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Assessment of the genetic diversity and population structure of *Jatropha curcas* accessions in Brazil using ISSR markers

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ABSTRACT. The oleaginous species *Jatropha curcas* (Euphorbiaceae) exhibits significant potential as a source of biodiesel, but the scarcity of information regarding genetic variability within the species limits its possible exploitation. We examined the genetic diversity and relationships among 97 accessions of *J. curcas* originating from the Brazilian states of Piauí, Maranhão, Ceará, Bahia, and Minas Gerais. One-hundred ISSR markers were tested and 11 selected for genotyping. Among 307 loci generated by these markers, 96 % were polymorphic. Genetic similarities between accessions were estimated from Jaccard coefficients and the corresponding similarity matrix, and genetic relationships were determined from the dendrogram constructed using the unweighted pair group method with arithmetic average (UPGMA) technique. Nei's genetic diversity indices varied between 0.0256and 0.1703, while Shannon's information indices ranged from 0.0374 to 0.1926. The number of alleles varied from 1.0619 to 1.3257 and the effective number of alleles ranged between 1.0438 and 1.1496. The

inter-population genetic coefficient differentiation was 0.6306, while analysis of molecular variance revealed that genetic divergence among populations was highly significant ($p< 0.001$), expressed by a fixation index (Φ_{ST}) of 0.4452. Pair-wise analysis of Φ_{ST} confirmed an inter-population diversity of 44.52% and an intra-population variation of 55.48%. UPGMA analysis allowed the separation of the accessions into four genotypic groups. We conclude that there is significant genetic diversity among the populations of *J. curcas* and that this variability is mainly due to intra-population genetic diversity.

Key words: Genetic divergence; Biodiesel; Oleaginous seeds; DNA marker

INTRODUCTION

Significant changes in climate that have become apparent in recent times have been attributed to global warming induced by the increased emission of greenhouse gases generated by human activities. A large part of greenhouse gas emissions arises from the combustion of gasoline and diesel fuel in cars, trucks and motorized equipment and, for this reason, there is growing pressure to adopt alternative clean-burning and renewable fuels such as biodiesel.

One of the more promising sources of biodiesel is *Jatropha curcas* (Euphorbiaceae), the seeds of which contain high levels of extractable oil. This perennial tree/shrub has a life span of more than 50 years and is native to tropical regions of the Americas, but is currently in the process of domestication in various areas of the world (Saturnino et al. 2005). In Brazil, *J. curcas* (known locally as "pinhão manso") is distributed widely and may be found in nearly all regions of the country, growing in areas with various types of edaphoclimatic characteristics (Arruda et al. 2004). This species is considered particularly important for the regional development of northeastern Brazil where poverty is most striking in the rural areas. In this context, there is considerable demand for technical and scientific information from researchers, breeders, farmers and governmental authorities who envisage large-scale cultivation of this crop for the production of biodiesel. However, despite the economic and social importance of *J. curcas*, knowledge regarding the genetic base and cultivation requirements of the species is still emerging. Furthermore, the plant materials currently employed in the production of commercial crops are genetically unknown (Laviola et al. 2011).

Molecular characterization is an efficient strategy for the determination of genetic variability between individual plants at the DNA level and for the assessment of genetic similarity between populations (Melchinger et al. 1994). Moreover, molecular markers can be used to create genetic maps enabling definitive conclusions to be drawn regarding genetic relationships between species, cultivars, and intra- and inter-specific hybrids (Borém and Caixeta 2009). In this context, inter-simple sequence repeat (ISSR) markers have been used widely in the determination of genetic diversity and population structure of a number of species (Christopoulos et al. 2010). These markers are amplified via polymerase chain reaction (PCR) from regions of genomic DNA located between two identical, adjacent and inversely oriented microsatellites (Assefa et al. 2003; Zietkiewicz et

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al. 1994), and they provide reliable and reproducible results (Gomes et al. 2012; Mendes et al. 2012).

Low genetic diversity among *J. curcas* populations has been reported following genotypic characterization by ISSR and by amplified fragment length polymorphism (AFLP) of accessions in an active germplasm bank (AGB) from China (Sun et al. 2008), and by AFLP and random amplified polymorphic DNA (RAPD) studies of specimens collected in a distinct geographical area of India (Pamidimarri et al. 2010). Additionally, ISSR and RAPD analyses of *J. curcas* accessions originating from different regions of Brazil revealed low genetic diversity (Rosado et al. 2009). In contrast, the results of ISSR analysis of nine Chinese populations of *J. curcas* suggested the existence of high genetic diversity (He et al. 2007), on Mexico has a wide genetic diversity of *J. curcas*, mainly in the state of Chiapas, revealed by AFLP (Pecina-Quintero et al. 2014), while ISSR assessment of 332 accessions from various regions of Brazil also indicated a high degree of polymorphism (91%) and genetic diversity (0.4340) (Grativol et al. 2011).

Our objective was to determine the genetic diversity and relationships among *J. curcas* populations in active germ plasm banks (AGBs) maintained at the research centers of Embrapa Mid-North and Embrapa Cotton located in the states of Piauí (PI) and Paraíba (PB), respectively, in northeastern Brazil.

