Crop Breeding and Applied Biotechnology 10: 364-369, 2010
Brazilian Society of Plant Breeding. Printed in Brazil

NOTE

Genetic similarity of *Jatropha curcas* accessions based on AFLP markers

Carlos Antonio Fernandes Santos[1]*, Marcos Antonio Drumond[1], Marciene Amorim Rodrigues[1] and Marcio Rannieri Viana Evangelista[1]

Received 11 February 2010

Accepted 16 July 2010

ABSTRACT - *The genetic relationships between accessions of Jatropha* (Jatropha curcas) *were determined based on AFLP marker. A set of 50 plants from 12 accessions of J. curcas was analyzed with molecular data from 164 loci generated from 17 AFLP primer combinations. Molecular variance of data was analyzed by total decomposition between and within accessions. An UPGMA dendrogram was constructed based on genetic distances estimated by Jaccard's similarity coefficient. The well-defined dendrogram showed a cophenetic value of 0.91. Groups of plants were observed in six of the 12 accessions studied with similarity of over 30 %, indicating high genetic variability. The variation among accessions was estimated to be 0.275, also indicating high variability. These results show that the genetic variability of the studied J. curcas accessions is structured according to the origin and that a greater number of populations should be sampled to increase the genetic diversity of the studied genebank.*

Key words*: Physic nut, dendrogram, AMOVA.*

INTRODUCTION

Physic nut (*Jatropha curcas* L.) is a shrubby tree plant of the family Euphorbiaceae, fast growing that reaches a height of 2-3 m or up to 5 m under special climate and soil conditions. The reproductive system of the species is allogamous due to monoecism, where the number of male is much larger than of female flowers, reaching a ratio of 29:1 (Solomon and Ezradanam 2002). *J. curcas* is a plant native to Central America and possibly to Brazil, but there are records of its occurrence in other countries such as Australia, South Africa and India. From the Caribbean Islands, where the species *J. curcas* may have been used by the Maya, it was probably brought by Portuguese traders from Cape Verde and Guinea Bissau to other countries in Africa and Asia (Jongschaap et al. 2007).

According to Peixoto (1973), the geographical distribution of jatropha in Brazil is rather widespread, in view of its robustness and strong drought-resistance, and adaptability to greatly varied soil and climate conditions, from the North of the country to the states of São Paulo and Paraná. It was also observed that *J. curcas* grows well both in tropical dry and humid equatorial zones, at sea level and up to 1,200 m altitude. Altitudes between 500 and 800m are most appropriate for cultivation. On arid and wind-exposed slopes, the plant height is no more than 2m. Different from initial reports, which attributed insect protection to the toxic and insecticide properties of the species, several groups of insects have overcome these natural barriers, causing damage in commercial plantations of *J. curcas* (Shanker and Dhyani 2006).

Jatropha is a potential species for biodiesel production since oil can be extracted by pressing (30-40 % of the total weight) from its seeds (Ram et al. 2008). There is growing interest in physic nut, because chemical or biological transesterification can easily convert oil from *J. curcas* seeds

[1] Embrapa Semiárido, C.P. 23, 56302-970, Petrolina, PE, Brazil. *E-mail: casantos@cpatsa.embrapa.br.

into biodiesel, promising low production costs. However, information in the literature on jatropha germplasm characterization, multi-site evaluation of different accessions and development of varieties with better agronomic performance is scarce (King et al. 2009).

For Basha and Sujatha (2007), the morphological characterization to assess the genetic diversity was not successful due to the strong environmental interaction with high-heritability traits, such as 100 seed weight, protein and oil content. Molecular markers were used in some studies with *J. curcas*, such as Random Amplification of Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR), to analyze 42 Jatropha accessions from different regions of India (Basha and Sujatha 2007) and more recently, Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeats (SSR), to differentiate toxic from non-toxic varieties (Pamidimarri et al. 2008a). Phylogenetic studies were also performed by RAPD and AFLP for seven species of the genus (Pamidimarri et al. 2008b).

