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Genetic diversity assessment of *Jatropha curcas* L. germplasm from Northeast India

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ABSTRACT

Genetic variability in the wild genotypes of *Jatropha curcas* L., collected from different parts of Northeast India, was analyzed using two different single primer amplification reactions (SPAR) methods, *viz.*, inter-simple sequence repeats (ISSR) and directed amplification of minisatellite DNA (DAMD). A total of 36 genotypes were used to investigate the existing natural genetic variation at intra-specific level. One hundred forty nine (149) amplification products were scored by ISSR and DAMD, both of which collectively showed 75.83% polymorphism with a mean intra-population genetic diversity (H_S) of 0.1309. However, their level of diversity at inter- and intra-population levels was significant, with the percentage of polymorphic loci (P) ranging from 22.82% to 44.30%, Shannon's information index (H_{pop}) from 0.1302 to 0.2541 and Nei's gene diversity (H_E) from 0.0831 to 0.1723 with mean Nei's gene diversity (H_T) 0.2202 and the overall estimate of gene flow being (N*m*) 0.8085. Analysis of molecular variance (AMOVA) showed that 68.88% of variation at intra-population level, whereas 31.12% variation was recorded at inter-population level. Cluster analysis also supported the existence of genetic diversity in the genotypes of *J. curcas* collected from Assam and Meghalaya provinces of Northeast India. Present investigation suggests the efficiency of SPAR methods to estimate the genetic diversity precisely which can define genetic relationship and population genetics of *J. curcas*.

1. Introduction

Jatropha curcas L. (Euphorbiaceae) commonly known as '*physic nut*' is a multipurpose tree of Latin American origin, distributed throughout the tropical and subtropical regions of the World including India [1]. *J. curcas* has been found to be highly promising species in terms of oil yield, which can be used as a source of energy in the form of biodiesel [2]. It is a highly medicinal, economically valuable and well-known as energy crop throughout the World [1–5]. It is used for preparation of the ayurvedic drug ''*dravanti*'' [3] and is a potential source of lubricants, soaps, candles and coloring dye besides being useful as purgative, emetic, abortifacients and astringents [4,5]. The economic importance of *J. curcas* generates a wide interest among researchers to evaluate the existing natural genetic diversity, for selection and breeding of superior genotypes both at inter- and intra-specific levels. Analysis of genetic diversity using molecular markers is a trusted and reliable approach which could provide useful baseline information for breeding programs of various wild as well as cultivated crops [6]. Among the various molecular markers employed to assess diversity studies, PCR-based markers such

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as RAPD (Random Amplified Polymorphic DNA) [7,8], ISSR (Inter-simple Sequence Repeats) [9], DAMD (Directed Amplification of Minisatellite DNA) [10] and AFLPs (Amplified Fragment Length Polymorphism) [11] are popular, as their application does not need any prior sequence information. On the other hand, microsatellite or simple sequence repeats (SSR), the markers for breeding applications, are quite expensive and time consuming [12]. Although various approaches have been carried out by researchers to assess genetic variation in populations of *J. curcas* [13–18], but not much work has been carried out from the populations of Northeast India. Ranade et al. [15] in their study have indicated that two accessions of Northeast India are genomically different as compared to those occurring in other parts of India. Besides this, the seed oil content of various *J. curcas* genotype was reported to exhibit variation in the range of 25.63–42.46% which is quite significant and reflect the underlying genetic variability (unpublished data). Therefore, the present study was taken up to assess and analyze the natural genetic diversity existing among *J. curcas* genotypes at intra-specific levels representing Assam and Meghalaya.

2. Materials and methods

2.1. Study sites and sample collection

Six populations inhabiting different regions *viz.*, Ri-Bhoi (R), West Garo Hills (WG), and South Garo Hills (SG) districts from Meghalaya, and Kamrup-Metro (KM), Nagaon (NG), and Sonitpur (SP) districts from Assam (Table 1; Fig. 1; Table S1) provinces of Northeast India were selected. Six individuals per population (36 individuals) were randomly selected for present study. Leaf samples from these plants were collected and immediately frozen in liquid nitrogen until DNA extraction was performed.