MATERIAL AND METHODS

Plant material

Young healthy leaves were collected during the active phase of growth from each of the 97 *J. curcas* accessions maintained at the AGBs of Embrapa Mid-North (Teresina, PI) and Embrapa Cotton (Campina Grande, PB) (Fig. 1, Table 1). Samples, stored in labeled plastic bags and cooled over ice, were transported to the Laboratory of Biotechnology and Molecular Biology, Embrapa Mid-North, and stored at -20 ºC until required for analysis.

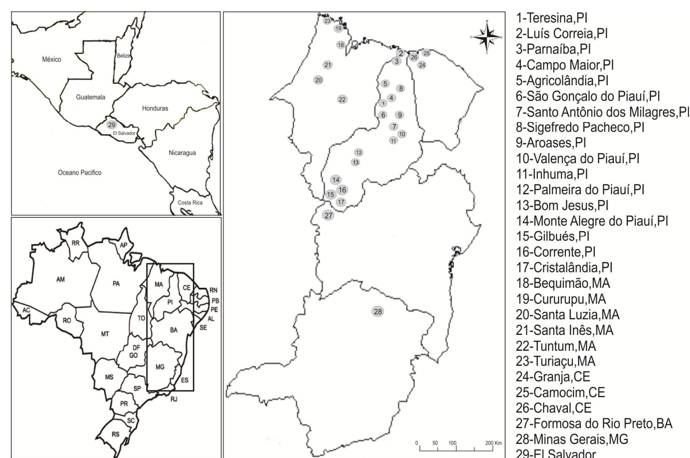

Figure 1. Origins of the *Jatropha curcas* accessions analyzed in the study. The maps show the location of El Salvador in Central America (top left), Brazilian states: Ceará (CE), Piauí (PI), Maranhão (MA), Bahia (BA) and, Minas Gerais (MG) (bottom left), and the towns nearest to the specific collection points (right).

Table 1- Origins of the *Jatropha curcas* accessions distributed according to the state and town of collection in Brazil.

Accession	Location	Coordinates
From Piauí state (PI)		
CMN26	Teresina	-
CMN10/26	Teresina	-
CMN27	Teresina	-
CMN29	Teresina	-
CMN31	Luís Correia	03°03'12,8"S; 41°18'56.6"W
CMN57	Luís Correia	03°4'46.1"S; 41°26'11.6"W
CMN58	Luís Correia	03°05'21.9"S;42°27'06.9"W
CMN59	Parnaíba	02°58'29.5"S;4°41'38.0"W
CMN60	Parnaíba	03°04'37.1"S;41°46'22.4"W
CMN6/64	Campo Maior	-
CMN12/66	Campo Maior	04°51'43.1"S;42°2' 0.9"W
CMN15/67	Campo Maior	04°51'3937"S;42°01'55.1"W
CMN8/80	Agricolândia	05°47'8.4"S;42°38'28.05"W
CMN28/78	Agricolândia	05°47'2.9"S;42°38'7.3"W
CMN3/98	São Gonçalo do Piauí	05°59'19.7"S;42°42'40.1"W
CMN20/99	São Gonçalo do Piauí	05°59'44.4"S;42°42'24.9"W
CMN101	Santo Antônio dos Milagres	06°2'41.3"S;42°42'37.6"W
CMN102	Santo Antônio dos Milagres	06°3'17.1"S;42°43'15.1"W
CMN103	Santo Antônio dos Milagres	06°3'19.4"S;42°43'19.6"W
CMN11/104	Santo Antônio dos Milagres	06°3'21.8"S;42°43'29"W
CMN178	Sigefredo Pacheco	04°59'6.4"S; 41°44'49.3"W
CMN9/179	Sigefredo Pacheco	4°59'8,8"S;41°44'37,8"W
CMN181	Sigefredo Pacheco	4°55'3,2"S;41°44'11,2"W
CMN207	Aroases	6°10'48,8"s;41°49'16,2"W
CMN208	Aroases	06°10'48.6"S;41°49'16"W
CMN209	Aroases	06°10'45.9"S;41°49'16"W
CMN212	Aroases	06°6'46.3"S; 41°47'47.1"W
CMN213	Aroases	06°6'47.4"S; 41°47'50.7"W
CMN214	Aroases	06°6'44.4"S;41°47'50"W
CMN219	Valença do Piauí	06°25'0.9"S; 41°45'3.1"W
CMN221	Valença do Piauí	06°24'55.4"S; 41°44'29.7"W
CMN222	Valença do Piauí	06°24'58.1"S; 41°44'30.8"W
CMN225	Inhuma	06°40'11.9"S; 41°43'3.1"w
CMN231	Inhuma	06°39125.9"S; 41°44'48.5"W
CMN234C	Inhuma	06°39'25.3"S;41°45'23"W
CMN259	Inhuma	6°39'26.5"S;41°45'23"W
CMN308	Palmeira do Piauí	08°42'35.5"S; 44°15'12.9"W
CMN309	Palmeira do Piauí	08°41'59.5"s; 44°16'19.4"W
CMN312	Bom Jesus	09°07'6.7"S; 44°25'58.7"W
CMN313	Bom Jesus	09°13'01"S; 44°29'25.2"W
CMN317	Monte Alegre do Piauí	09°33'16"S; 44°53'49.9"W
CMN318	Monte Alegre do Piauí	09°33'44.8"S; 44°57'55.8"W
CMN319	Monte Alegre do Piauí	09°44'21.7"S; 45°10'29.4"W
CMN321	Monte Alegre do Piauí	09°45'20.3"S; 45°18'4.8"W
CMN322	Gilbués	09°49'21.7"S; 45°24'45.3"W
CMN323	Gilbués	09°49'38.6"S; 45°25'7.6"W
CMN326	Corrente	10°27'10"S; 45°09'43.1"W
CMN327	Corrente	10°26'8.6"S; 45°09'36.1"W
CMN328	Cristalândia	10°37'55.1"S; 45°10'37.9"W
CMN330	Cristalândia	10°43'10.6"S; 45°11'14.4"W
CMN331	Cristalândia	10°43'22.6"S; 45°11'23.1"W
From Maranhão state (MA)		
CMN1	Bequimão	2°25'21.1"S; 44°46'33.8"W
CMN2	Bequimão	2°25'19.9"S; 44°46'46.33"W
CMN3	Bequimão	2°25'15.1"S; 44°46'34.3"W
CMN4	Bequimão	2°24.1'17"S;44°44'16"W
CMN5	Bequimão	2°30' 20.9"S; 44°46'49.8"W
CMN7/7	Bequimão	-
CMN8	Bequimão	1°50'30.5"S; 44°46'46.9"W
CMN9	Cururupu	01°50'30.5"S; 44°52'36.9"W
CMN10	Cururupu	01°48' '7.7"S; 44°42'59"W
CMN11	Cururupu	01°48' 9.8"S; 44°42' 52.2"W
CMN13	Cururupu	01°40'14.7"S; 44°47'4.6"W
CMN17	Santa Luzia	02°29'16.9"S; 45°47'5.9"W
CMN18	Santa Luzia	02°29'10.6"S; 45°47'2.2"W
CMN14/18	Santa Luzia	-
CMN19	Santa Inês	3°39'23.9"S;45°22'35"W
CMN 21	Santa Inês	03°39'19.7"S; 45°26'35.7"W
CMN 22	Santa Inês	03°41'33.8"S;45°24'15.8"W

