Behavior of genetic diversity in F1 crosses of selected accessions of *J. curcas*

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ABSTRACT

Distinguishing accessions by their similarity is important for genetic improvement, given their usefulness for increasing and preserving the genetic diversity of crops. Some studies have assessed the similarity between accessions of *Jatropha curcas* from different regions or origins, but it is not yet clear how the similarity between accessions behaves in crosses, when different accessions are selected as maternal or paternal parents. Thus, this work used ISSR molecular markers to evaluate accessions resulting from the crossing of six parents of different origins. Of the 15 crosses studied, 7 (46%) had greater similarity with their paternal parent, 2 (13%) had greater similarity with the maternal parent and 6 (41%) showed no close similarity to any parent. These results suggest that the similarity between accessions derived from crossings is similar to that found in species such as *Gossypium hirsutum*, and several cycles of retro-crossing would be needed to maintain the characteristics of a maternal parent.

1. Introduction

Jatropha curcas is a perennial shrub that belongs to the Euphorbiaceae family, it can be vegetative propagated and sexually by cross or selfcrosing, its genome size and characterization were previous reported in 416 Mb with a karyotype 2n = 22, and so other phenotypic descriptions were documented (Avendaño-Arrazate and Zamarripa-Colmenero, 2012; Carvalho et al., 2008; Orwa et al., 2009; Sunil et al., 2013); it has become important for the quality of the oil extracted from its seeds, which can be used in the manufacture of biofuels (Patil and Deng, 2009). However, this plant has not been domesticated yet, and only a few studies have focused on the genetic improvement of the species (Montes and Melchinger, 2016). These studies have focused on exploring the genetic diversity of different accessions distributed throughout the world and on selecting those with better agronomic characteristics (Santos et al., 2016; Silva et al., 2015; Yang et al., 2010). However, the use of these accessions in commercial plantations has resulted in phytosanitary problems and low productivity (Valdés et al., 2014). These problems have been addressed by crossing the accessions identified as the most productive with the accessions that are best adapted to the conditions prevailing in the places where the crops are grown. This has given rise to a new generation of F1 crosses that combine the productivity of a parent with the adaptation of another (Montes and Melchinger, 2016). One way to achieve this, is to increase the genetic diversity of the individuals used to produce F1 crosses by choosing genetically distant parents (Washio et al., 1968), which

increases the heterogeneity of the resulting lines. Montes and Melchinger (2016), mention that the use of molecular markers and genetic distances to obtain this type of data for accessions can accelerate the genetic improvement of *J. curcas*. However, before doing this, it is necessary to understand the relationship between the similarity of accessions and the product of their crosses. This work analyzed the behavior of ISSR molecular markers in F1 crosses of selected accessions of *J. curcas*, in order to understand the behavior of molecular markers in crossing systems of selected accessions for purposes of genetic improvement.

2. Materials and methods

2.1. Plant material

Seeds were collected from different regions of the species origin center, and growth in experimental fields, where they were characterized by its productivity at the second year of stablished, from where six parents were chosen to follow a second phase breeding program. Five seeds of each cross where grown under same conditions as parents and lines were characterized by its productivity at the second year of stablished (Data not shown), after that, one line per cross candidate for variety materials where chosen for this work, they were vegetative propagated within their parents and three accessions that were not used in the second phase as parents in the breeding program as controls to define a better separation of crosses from parents. In total twenty-four

Table 1
Plant material used. Type of accessions and origin.

Accession	Type of accession	Origin[a]
JatroP1	Parental	Nicaragua
JatroP2	Parental	Nicaragua
JatroP3	Parental	Chiapas
JatroP4	Parental	Chiapas
JatroP5	Parental	Veracruz
JatroP6	Parental	Chiapas
JatroT1	Control	Veracruz
JatroT2	Control	Chiapas
JatroT3	Control	Yucatan
JatroC1-1Fx2P	Cross	Crossing JatroP1xJatroP2
JatroC2-5Fx2P	Cross	Crossing JatroP5xJatroP2
JatroC3-2Fx4P	Cross	Crossing JatroP2xJatroP4
JatroC4-1Fx4P	Cross	Crossing JatroP1xJatroP4
JatroC5-1Fx5P	Cross	Crossing JatroP1xJatroP5
JatroC6-2Fx6P	Cross	Crossing JatroP2xJatroP6
JatroC7-1Fx6P	Cross	Crossing JatroP1xJatroP6
JatroC8-2Fx3P	Cross	Crossing JatroP2xJatroP3
JatroC9-1F-3P	Cross	Crossing JatroP1xJatroP3
JatroC10-4F-5P	Cross	Crossing JatroP4xJatroP5
JatroC11-4Fx6P	Cross	Crossing JatroP4xJatroP6
JatroC12-4F-3P	Cross	Crossing JatroP4xJatroP3
JatroC13-5Fx6P	Cross	Crossing JatroP5xJatroP6
JatroC14-5Fx3P	Cross	Crossing JatroP5xJatroP3
JatroC15-6Fx3P	Cross	Crossing JatroP6xJatroP3

[a] First parental of crosses correspond to flower receptor of parental on crosses origin.

accessions of *J. curcas*, that did not show visually differences phenotypically were evaluated; three of them were used as controls and six as parents. Parental accessions were selectively crossed, classifying them as flower receptor (First parent of crosses in Table 1) or pollen donors (Second parent of crosses in Table 1). From them, 15 F1 crosses were obtained (Table 1).