2.2. DNA isolation and PCR amplification

Total genomic DNA was extracted from young leaves following the standard CTAB method with few minor modifications [19]. Genomic DNA was quantified against a known quantity of unrestricted Lambda (λ) DNA by electrophoresis using 0.8% agarose gel.

2.3. PCR optimization and primer survey

Amplifications with ISSR and DAMD primers containing varying concentrations of (i) template DNA (20–60 ng), (ii) Taq DNA polymerase (0.5–2 U), and (iii) Mg^{++} salt (0–5 mM) were used to optimize reaction conditions of the Polymerase Chain Reaction (PCR). Of the five different concentrations of template DNA, 50 ng was found to be the most ideal as it yielded maximum number of reproducible bands. In addition, 1.5 mM of $MgCl_2$, and 0.6 U Taq DNA polymerase have given ideal results among 90 various concentrations tested. Thirty ISSR (Operon Technologies, USA) and 8 DAMD (Bangalore Genei, India) primers were assayed to identify primers which yielded reproducible and polymorphic patterns.

2.4. ISSR and DAMD amplification reactions

All the PCR reactions were carried out in 25 μl volumes containing 50 ng of template DNA, 200 μM of each of the four dNTPs, 1X PCR buffer (10 mM Tris pH 9.0, 50 mM KCl), 1.5 mM $MgCl_2$, 0.6 U Taq DNA polymerase (Bangalore Genei, India) and 10 pmol of primer (in case of ISSR) and 20 pmol (in case of DAMD). The reaction programmes were set at 94 °C for 4 min followed by 40 cycles of 92 °C for 30 s, 1 min at annealing temperature (42–58 °C depending on the primer's Tm and/or according to GC contents), 2 min elongation at 72 °C and a final extension at 72 °C for 7 min in a thermal cycler 2720 (Applied Biosystems, USA). For DAMD amplification, reactions were carried out at 94 °C for 2 min followed by 40 cycles of 92 °C for 1 min, 2 min at 55 °C, 2 min elongation at 72 °C and a final extension at 72 °C for 5 min [20]. After completion of the amplification, 2.5 μl 10X blue dye was added to the samples, and the amplified DNA was analyzed on 2% agarose gel in 1X TAE buffer at 65–70 V for 4–5 h.

2.5. Scoring and data analysis

Only clear and non-ambiguous amplicons were scored across all samples. These fragments were scored independently as either present (1) or absent (0) in each population and a binary data matrix was constructed. Molecular weights of the bands were estimated by using 0.5 kb and 0.1 kb DNA ladders (Bangalore Genei, India) as standards for DAMD and ISSR respectively. The ISSR and DAMD-PCR fragments were analyzed as alleles, under the following assumptions. Firstly,

Table 1 – Details of populations of *J. curcas* sampled.

Assigned name of Population	Area of plant collection	No. of plants collected per population	Collection name	Latitude (N)	Longitude (E)	Altitude (M)
Meghalaya						
Pop1	Ri-Bhoi	6	SK-RB1 to SK-RB6	25°51′41.82″	91°52′49.74″	537.0
Pop2	South Garo Hills	6	SK-SG1 to SK-SG6	25°12′20.22″'	90°18′44.88″	82.5
Pop3	West Garo Hills	6	SK-WG1 to SK-G6	25°26′37.62″	90°12′37.56″	277.9
Assam						
Pop4	Kamrup-Metro	6	SK-KM1 to SK-KM6	26°6′16.56″	92°0′12.48″	60.1
Pop5	Nagaon	6	SK-NG1 to SK-NG6	26°25′31.80″	92°50′57.42″	62.3
Pop6	Sonitpur	6	SK-ST1 to SK-ST6	26°39′37.86″	92°49′47.94″	59.3