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Accession	Location	Coordinates
CMN368	Tuntum	05°24'4.9"S;44°49'58.5"W
CMN369	Tuntum	05°22'53.2"S; 44°47'15.2"W
CMN370	Tuntum	05°16'07"S;44°37'56.3"W
CMN16	Turiaçu	01°38'58.2"S;45°3'11.2"W
CMN2/16	Turiaçu	-
From Ceará state (CE)		
CMN34	Granja	03°7'28.1S; 40°49'30.8"W
CMN38	Granja	03°10'38.8"S;41°06'40.1"W
CMN39	Granja	03°16'10.9"S;41°08'01.0"W
CMN41	Granja	03°10'23.3"S;41°12'23.8"W
CMN42	Granja	03°13'43.1"S;41°12'36.2"W
CMN43	Granja	03°16'3.5"S;40°59'20.4"W
CMN45	Camocim	02°59'25.1S;40°54'47.7"W
CMN46	Camocim	02°57'11.9"S;40°55'51.7"W
CMN47	Camocim	02°56'1.7"S;40°55'42.5"W
CMN48	Camocim	02°55'45.7"S;40°55'41.2"W
CMN49	Camocim	2°55'11.8"S;40°53'11.7"W
CMN10/49	Camocim	-
CMN50	Camocim	02°53'37.6"S;40°55'13.3"W
CMN51	Chaval	03°02'51.1"S;41°14'18.4"W
CMN56	Chaval	03°04'44.3"S;41°15' 9.0"W
From Bahia state (BA)		
CMN332	Formosa do Rio Preto	11°02'48.6"S;45°11'44.6"W
CMN333	Formosa do Rio Preto	11°00'40.7"S;45°16'26.6"W
CMN334	Formosa do Rio Preto	11°01'1.9"s;45°16'50.7"W
CMN336	Formosa do Rio Preto	10°59'53"S;45°16'54.1"W
From Minas Gerais state (MG)		
CMN J	Nova Porteirinha	-
CMN NP	Nova Porteirinha	-
CMN SS	Nova Porteirinha	-
From El Salvador[a]		
CNPA PM VII P3	Mundo Novo	07°35'31.3"S; 37°11'41.0" W
CNPA PM VII P4	Mundo Novo	07°35'31.3"S; 37°11'41.0" W

[a] Plant material originating from El Salvador but obtained from producers in Mundo Novo, state of Paraíba (PB).