Sun et al. (2008) found little genetic variability among 54 *J. curcas* accessions from different regions of China and two from Malaysia, evaluated with 70 AFLP markers and two alleles of a microsatellite, using the groups formed as reference. In contrast to this result, Wei et al. (2007) reported high variability in Chinese *J. curcas* accessions, based on the diversity index $\hat{\phi}_{ST}$.

The aim of this study was to determine the genetic relationships based on AFLP markers, of 12 jatropha accessions from different regions of Brazil and other countries with a view to guide the management of the species and improvement strategies of the genetic resources.

MATERIAL AND METHODS

Five plants of 12 accessions of the jatropha genebank of Embrapa Semiárido, Petrolina, PE, were used from: Triunfo-PB (TR) Quixeramobim-CE (CE), Dominican Republic (DR), Bebedouro-SP (SP), Janaúba-MG (MG), Tanzania (TZ), Para 2 (PA2), Para 3 (PA3), Para 1 (PA1), Juazeiro-BA (BA), Paraguay (PG), Petrolina-PE (PE). Healthy leaves of each plant were deep frozen at -80 °C until DNA extraction.

DNA was extracted from the leaves according to Doyle and Doyle (1990), with the following modifications: the first and second centrifugation were performed at 6,000 rpm and 10,000, respectively, the concentration of beta-mercaptoethanol was changed to 2% and the samples incubated for 30 minutes at 60 °C. After resuspending the final pellet in TE buffer (10 mM Tris pH 8.0, 1 mM EDTA), the DNA solution was treated with RNAse to remove co-isolated RNAs. The quantification and DNA integrity were checked on agarose gel, followed by dilution of genomic DNA to 40 ng mL^{-1}.

Approximately 200 ng DNA from each genotype was double-digested for 2.5 h with 0.65 units of the endonucleases *EcoRI* and *MseI*. The adapters were ligated with 1.25 units of *T4* DNA Ligase enzyme (Fermentas). The thermal cycler for pre-selective and selective amplifications was set according to Vos et al. (1995). The pre-selective amplification was performed in a final volume of 15 µL containing: 1.5 µM of the primers *EcoRI* and *MseI*, each with one selective nucleotide, 0.2 µM dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.5 units of *Taq* DNA Polymerase (Fermentas) and 2 µL linked DNA diluted to 1:5. An aliquot of 2 µL pre-amplified DNA diluted 20 times was used as template in the selective amplification, in a volume of 10 µL containing 0.2 mL of primer *EcoRI* and 0.3 mM of primer *MseI* plus three selective nucleotides, 0.2 mM dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl2, 0.5 units of *Taq* DNA Polymerase (Fermentas). The reactions were maintained for 3 minutes at 94 °C in the presence of formamide and immediately placed on ice prior to application on polyacrylamide gels. The gels were silver-nitrate stained, as described by Creste et al. (2001). All reactions were conducted in the genetics laboratory of Embrapa Semiárido.

The mean number of bands per primer combination was determined, as well as the percentage of polymorphic bands. The Jaccard similarity index was used to estimate the genetic similarity between plants of the species studied. The similarity matrix was used to construct the dendrogram based on Unweighted Pair-Group Method with Arithmetic Mean – UPGMA, using NTSYS software (Rohlf 1989). The adjustment of the dendrogram was evaluated by the coefficient of co-phenetic correlation, which compares the co-phenetic matrix with the matrix of genetic similarities.

Some plants of the accessions EC, SP, MG, PA2, PA3, PA1, BA, and SP were excluded from the analysis due to the number of more than 10% failure of the total number of registered polymorphic bands. Warburton and Crossa (2000) suggested the removal of plants or markers when the number of failures exceeds 15%.

The variance of molecular data (AMOVA) was analyzed by the decomposition of the total variation in

components between and within accessions using the squared Euclidean distance, as described by Excoffier et al. (1992). The significance of genetic parameters was determined by the method of randomization (1000 permutations). The interpretation of the parameter of genetic diversity $\hat{\phi}_{ST}$ was similar to Wright's F statistic, considering that there is no genetic differentiation when F = 0 and that there is fixation of alternative alleles and high differentiation among populations when F = 1. The program Arlequin (Schneider et al. 2000) was used in these tests. The gene flow was estimated by the number of migrants, based on the parameter $\hat{\phi}_{ST}$.

