Molecular characterization of worldwide accessions of *Jatropha curcas* L.

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Abstract

Jatropha curcas L. (2n=2x=22) is a multipurpose perennial small tree or large shrub belongs to *Euphorbiaceae* family. It is native in the Central America and is distributed in many tropical and subtropical regions of America, Africa and Asia. Jatropha is cited as one of the best candidates for future biodiesel production, but it is still considered a wild species and at very limited information is available on the genetic variability in this species. The objectives of this study are to collect diverse genetic materials of Jatropha and assess the genetic variability using molecular markers. Seven Jatropha populations were collected from Cuba, Cape Verde, Brazil, Senegal (from three different areas) and Mozambique. Jatropha specific Simple Sequence Repeat (SSR) (32) and two primer pairs for specific sequences were collected from public databases and were assayed on 64 genotypes. Several primers were found highly conserved and monomorphic throughout all the genotypes, but polymorphic markers which can differentiate populations were also recorded. Some monomorphic fragments were sequenced and one SNP was found on Cape Verde and Cuba accessions. This mutation has been also examined with the High Resolution Melting (HRM) analysis and the graphic of melting temperature differences is shown. Genetic similarity among and within populations will be calculated by the procedure SIMQUAL using the DICE similarity coefficient. Detailed clustering analysis based on microsatellite markers variation is also presented and discussed.

Introduction

Jatropha (*Jatropha curcas* L.) is a perennial shrub to small evergreen tree of up to 6 m tall and belongs to the family Euphorbiaceae (Fig. 1). It is a native of tropical America which was subsequently introduced into Africa and Asia and is now cultivated worldwide. Jatropha has vital importance as a potential biodiesel crop in more than 50 countries. Jatropha seeds contain 46–58% of oil on kernel weight and 30–40% on seed weight.

Figure 1. Plants and female and male flowers of Jatropha

A high degree of genetic variability is expected within and among populations. Selection of superior genotypes is an important step in breeding outcrossing species. Exploration of genetic variability in Jatropha is very limited. Thus the objective of our study is to assess the genetic variability in Jatropha populations collected from different geographic locations using molecular markers.

Materials and Methods

Plant materials. Seven Jatropha populations were collected from different worldwide areas (Fig. 2). Each population was represented by 8-16 genotypes. All genotypes were used for genetic similarity analysis (Table 1). Only 2-4 genotypes *per* population were used for sequence analysis. Ten genotypes belonging to 6 populations (population S3 was excluded from the analysis) were used for HRM analysis

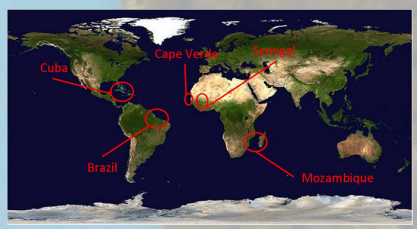

Figure 2. Locations where Jatropha populations were collected for this study

Name	Origin	Genotypes	Source
BRA	Brazil	8	Prof. F. Cotana*
CB	Cuba	8	Prof. F. Cotana*
CV	Cape Verde	8	Prof. F. Cotana*
S1	Senegal	8	Prof. F. Cotana*
S2	Senegal	8	Prof. F. Cotana*
S3	Senegal	8	Dr. P. Versiglioni†
M1	Mozambique	16	Dr. O. Davolio Marani¶ Prof. A. Zazzerini*

*University of Perugia, Italy, †Senegal, ¶Mozambique.

Table 1. Jatropha populations, their origin and source of collection

Genotyping. DNA was extracted from young leaves using Genelute Plant Genomic DNA kit (Sigma-Aldrich). A set of 34 Jatropha SSR primer pairs was chosen based on published reports and used for genotype analysis. PCR products were resolved in ABI 3730 Sequencer. Scoring was made using GeneMapper (V 3.7).

Sequencing. PCR products from 10 selected primer pairs from 16 genotypes were purified with Quiaquick PCR Purification Kit and sequenced. Sequence data were then analyzed with DNASTAR software.