Extraction and quantification of DNA

DNA was extracted using Invisorb® Spin Plant Mini Kits (Stratec Molecular, Berlin, Germany) following the procedure recommended by the manufacturer. Aliquots of extracted DNA were subjected to electrophoresis on 0.8% agarose gel in Tris-borate-EDTA (0.5 × TBE) buffer and subsequently stained with GelRed™ (10,000 ×; Biotium, Hayward, CA, USA). The quantity and quality of extracted genomic DNA were determined by comparison with λ DNA standards (100 ng) using a NanoDrop 2000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). DNA samples were diluted to 7.0 ng/µL and stored at - 20°C until required for ISSR analysis.

PCR amplification and selection of primers for ISSR

The reaction mixture employed in PCR amplifications contained 1.5 × buffer (30 mM Tris-HCl, 75 mMKCl; Ludwig Biotec, Alvorada, RS, Brazil),2.5 mM $MgCl_2$ (Ludwig Biotec), 0.8 mM dNTPs, 0.25 µM primer, 1U Taq DNA polymerase (Ludwig Biotec), 1 µL DNA template (7.0 ng/µL) and ultrapure distilled water to a final volume of 10 µL. Amplification reactions were carried out in a Veriti 96 Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: initial denaturation for 90 s at 94ºC, 40 cycles each comprising denaturation for 40 s at 94ºC, annealing (temperature varied according to the melting temperature of the primer; Table 2), extension

for 2 min at 72ºC, and final extension for 5 min at 72ºC. The resulting amplicons were separated by electrophoresis on a 1.5% agarose gel in 0.5 ×TBE buffer for 6 h at 110 V, stained with GelRed, visualized under a UV transilluminator and subsequently photographed. The sizes of the amplicons were estimated in comparison with Invitrogen 1 kb DNA ladder (Life Technologies do Brasil, São Paulo, SP, Brazil).

Table 2. Characteristics of the 11 primers selected for use in the ISSR reactions.

Primer	Tm[a] (ºC)	Ta[b] (ºC)	Sequence 5' - 3'[c]	GC[d](%)
UBC 813	45.70	48.00	CTC TCT CTC TCT CTC TT	47.06
UBC 818	51.00	55.00	CAC ACA CAC ACA CAC AG	52.94
UBC 825	51.40	55.00	ACA CAC ACA CAC ACA	46.67
UBC 827	53.00	56.00	ACA CAC ACA CAC ACA CG	52.94
UBC 834	49.20	51.00	AGA GAG AGA GAG AGA GYT	50.00
UBC 844	48.60	50.00	CTC TCT CTC TCT CTC TRC	55.55
UBC 849	51.40	55.00	GTG TGT GTG TGT GTG TYA	50.00
UBC 855	53.10	57.00	ACA CAC ACA CAC ACA CYT	50.00
UBC 856	52.80	57.00	ACA CAC ACA CAC ACA CYA	50.00
UBC 885	48.30	52.00	BHB GAG AGA GAG AGA GA	58.82
UBC 889	50.10	52.00	DBD ACA CAC ACA CAC AC	58.82

[a] Melting temperature.
[b] Annealing temperature.
[c] Y = C,T; B = C,G,T; D = A,G,T; H = A,G,T; R = A,C,G.
[d] Guanine-cytosine base pairs.

In order to select the most suitable primers for ISSR reactions, DNA samples from four *J. curcas* accessions were initially amplified using 100 primers obtained from the University of British Columbia, Vancouver, Canada. Eleven primers (Table 2) were chosen based on resolution and high levels of polymorphism, and these were subsequently employed in the PCR amplification of DNA samples derived from all 97 *J. curcas* accessions.

Phylogenetic analysis

The number of well-resolved and intense polymorphic bands generated by each of the 11 primers was determined by visual inspection. Each band was considered to represent a single trait, and a binary matrix was created in which '1' indicated the presence and '0' the absence of the band. Genetic similarities between *J. curcas* accessions were estimated from Jaccard coefficients and the corresponding similarity matrix (Rohlf, 2000). A dendrogram was constructed using the unweighted pair group method with arithmetic average (UPGMA) clustering technique. The cophenetic correlation coefficient (*r*) was calculated from the similarity matrix and from the dendrogram. The bootstrap confidence index was also calculated from the binary matrix of amplified fragments and from the dendrogram after 1000 permutations. Analyses were performed with the aid of the software PAST version 1.34 (Hammer et al. 2001).

Intra- and inter-population analyses were carried out using the population genetic analysis program Popgene (version 1.31) (Yeh et al. 1999). Summary statistics included the total number of loci, the percentage of polymorphic loci (**P**), the observed number of alleles (*Na*), the effective number of alleles (*Ne*), Nei's genetic diversity index (H_e) (Nei, 1973) and Shannon's information index (H_o). Population differentiation was determined by analysis of molecular variance (AMOVA) and Arlequin software version 3.1 (Excoffier, 2006). The magnitude of genetic differentiation was expressed by the genetic distance coefficient among populations (G_{ST}) and the fixation index (Φ_{ST}). In order to define groups of genetically related individuals, Bayesian analysis was performed by the delta K method using Structure software version 2.3.4

(Pritchard et al. 2000) with 300,000 iterations after 50,000 rounds. The results obtained following three replications were displayed using Distruct software version 1.1 (Pritchard et al. 2000).