RESULTS AND DISCUSSION

This is the first study to apply AFLP markers to analyze the genetic diversity among Brazilian *J. curcas* accessions. Aside from being a highly promising species for biodiesel production, jatropha can also be grown on marginal soils and Brazil is one of the centers of diversity (Jongschaap et al. 2007).

A total of 283 AFLP bands was obtained, of which 164 were polymorphic and 119 and monomorphic, in 17 primer combinations (PC) EcoR1/Mse1, with a mean of 9.6 polymorphic markers/PC. The PCs produced the following numbers of polymorphic bands: ACT/CAT (13), AAG/CAT (9), AGC/CAC (12), AAG/CTC (12), ACA/CAT (14), AGG/CTC (7), ACC/CTC (10), ACC/CCC (9), ACT/CTG (10), AAC/CTC (8), AGG/CAT (7), AAG/CAA (13), ACA/CTC (10), AAG/CTA (6), ACA/CCC (5), ACT/CAA (9), and AAC/CTT (10). The mean of 9.6 differents polymorphic markers/PC was lower than that reported by Vos et al. (1995) and close to the mean of 10 polymorphic AFLP fragments reported by Santos et al. (2008), who used silver-nitrate staining for umbu tree, under the same experimental conditions. Means of 1.4 and 7 monomorphic and polymorphic AFLP bands, respectively, were reported by Sun et al. (2008), who used silver-nitrate staining, in *J. curcas* accessions in China.

The cophenetic correlation was 0.91, indicating that there was a low distortion of the clustering of accessions/origin by the dendrogram (Figure 1). The similarity of the plants and TZ_3 and TZ_4 was highest (93 %), while PE_2 was the most divergent. The similarities of the 12 studied accessions ranged from 30 to 93%, suggesting high genetic variability in the *J. curcas* accessions evaluated at Embrapa Semiárido.

Specific groupings in the dendrogram of all plants of the accessions TR, PA2 and TZ were observed as well as groups with more than 75% of plants of the accessions EC, SP and EP (Figure 1). The specific groups of plants of six of the 12 accessions studied should reflect the allogamous character of the crossing system of the species, with less variability within than among accessions, as pointed out by Reis and Grattapaglia (2004) for *Myracrodruon urundeuva*.

Two hypotheses can be proposed to explain the clustering of the said six accessions with their specific plants. The first based on the predominantly allogamous mating system of *J. curcas*, resulting in less variability within than between accessions (Reis and Grattapaglia 2004). The second would explain that the accessions differed according to the place of origin. It is proposed to enrich the genebank by the introduction of a greater number of germplasm/seeds come from locations other than those studied.

Dahmer et al. (2009) found chromosome stability in five *J. curcas* populations (2n = 2x = 22). This led the authors to conclude that the species is diploid, in contrast to reports suggesting autotetraploid species (Carvalho et al. 2008), and that the chromosomal stability is an advantage due to regular meiosis, to perform manual crosses between different accessions of the species.

Contrary to what was observed here, Basha and Sujatha (2007) report an absence of geographic variation in 42 *J. curcas* accessions from different regions of India and great similarity, (70-96%) of the accessions in RAPD and ISSR analyses. Also in Indian genotypes, Ranade et al. (2008) measured high genetic similarity with RAPD and Directed Amplification of Minisatellite DNA (DAMD), although specific groupings in the dendrogram were observed, according to the place of origin of the genotypes.

The estimate of variation among accessions was 0.275 ($\hat{\phi}_{ST}$) (Table 1) and is considered high, according to the classification proposed by Wright (1951), since the interpretation of the parameter is similar to Wright's F statistic. For tree species, the genetic divergence among populations is high when $\hat{\phi}_{ST}$ varies between 15 and 25% of the total variation, moderate between 5-15% and low if $d \leq 5\%$ (Kageyama et al. 2003).

