf petiole length, number of leaf lobes, and seed coat color and contributed to 28.2% of the total variation. The third factor had high contributing loadings from average branch length, internode length, number of leaf lobes, and flower color, and contributed to 20.9% of total variation. The first three principal components in the collection with eigen values were able to explain 89.2% of total variation for morphological traits. The variance accumulated by the last components of the base collection accounted for a small amount. According to Mardia et al. (1979), the total variance accumulated by principal component close to 80% explains satisfactorily the variability manifested between individuals. It is concluded that leaf petiole length, leaf length, leaf breadth, branch length, stem shape, and seed coat color could be used as characters to distinguish the germplasm entries.

Morphological Diversity

The clustering of Jatropha accessions based on the variations across morphological traits indicated that five different clusters, with the cluster size variation from 1 to 5. (Fig. 1) The maximum number of accessions was included in cluster I having 5 accessions and the minimum number in clusters II, IV, and V having 1 accession. The cluster I consisted of *J. curcas* (*CJC 18*), *J. curcas* (*CJC 20*), *J. curcas* (*CJC 22*), *J. curcas (CJC21*), *and J. curcas* (*CJC 25*). The cluster II consisted of *J. integerrima*. The cluster III consisted of *J. ramanadensis*, *J. gossypiifolia*, and *J. podagrica*. The cluster IV consisted of consisted of *J. tanjorensis*. The cluster V consisted of *J. villosa* and *J. glandulifera*.

ISSR marker diversity

A total of 157 markers were produced out of which 156 were polymorphic. The polymorphism percentage was 99.31. The number of markers produced by different primers ranged from 5 to 12 with an average 7.47 markers per primer. The maximum number of amplified product (12) was observed in the profiles of the primer UBC 884. The minimum number of amplified product (5) was observed in the profiles of primer UBC 807, UBC 843, UBC 867, and UBC 896. ISSR profiles of the representative primer UBC 841 and UBC 826 are shown in Figs. 2 and 3.

The Jaccard's similarity coefficient for the ISSR data set varied from 0.10 to 0.73. Recently Basha and Sujatha (2007) had reported low levels of molecular diversity among Indian accessions of *J. curcas* germplasm indicating a narrow genetic base, the level of polymorphism produced by 400 RAPD and 100 ISSR primers was very low. This was proven by the present ISSR study, in which the cultivars of *J. curcas* were found to be in a single cluster. Although they were found to be in a single cluster, a gene specific allele was found in *J. curcas* (*CJC 21*) with the UBC 826 primer at 700 bp allele. The ISSR marker profiles resulted in six clusters. The cluster I was highly heterogeneous. The cluster I consisted of *J. curcas* (*CJC 18*), *J. curcas* (*CJC 20*), *J. curcas* (*CJC 22*), *J. curcas* (*CJC 21*), *and J. curcas* (*CJC 25*). The cluster II consisted of *J. tanjorensis*. The cluster III consisted of *J. gossypiifolia*. The cluster IV consisted of *J. glandulifera*. The cluster V consisted of *J. podagrica*. The cluster VI consisted of accessions *J. ramanadensis*. The cluster VII consisted of accessions *J. villosa*. The cluster VIII consisted of accession *J. integerrima* (Fig. 4).

On morphological analysis *J. ramanadensis*, *J. gossypiifolia*, and *J. podagrica* formed a single cluster. Similarly *J. villosa* and