RESULTS

Genetic diversity

The set of 11 primers selected for ISSR analysis of the *J. curcas* accessions generated a total of 307 loci, of which 294 (95.76%) were polymorphic (Table 3). The average number of loci per primer was 27 and the sizes of the fragments ranged between 300 and 5000 bp. Generally, the greater the number of loci generated by a primer the higher the percentage polymorphism. Thus, primer UBC818 generated the largest number of polymorphic loci (51 bands) and exhibited 100% polymorphism, whereas the smallest number of polymorphic loci (7 bands) representing 58.33% polymorphism was obtained with primer UBC889. Such large amplitude of variation demonstrates not only a high level of diversity among the *J. curcas* accessions maintained in the AGBs of Embrapa Mid-North and Embrapa Cotton, but also the efficiency of the selected ISSR primers as shown in Figure 2.

Table 3. Characteristics of the loci amplified using the 11 selected ISSR primers.

Primer	Number of loci		Polymorphism (%)	Size of fragments (bp)
	Total	Polymorphic		
UBC 813	12	10	83.33	880-2000
UBC 818	51	51	100.00	450-3800
UBC 825	49	49	100.00	450-3500
UBC 827	41	41	100.00	450-5000
UBC 834	10	10	100.00	490-850
UBC 844	15	15	100.00	820-4000
UBC 849	31	29	93.55	850-3000
UBC 855	29	29	100.00	310-1680
UBC 856	43	43	100.00	1400-4000
UBC 885	14	10	71.42	300-1650
UBC 889	12	7	58.33	490-3000
Mean	28	27	91.51	-

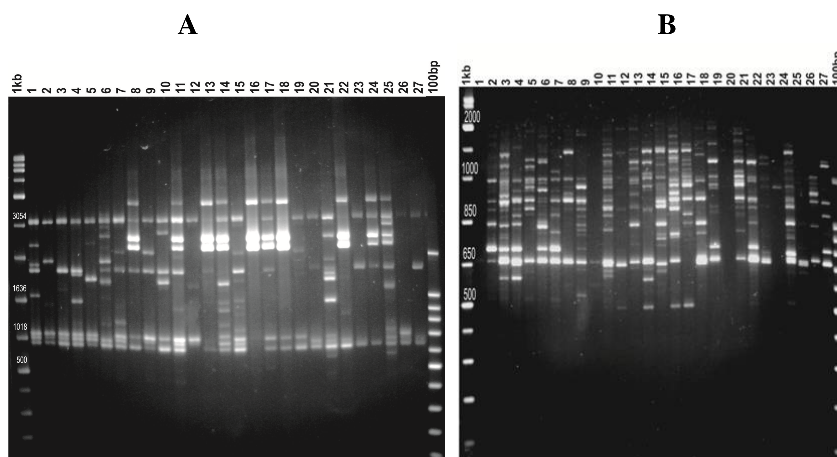

Figure 2. Electrophoretic profiles of ISSR amplifications generated by primers UBC 889 (A) and UBC 856 (B) with DNA derived from 27 of *Jatropha curcas* accessions Lanes 1 to 25 relate to accessions obtained from Piauí state while lanes 26 and 27 relate to material originating from El Salvador.

Table 4 presents the genetic parameters of the 97 *J. curcas* accessions grouped into 29 populations according to specific location of collection (*cf*. Table 1).Values of the Nei genetic diversity (H_e) index varied between 0.0256 and 0.1703 for s from Corrente (Piauí) and Bequimão (Maranhão) populations, respectively. For the same populations, the Shannon information index (H_o) varied between 0.0374 and 0.1926, respectively, and the percentage of polymorphic loci (*P*) varied between 6.19 and 32.57%, respectively. The largest variation in number of alleles (N_a) was detected between Corrente and Bequimão populations (1.0619 and 1.325, respectively), whereas the greatest variation in N_e was observed between Corrente and Santo Antônio dos Milagres (Piauí) populations (1.0438 and 1.1496, respectively). All loci were assumed to be in Hardy-Weinberg equilibrium.

Table 4. Genetic parameters of 29 *Jatropha curcas* populations.