This estimate suggests that the species has restricted gene flow, less than one migrant per generation (Nm = 0.66) (Table 1). Although jatropha is a cross-pollinated species (Solomon and Ezradanam 2002), the gene flow between populations was considered small and restricted,

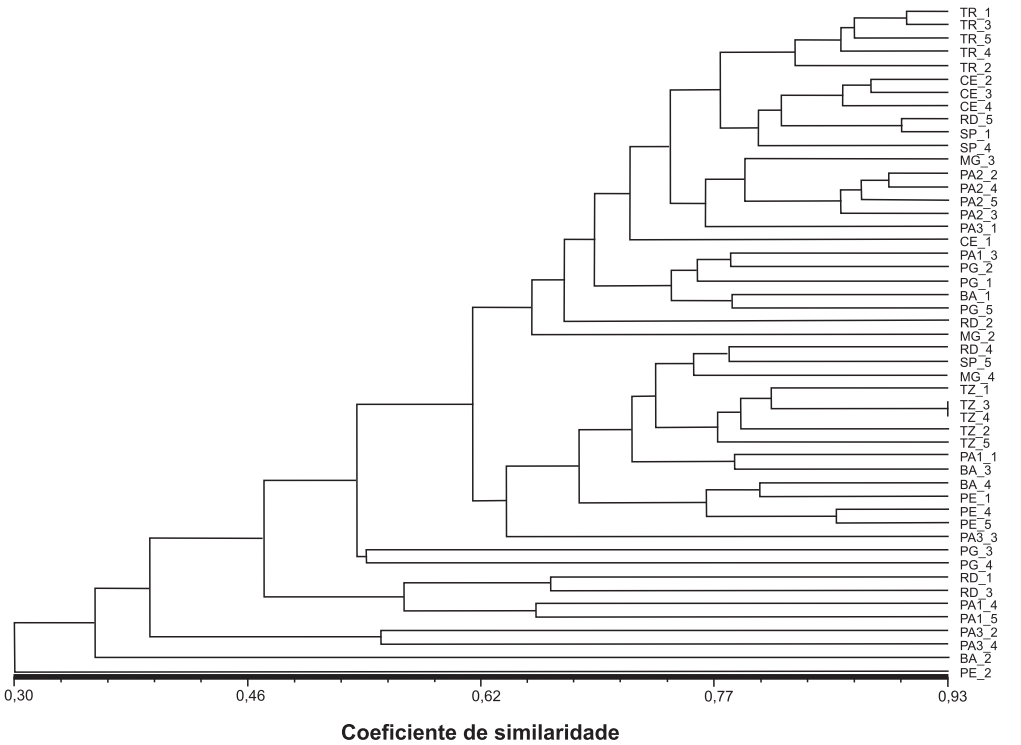

Figure 1. UPGMA dendogram of the similarity coefficient of Jaccard of 50 jatropha plants of 12 accessions from different origins, analyzed with 164 AFLP markers. Co-phenetic value (0.91). Triunfo-PB (TR), Quixeramobim-CE (CE), Dominican Republic (RD), Bebedouro-SP (SP), Janaúba-MG (MG), Tanzânia (TZ), Para 2, (PA2), Para 3 (PA3), Para 1 (PA1), Juazeiro-BA (BA), Paraguai (PG), Petrolina-PE (PE).

Table 1. Analysis of molecular variance (AMOVA) and estimate of gene flow (Nm)[1] of 12 jatropha accessions evaluated with 164 AFLP markers.

Sources of variation	df	SS	Total variation (%)	P[2]	Statistic $\hat{\phi}_{ST}$	Nm
Among accessions	11	557.8	27.53	(< 0.001)	$\hat{\phi}_{ST} = 0.2753$	0.66
Within accessions	38	747.1	72.47	(<0.001)	$1\text{-}\hat{\phi}_{ST} = 0.7247$	
Total	49	1304.9				

[1] $Nm = [(1-\hat{\phi}_{ST})/(4\hat{\phi}_{ST})]$; [2] Probability based on 1000 permutations.

probably as a result of the geographical isolation of the accessions studied. Wei et al. (2007) also reported a high $\hat{\phi}_{ST}$ value (0.54) and limited gene flow (Nm = 0.466), leading to the conclusion that the high variability among populations was not only caused by a limited gene flow, but also by genetic drift

For six *Rubus* species native to 27 locations in Colombia, Marulanda et al. (2007) reported variation among accessions of 0.196, indicating great variability among species. Santos et al. (2008) also found great variability among populations of umbu (*Spondias tuberosa*), a species endemic to the semi-arid region of Brazil, leading the authors to conclude that there are barriers to free mating between different populations.