Diversity analysis. Similarity matrices for the genotypes were calculated using NTSYS-PC 2.10 (Applied Biostatistics). The genetic similarity among genotypes was calculated by the SIMQUAL procedure using the DICE similarity coefficient. For clustering, the SAHN module was used.

HRM. The LightScanner PCR protocol was used to amplify the fragments and the data were analyzed by the LightScanner software program (version 2.0) following the small amplicon genotyping parameters.

Results

Genetic variability. A total of 62 SSR bands from 34 primer pairs were used for evaluation of genetic diversity among the seven Jatropha populations. Populations from Cuba (CB) and Cape Verde (CV) were clustered together distinct from other populations (Fig. 4). Populations from South America and Africa were genetically similar.

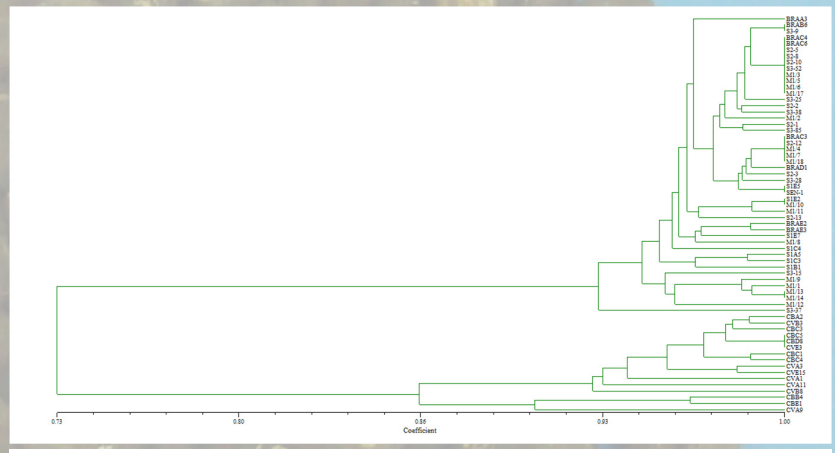

Figure 4. Dendogram showing the genetic diversity in Jatropha accessions assessed by microsatellite markers. Similarity coefficients obtained through DICE module of the NTSYS analysis using marker data obtained from the Jatropha-specific SSR markers.

Sequence analysis. Products from 10 monomorphic microsatellite loci were sequenced to verify any variability. Fot 3 of these, no good quality sequences were obtained. Six of the remaining amplicons generated the same sequences in all 16 genotypes belonging to 7 Jatropha populations. Only in locus Jcps9 a SNP variation between CV - CB and other populations was identified (Fig. 5).

HRM. The difference in melting temperature for Jcps9 amplicons allowed to separate genotypes into two distinct groups. The grey curves are of CV-CB group and the red curves of the other populations (Fig. 6A-B). The results were double tested (10 genotypes -20 curves).

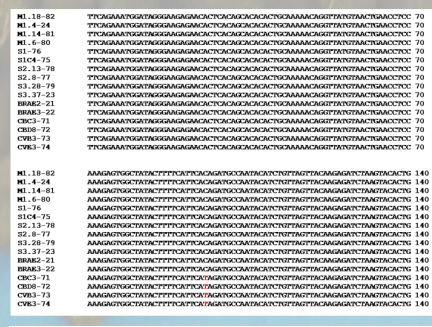

Figure 5. Sequences for locus Jcps9 in 16 Jatropha genotypes.SNP variation marked in red font.

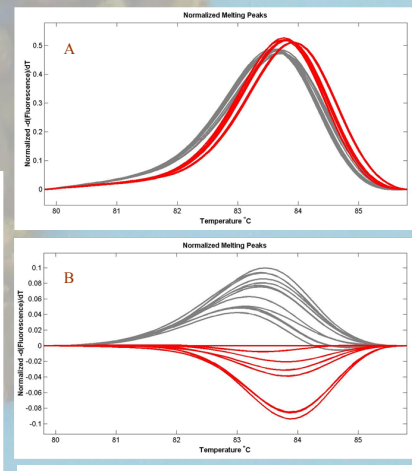

Figure 6 A. Normalized melting curves of jatropha genotypes and B. Grouping of the melting curves using BRAC6 as x-axis.

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