Population[a]	*P* (%)	*Na*	*Ne*	*He*	*Ho*[f]
Teresina, PI	19.22	1.1922	1.1310	0.0749	0.1101
LuísCorreia, PI	18.89	1.1889	1.1219	0.0713	0.1060
Parnaíba, PI	11.07	1.1107	1.0783	0.0459	0.0670
Campo Maior, PI	21.82	1.2182	1.1327	0.0795	0.1193
Agricolândia, PI	16.94	1.1694	1.1198	0.0702	0.1024
São Gonçalo do Piauí, PI	9.12	1.0912	1.0645	0.0378	0.0552
Santo Antônio dos Milagres, PI	25.73	1.2573	1.1496	0.0890	0.1346
Sigefredo Pacheco, PI	21.82	1.2182	1.1242	0.0764	0.1160
El Salvador	7.49	1.0749	1.0530	0.0310	0.0453
Aroases, PI	19.22	1.1922	1.0853	0.0538	0.0849
Valença do Piauí, PI	13.36	1.1336	1.0725	0.0455	0.0697
Inhuma, PI	25.08	1.2508	1.1281	0.0799	0.1235
Palmeira do Piauí, PI	12.05	1.1205	1.0852	0.0499	0.0729
Bom Jesus, PI	7.49	1.0749	1.0530	0.0310	0.0453
Monte Alegre do Piauí, PI	14.98	1.1498	1.0783	0.0486	0.0748
Gilbués, PI	13.36	1.1336	1.0944	0.0553	0.0808
Corrente, PI	6.19	1.0619	1.0438	0.0256	0.0374
Cristalândia, PI	15.64	1.1564	1.0891	0.0548	0.0832
Bequimão, MA	32.57	1.3257	1.1422	0.1703	0.1926
Cururupu, MA	18.57	1.1857	1.0848	0.0548	0.0864
Santa Luzia, MA	18.24	1.1824	1.1054	0.0645	0.0976
Santa Inês, MA	15.64	1.1564	1.0994	0.0585	0.0872
Tuntum, MA	14.01	1.1401	1.0890	0.0524	0.0781
Turiaçu, MA	8.79	1.0879	1.0622	0.0364	0.0532
Granja, CE	19.22	1.1922	1.0829	0.0528	0.0837
Camocim, CE	20.52	1.2052	1.0967	0.0606	0.0946
Chaval, CE	7.49	1.0749	1.0530	0.0310	0,0453
Formosa do Rio Preto,BA	22.48	1.2248	1.1273	0.0762	0.1157
Minas Gerais, MG	15.31	1.1531	1.0792	0.0508	0.0783

[a] Piauí (PI), Maranhão (MA), Ceará (CE), Paraíba (PB), Bahia (BA) and, Minas Gerais (MG) Brazilian states.
P: polymorphic loci.
Na: observed number of alleles.
Ne: effective number of alleles.
He: Nei's genetic diversity.
Ho: Shannon's information index.

Cluster analysis

Genetic relationships among the 97 *J. curcas* accessions were established based on Jaccard coefficients calculated from the 307 amplified loci, and the associated dendrogram was constructed using the UPGMA method. Comparison of individual accessions pair-wise revealed that CMN 10/26 and CMN 26 presented the highest genetic similarity coefficient (0.915),while the lowest coefficients were observed between CMN 58 and CMN NP (0.132), CMN 7/7 and CMN NP (0.133), CMN 333 and CMN 10/26 (0.160), CMN 333 and

CMN 58 (0.161), CMN 16 and CMN 7/7 (0.166), CMN NP and CMN 10/26 (0.168), CMN 333 and CMN 26 (0.170), and CMN NP and CMN 12/66 (0.188). These results reveal that significant genetic variability is present in the *J. curcas* accessions available in the AGBs of Embrapa Mid-North and Embrapa Cotton, a feature that can be exploited in future breeding programs.

The average similarity coefficient considering all the loci was 0.35; this value was taken as the cut-off point in the dendrogram displayed in Figure 3. In this manner, the data obtained from ISSR analysis enabled four distinct genotypic groups to be distinguished among the *J. curcas* accessions. Group I comprised 25 accessions incorporating six populations originating from Bahia, Ceará, Maranhão and Minas Gerais states; group II contained 23 accessions encompassing six populations from Piauí and Maranhão states; group III included 25 accessions covering eight populations from Piauí state (center -north and southwest mesoregions); and group IV comprised 25 accessions also from Piauí state (eight populations from the north and center-north mesoregions and one from El Salvador).