The results of the *J. curcas* dendrogram (Figure 1) suggest that the accessions differ depending on the region of origin; the dissimilarity among plants from a given origin is smaller than the genetic variability of the species among accessions. We suggest the introduction or collection of seed germplasm from a greater number of locations to increase genetic variability in the study collection. The same trend was observed in the analysis of molecular variance, which shows a wide variation between jatropha accessions ($\hat{\phi}_{ST}$ = 0.275), indicating that a larger number of populations should be sampled to increase the genetic diversity in the species.

For crosses in breeding programs, the results obtained in this work suggest the selection within accessions to explore the intra-population variability, followed by crosses among different accessions to explore the inter-population variability, as e.g., among selections within accession Tanzania with selections within accession Triumph, provided the good agronomic performance.

Similaridade genética de acessos de *Jatropha curcas* por meio de marcadores AFLP

RESUMO - *Foram determinadas as relações genéticas entre acessos de pinhão-manso* (Jatropha curcas)*, com base no marcador AFLP. Um conjunto de 50 indivíduos de 12 acessos de pinhão-manso foi analisado com dados moleculares de 164 locos AFLP gerados de 17 combinações de iniciadores. A análise da variância de dados moleculares foi realizada pela decomposição total entre e dentro de acessos. Foi construído um dendrograma UPGMA com base nas distâncias genéticas estimadas pelo coeficiente de similaridade de Jaccard. O dendrograma apresentou boa definição, com valor cofenético de 0,91. Foram observados agrupamentos dos indivíduos de seis dos 12 acessos estudados os quais, apresentaram similaridade superior a 30%, evidenciando a alta variabilidade genética. A variação entre acessos foi estimada em 0,275, indicando alta variabilidade genética. A variabilidade genética dos acessos de pinhão-manso está geneticamente estruturada em função da origem e um maior número de populações deve ser amostrado para ampliar a diversidade da coleção de germoplasma.*

Palavras-chave: *pinhão-manso, dendrograma, AMOVA.*

ACKNOWLEDGEMENTS

The authors are indebted to the FINEP for financial support.

REFERENCES

Basha SD and Sujatha M (2007) Inter and intra-population variability of *Jatropha curcas* L. characterized by RAPD and ISSR markers and development of population-specific SCAR markers. **Euphytica 156**: 375-386.

Carvalho CR, Clarindo WR, Praça MM, Araújo FS and Carels N (2008) Genome size, base composition and karyotype of *Jatropha curcas* L., an important biofuel plant. **Plant Science 174**: 613-617.

Cortesão M (1956) **Culturas tropicais**: plantas oleaginosas. Editora Clássica, Lisboa, 231p.

Creste S, Tulmann Neto A and Figueira A (2001) Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining. **Plant Molecular Biology Reporter 9**: 299-306.

Dahmer N, Schifino-Wittmann MT and Dias LAS (2009) Chromosome numbers of *Jatropha curcas* L.: an important agrofuel plant. **Crop Breeding and Applied Biotechnology 9**: 386-389, 2009.

Doyle JJ and Doyle JL (1990) Isolation of plant DNA from fresh tissue. **Focus 12**: 13-15.

Excoffier L, Smouse PE and Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. **Genetics 131**: 479-491.

Jongschaap REE, Corré, WJ, Bindraban, PS and Brandenburg WA (2007) **Claims and facts on *Jatropha curcas* L.** Global *Jatropha curcas* evaluation, breeding and propagation programme. Available at http://library.wur.nl/way/bestanden/clc/1858843.pdf. Assessed in January 2010.