2.2. DNA extraction

Leaf samples were taken from the experimental field, and a sample of leaf tissue (0.5 g) were frozen in nitrogen at the moment of sampling the leaves and maintained at −80 °C until its utilization. Genomic DNA extraction was performed following the CTAB protocol described by Sunil et al. (2011), with modifications. The lysis solution consisted of 100 mM of Tris (pH 8), 20 mM of EDTA, 1.6 M of NaCl, 2% CTAB, 2.5% PVPP and 0.5% ß-mercaptoethanol. The solution was preheated to 60 °C for 30 min, and then the leaf material was macerated in the lysis solution (0.1 g of leaf per ml of lysis solution). The macerated material was transferred to a new microcentrifuge tube and incubated for 30 min at 60 °C with continuous inversions, then allowed to cool at room temperature. Afterwards, 500 μl of chloroform-alcohol isoamyl were added and mixed by inversion until the mixture was emulsified. It was then centrifuged at 8000*g* for 15 min and the upper phase was separated and transferred into a new tube. One hundred and thirty microliters of precipitate solution from the GeneElute Plant Genomic DNA mini prep kit (SIGMA-ALDRICH®) were then added to the mixture, which was transferred to the kit's filtration column and centrifuged; afterwards, the column was discarded. After adding 700 μl of the kit's bonding solution, the mixture was transferred to the DNA retention column, where it was centrifuged, and the filtrate was discarded. Finally, two washes were performed with the kit's washing solution and the DNA was eluted in 100 μl of deionized water. The extracted DNA was quantified by spectroscopy and its quality was checked by measuring the A260/A280 absorbance, looking for an absorbance ratio between 1.8 and 2.0.

2.3. Amplification of molecular markers

The molecular markers used were of the ISSR type; the primers were

previously reported by Gupta et al. (2008), who used a 20 ng sample of extracted DNA to amplify primers by PCR.

The PCR assay was performed using the following program: 5 min at 95 °C for the DNA denaturation step, 40 cycles of 1 min at 95 °C, 1 min at 45 °C and 2 min at 72 °C for the ISSR fragment amplification step, with a final elongation step for 10 min at 72 °C.

2.4. Identification of molecular markers

PCR products were separated by electrophoresis on 1% agarose gel using 1X TAE buffer (Tris-Acetic Acid-EDTA) under conditions of 0.4 V per cm^2 of agarose gel in a Thermo-Fisher Agarose gel box Owl A5, stained with ethidium bromide at a concentration of 0.05 μg/ml and running for 90 min. The amplified pattern was photodocumented using UV light and digitized.

Afterwards, the digital image with the banding pattern was analyzed with the software GelAnalyzer 2010a to characterize the bands amplified for each primer based on the molecular mass marker (1Kb plus DNA ladder; Invitrogen®). Each band was considered as a position and counted as an individual and separate character.

The number of polymorphic characters in each primer was identified, and the polymorphic information content of the oligo (PIC) and its resolving power (RP) were analyzed. The oligos that had a minimum PIC value of 0.3 or a RP value of 7 were preserved for analysis (Botstein et al., 1980; Prevost and Wilkinson, 1999). The Shannon-Weaver diversity index was calculated for the total number of characters obtained from the primers to assess a diversity index of the accessions evaluated, in a range from 0 to 4.64 (Hennink and Zeven, 1990).

2.5. Analysis of molecular markers in the crossing system

A binary matrix was generated with the frequency of the genetic characters obtained. This matrix was used to calculate the similarity between accessions using the Dice index (Dice, 1945). Afterwards, the accessions were grouped by the "Unweighted Pair Group Method with Arithmetic Mean" (UPGMA), using the PAST v.3.17 software (Hammer et al., 2001). The same binary matrix was used to perform a main component analysis (PCA) on the genetic characters of the accessions, using PAST v.3.17 software (Hammer et al., 2001).

3. Results and discussion

3.1. Amplification of molecular markers

The first step was to identify a type of molecular marker that complied with UPOV guidelines for molecular markers (UPOV, 2011). Various types of molecular markers have been reported for *J. curcas* (RFLP, SNP, ISSR etc.) but not all satisfy UPOV criteria, especially because of their lack of reproducibility and repeatability. Of the different available markers, ISSR markers have the best reproducibility and repeatability rates; because of that, other studies have proposed them as tools for the exploration of genetic diversity and to discriminate between accessions and hybrids (Gupta et al., 2008; Izzatullayeva et al., 2014; Karanja et al., 2009; Soonthornyatara et al., 2015; Tonk et al., 2014).

The present study evaluated 25 primers previously reported by Gupta et al. (2008); of these, six primers had adequate reproducibility and repeatability rates (Table 2). The other primers were not consistent in character amplifications, since each primer were evaluated three times from sample collection. Some primers do not amplify characters, others do not amplify polymorphic characters, that was the case of ISSR8 that amplify 2 bands and ISSR 12 that amplify 5 bands, so others amplify characters that were not reproducible between each repetition (Data not shown), this primer were discarded for further evaluations due to null information provided.