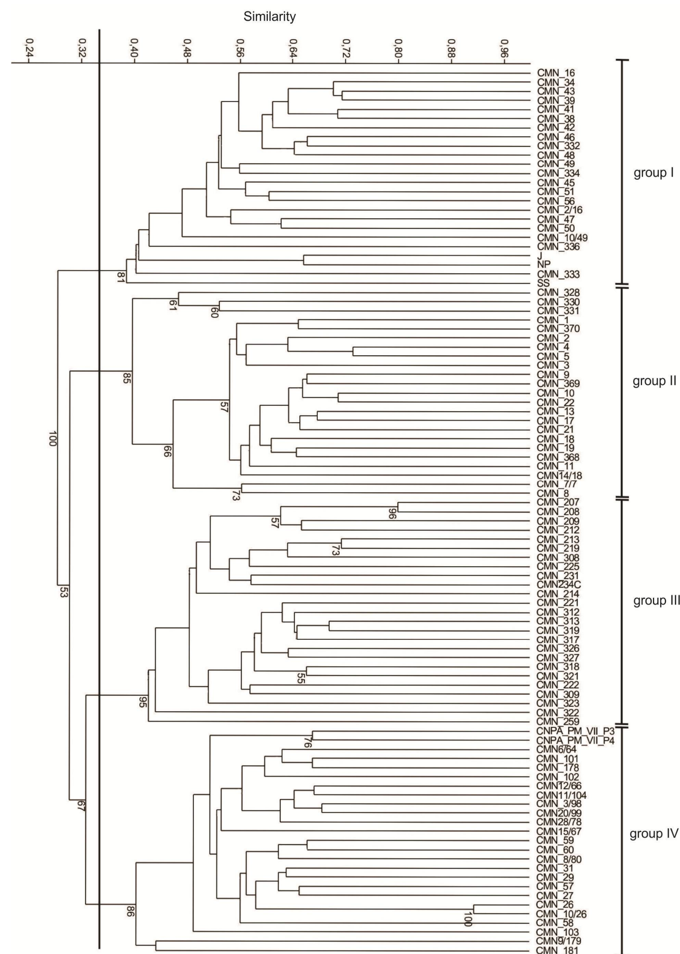

Figure 3. UPGMA dendrogram based on the 11 selected ISSR polymorphic markers showing similarity relationships between 97 *Jatropha curcas* accessions maintained at Embrapa Mid-North and Embrapa Cotton.

Population structure

The genetic distance coefficient between populations (G_{ST}) was 0.6306, while AMOVA revealed that the genetic divergence among the 29 *J. curcas* populations was highly significant, as expressed by a Φ_{ST} value of 0.4452 (P< 0.001; Table 5). Pair-wise AMOVA showed an inter-population variation of 44.52% and an intra-population variation of 55.48% (Table 5). Considering individual populations pair-wise, the smallest genetic distance (-0.132) was observed between Campo Maior and Agricolândia populations in Piauí state, while the greatest distance (0.679) was between Parnaíba and Chaval populations located in Piauí and Ceará states, respectively. Bayesian analysis showed a clustering structure encompassing four well-defined groups (K = 4) as shown in Figure 4.

Table 5. AMOVA of 29 *Jatropha curcas* populations.

Source of variation	Degrees of freedom	Sum of squares	Components of variance	Variation(%)	Φ_{ST}[a]	*p*value[b]
Inter-population	28	1671.647	13.16	44.52	0.4452	0.001
Intra-population	67	1098.988	16.40	55.48	-	-
Total	95	2770.635	29.56582	100.00	-	-

[a] Fixation index.
[b] Significance after 1000 permutations.

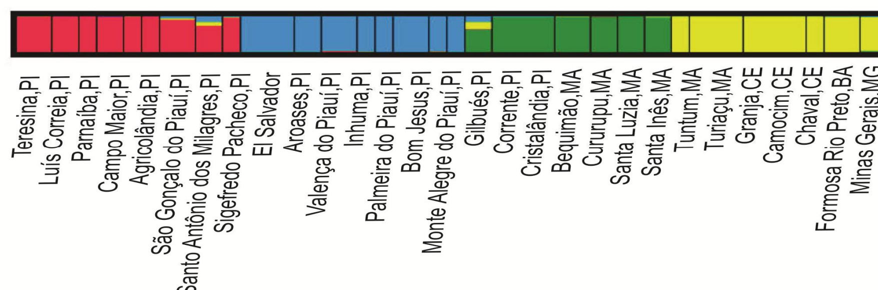

Figure 4. Population structure in *Jatropha curcas* as determined via Bayesian analysis (K = 4) of 29 populations originating from Ceará (CE), Piauí (PI), Maranhão (MA), Bahia (BA) and Minas Gerais (MG) Brazilian states, and from El Salvador in Central America. Each population is represented by colored columns, while the size of the sample population is defined by the width of the column.

DISCUSSION

The characterization of the genetic diversity of a species is essential for the success of breeding programs. In this context, the cultivation and genetic improvement of *J. curcas* has been limited by the lack of information regarding its genetic variability (Grativol et al. 2011).

Our ISSR analysis of *J. curcas* populations available in the AGBs of Embrapa Mid-North and Embrapa Cotton revealed a high degree of polymorphism among the accessions. The analysis was facilitated by the use of a horizontal electrophoresis system (20 x 25 cm) with wide comb pieces, which provided longer running times and superior separation of bands, coupled with the application of a dye with strong DNA-binding affinity allowing better visualization. The sizes of the amplified fragments were variable (300 to 5000 bp), although the values were similar to those obtained in previous studies, namely, 100 to 3500

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bp (Basha et al. 2009) , 250 to 3000 bp (Sunil et al. 2008) and 100 to 2500bp (Diaz et al. 2017).

The efficiency of a molecular marker may be assessed by the amount of polymorphism that it is able to detect (Grativol et al. 2011). In previous investigations, ISSR markers have proven to be particularly efficient. Thus, in a study on the genetic diversity of *Jatropha* species, Kumar et al. (2009) reported that eight ISSR primers generated 100% polymorphic bands and that the total polymorphism was 98.14%. Additionally, in a study of the genetic diversity and structure of nine natural *J. curcas* populations from China (He et al. 2007), application of 10 ISSR primers allowed the detection of a total polymorphism of 97.04%.