Kageyama PY, Sebbenn AM, Ribas LA, Gandara FB, Castellen M, Perecin MB and Venconsky R (2003) Diversidade genética em espécies arbóreas tropicais de diferentes estágios sucessionais por marcadores genéticos. **Scientia Forestalis 64**: 93-107.

King AJ, He W, Cuevas, JA, Freudenberger M, Ramiaramanana D and Graham IA (2009) Potential of *Jatropha curcas* as a source of renewable oil and animal feed. **Journal of Experimental Botany 60**: 2897-2905.

Marulanda ML, Aguilar SB and Lopez AM (2007) Genetic diversity of wild and cultivated *Rubus* species in Colombia using AFLP and SSR markers. **Crop Breeding and Applied Technology 7**: 242-252.

Pamidimarri DVNS, Singh S, Mastan SG, Patel J and Reddy MP (2008a) Molecular characterization and identification of markers for toxic and non-toxic varieties of *Jatropha curcas* L. using RAPD, AFLP and SSR markers. **Molecular Biology Reports 36**: 1357-1364.

Pamidiamarri, DVNS, Pandya N and Reddy MP (2008b) Comparative study of interspecific genetic divergence and phylogenic analysis of genus *Jatropha* by RAPD and AFLP. **Molecular Biology Reports 36**: 901-907.

Peixoto AR (1973) **Plantas oleaginosas arbóreas**. Editora Nobel, São Paulo, 284p.

Ram SG, Parthiban KT, Kumar RS, Thiruvengadam V and Paramathma M (2008) Genetic diversity among Jatropha species as revealed by RAPD markers. **Genetic Resources and Crop Evolution 55**: 803-809.

Ranade AS, Srivastava AP, Rana TS, Srivastava J and Tuli R (2008) Easy assessment of diversity in *Jatropha curcas* L. plants using two single-primer amplification reaction (SPAR) methods. **Biomass and Bioenergy 32**: 533-540.

Reis AMM and Grattapaglia D (2004) RAPD variation in a germplasm collection of *Myracrodruon urundeuva* (Anacardiaceae), an endangered tropical tree: recommendations for conservation. **Genetic Resources and Crop Evolution 51**: 529-538.

Rohlf FJ (1989) **NTSYS-pc numerical taxonomy and multivariate analysis system**, version 1.80. Exeter Software, Setauket.

Santos CAF, Rodrigues MA and Zucchi MI (2008) Variabilidade genética do umbuzeiro no Semi-Árido brasileiro, por meio de marcadores AFLP. **Pesquisa Agropecuária Brasileira 43**: 1037-1043.

Schneider S, Roessli D and Excoffier L (2008) **Arlequin ver.3.11, 2000: a software for population data analysis**. University of Geneva, Genetic and Biometry Laboratory, Geneva. Available at 2007. http://anthropologie.unige.ch/arlequin/. Assessed in November 2009

Shanker C and Dhyani SK (2006) Insect pests of *Jatropha curcas* L. and the potential for their management. **Current Science 91**: 162-163

Solomon AJ and Ezradanam V (2002) Pollination ecology and fruiting behavior in a monoecious species, *Jatropha curcas* L. (Euphorbiaceae). **Current Science 83**: 1395-1398.

Sun QB, Li LF, Li Y, Wu GF and Ge XJ (2008) SSR and AFLP markers reveal low genetic diversity in the biofuel plant *Jatropha curcas* in China. **Crop Science 48**: 1865-1871.

Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M and Zabeau M (1995) AFLP: A new technique for DNA fingerprinting. **Nucleic Acids Research 23**: 4407-4414.

Warburton M and Crossa J (2000) **Data analysis in the CIMMYT applied biotechnology center: for fingerprinting and genetic diversity studies**. CIMMYT, Mexico, 23p.

Wei GLH, Lan W, Wei Y and Lin TC (2007) ISSR analysis of genetic diversity of *Jatropha curcas* L. **Chinese Journal of Applied & Environmental Biology 13**: 466–470.

Wright S (1951) The genetical structure of populations. **Annals of Eugenics 15**: 395-420.