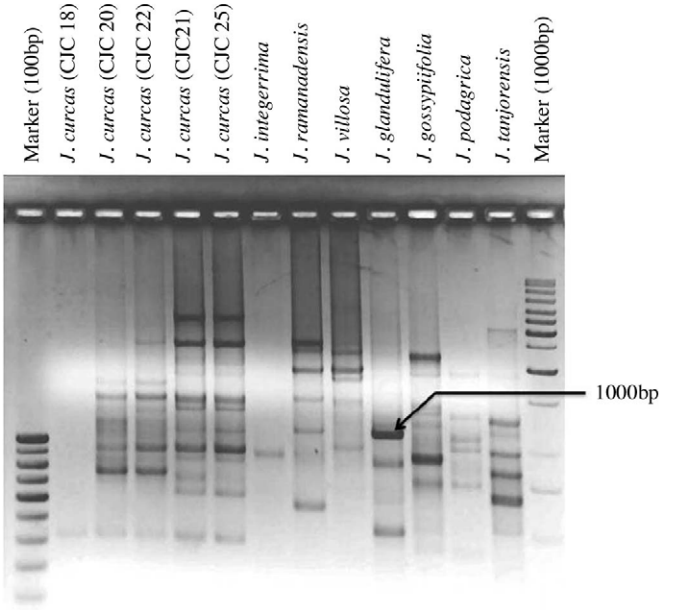

Fig. 2. ISSR profile of the primer UBC 841

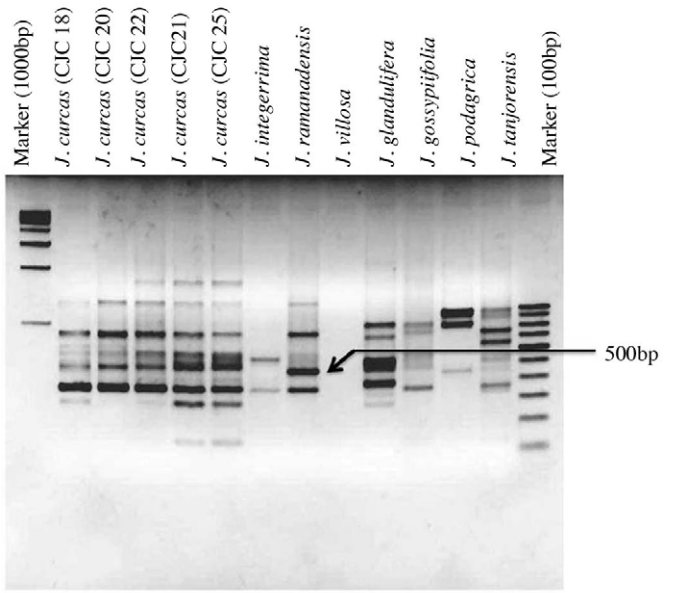

Fig. 3. ISSR profile of the primer UBC 826

J. glandulifera formed one cluster. This is because of similar phenotypic traits among them. But ISSR analysis differentiated all of them into different clusters indicating their diversity at the molecular level.

Though the marker related studies for *J. curcas* have been reported using ISSR (Basha and Sujatha 2007), RAPD and AFLP (Sudheer Pamidimarri 2008), AFLP (Tatikonda et al 2009), Biochemical, RAPD, ISSR and SSR (Basha et al. 2009), all of these studies reported low levels of molecular diversity among accessions of *J. curcas* germplasm indicating a narrow genetic base. And also, all of these studies are focused to characterize the toxic and non-toxic varieties of *J. curcas* accessions at the molecular level. But in the present study, ISSR markers have

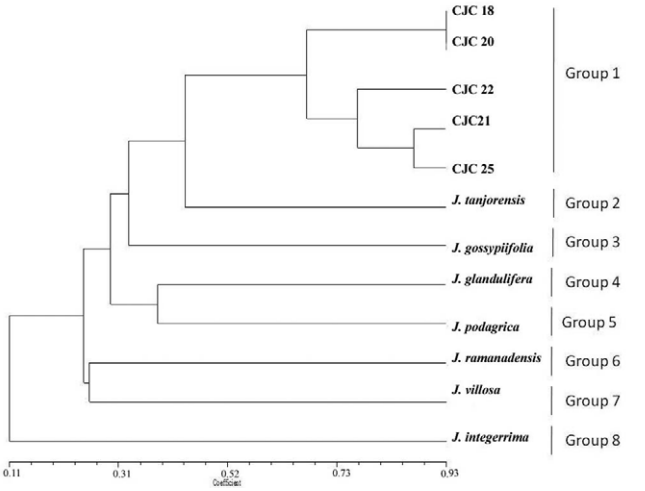

Fig. 4. Dendrogram of 12 Jatropha accessions based on Jaccard's similarity coefficient for ISSR data.

been used to group eight Jatropha accessions at intra- and inter-specific levels which provides valid guidelines for collection, conservation, and characterization of Jatropha genetic resources. The polymorphisms detected with ISSR primers in the present study (99.31) across eight species were considerably higher than the polymorphism detected by ISSR primers by Basha et al. 2009 (35.5%) and all other previous studies, done by using molecular markers (AFLP, RAPD, SSR). Hence, it is inevitable to exploit the wild relatives to broaden the genetic base as *J. curcas* is readily crossable with most of the species when used as female parent (Dehgan 1984).

Previously, RAPD analysis (Ganesh Ram et al. 2007) failed to differentiate the diversity among Jatropha species namely J. ramanadensis, *J. tanjorensis*, *J. podagrica*, *J. integerrima*, *J. villosa*, *and J. gossypiifolia*. But the present ISSR analysis differentiated the Jatropha species into different clusters with gene-specific allele for each species, indicating the advantage of the ISSR marker over the RAPD marker. And, also in ISSR analysis (Senthil et al. 2009) only molecular analysis was taken into account. The combination of morphological and molecular genetic analysis is more reliable and consistent.

Species-specific diagnostic markers

The primers UBC 841 (500 bp *J. ramanadensis*, 700 bp *J. tanjorensis*), UBC 812 (400 bp *J. glandulifera*, 700 bp *J. curcas*), UBC 840 (200 bp *J. podagrica*), and UBC 885 (1000 bp *J. ramanadensis*, 700 bp *J. podagrica*), UBC 826 (1000 bp *J. glandulifera*,700 bp *J. curcas*), UBC 840(200 bp *J. glandulifera*) detected species-specific diagnostic markers suitable for discriminating species of Jatropha. These species-specific ISSR markers could potentially be used for identifying a Jatropha species from any mixed population comprising other members of the Jatropha complex. These species specific ISSR markers will be the potential target for the development of new SCAR makers which will be useful for the large-scale screening of the Jatropha accessions.

The artificial hybridization between *J. curcas* and *J. gossypiifolia* showed a very high degree of incompatibility due to post

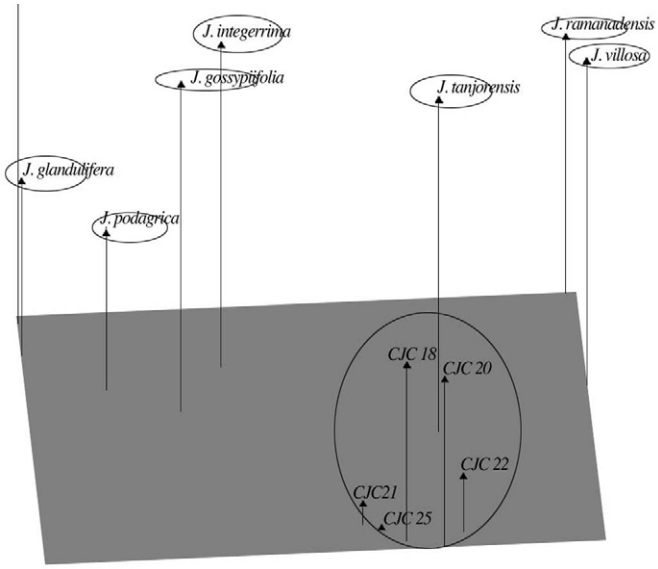

Fig. 5. Three-dimensional plot of Jatropha accessions by principal coordinate analysis using the Jaccard's similarity coefficients ISSR markers

fertilization barriers (Sujatha 1997). Sujatha and Prabakaran (1998) indicated that *J. tanjorensis* is an inter-specific cross of *J. curcas* and *J. gossypiifolia*. In the present study, the morphological data based on dendogram reveals that *J. tanjorensis* was found on a unique cluster, because of its sterile nature. Similarly the ISSR data of *J. tanjorensis* also supported the above facts. Hence, the present ISSR profile supports the facts indicated by Sujatha and Prabakaran (1998) about the origin of *J. tanjorensis*. The genotypes such as *J. villosa and J. glandulifera* that could not be distinguished by morphological data are differentiated by ISSR markers, with species-specific markers. The polymorphism observed in ISSR markers among the Jatropha accessions in the present study demonstrated the effectiveness of this method in determining genetic variation. The ISSR markers used in the study were found to be highly informative for revealing the genetic diversity among the genotypes studied, thus suggesting their potentiality in future genetic diversity analysis and also in identifying biofuel energy-efficient genotypes. Availability of unique or rare fragments present in different accessions (which are indicated in species specific diagnostic markers) together with genetic dissimilarly data would be very useful for improvement of the species through conventional breeding methodologies as well as molecular breeding approaches such as marker-assisted selection (MAS).

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