Although *J. curcas* is an allogamous species, variabilities as low as 33.5% (Basha and Sujatha 2007) and 34.0% (Chen et al. 2011) have been determined using ISSR markers, whilst variabilities of 26.99% (Shen et al. 2010), 34.0% (Chen et al. 2011) and 42% (Basha and Sujatha 2007) have been obtained with RAPD markers. Low genetic diversity was observed in three populations of *J. curcas* in the state of São Paulo when using AFLP markers (Pioto et al. 2015). Even with the use of codominant markers such as SSR, low genetic diversity was also observed among 92 accessions originating in Mozambique, Ethiopia, Tanzania, Brazil, Honduras and Indonesia (Santos et al. 2016).Such low genetic diversity was attributed to the intense diffusion of plant material via vegetative propagation, which increases the possibility that germplasm banks store plants of identical provenance. Alternatively, low genetic diversity may be attributed to the occurrence of apomixis in *J. curcas*, as highlighted by an Indian study revealing an apomixis rate of 32% (Bhattacharya et al. 2005). However, an evaluation by (Juhász et al. 2009) of the reproductive processes of *J. curcas* in Brazil showed that fruits were generated via apomixis (5%), natural self-pollination (20%), geitonogamy (79%), xenogamy with pollen from a cross-breeding plant (80%) and xenogamy with a mixture of pollen (88%), indicating the predominance of cross-pollination. Another Brazilian study revealed that fruit formation via xenogamy attained 80% in *J. mutabilis* and 95% in *J. mollissima* (Santos et al. 2005). Moreover, in the case of *J. curcas*, the lack of synchronization in the opening of male and female flowers would promote cross-fertilization (Heller, 1996; Luo et al. 2007). Although the reproductive biology of accessions was not determined in our study, it is likely that cross-pollination predominated, thereby favoring variability among the accessions. In general, however, knowledge regarding the reproductive systems of plant species, including endogamy and predominant reproductive processes, is critical for plant improvement, sustainable management and conservation (Bueno et al. 2006).

The origin of *J. curcas* has yet to be established with certainty, but some evidence suggests that the source may be Mexico and Central America (Basha and Sujatha 2007). Few reports refer to Brazil as the source but, based on the earlier demonstration (Grativol et al. 2011) of high polymorphism and the confirmation obtained in our study, it would appear that the country represents the region of highest diversity with regard to this species. However, further studies are required to verify this proposal, especially analyses of *J. curcas* populations from other regions.

The number of polymorphic loci detected by different types of molecular markers has been used to quantify genetic diversity. The ISSR markers employed in the present study gave rise to *P* values varying from 6.19% in Corrente, Piauí populations, to 32.57% in Bequimão, Maranhão populations thereby demonstrating that the accessions have different

origins and that the study populations are sources of variability. An earlier study of the genetic diversity of *J. curcas* conducted using ISSR and RAPD markers (Gupta et al. 2008) obtained *Na* values ranging from 0.426 to 1.765 and *Ne* values ranging from 0.355 to 1.407. Additionally, a study involving AFLP markers (Ovando-Medina et al. 2011) revealed *Na* and *Ne* values in the ranges 1.434 – 1.842 and 1.181 – 1.398, respectively, for five *J. curcas* populations from the Chiapas state in Mexico.

In our study, the lowest genetic diversity indices (*He* = 0.0256 and *Ho* = 0.0374) were found among the populations from Corrente, whereas the largest variabilities (*He* = 0.1703 and *Ho* = 0.1926) were detected among the populations from Bequimão. These results suggest that accessions in the Bequimão populations could provide suitable parents for breeding programs. Genetic variation indices reported previously for *J. curcas* were *He* = 0.0498 - 0.1699 and *Ho* = 0.0782 – 0.2450 (Grativol et al. 2011).

The similarity matrix confirmed variability among *J. curcas* accessions with a cophenetic correlation (*r*) of 0.91, indicating high consistency of the data and enhanced reliability of the inferences drawn from the dendrogram constructed using the UPGMA method. According to Sokal and Rohlf (1962), *r* values greater than 0.80 indicate good fit between the original distance matrices and analysis of the resulting clusters. Thus, combinations of accessions may be employed as parents for breeding purposes as well as for conservation and for extension of the genetic base.

The amplitude of variation coefficients of similarity within the groups was large, particularly in groups I and IV. Within group I, the coefficients ranged from 0.1319 (CMN NP and CMN 58) to 0.7143 (CMN 39 and CMN 43), whereas in group IV the coefficients varied from 0.264 (CMN 181 and CMN 10/26) to 0.916 (CMN 10/26 and CMN 10). The variation in group I can be attributed to the fact that a large number of populations derived from different states, while in group IV the variation is associated with the assemblage of populations from the north and center-north mesoregions of Piauí state together with the population of El Salvador. These results highlight the extent of genetic diversity among *J. curcas* populations from Piauí state, since they were grouped together with the accessions originating from the area where this species supposedly arose. Within group II, the Cristalândia, Piauí population was grouped with the populations from Maranhão state, indicating that there has been an exchange of material between these two states and confirming the existence of genetic differentiation between the Cristalândia population and other populations from Piauí state. The similarity coefficients within group II varied from 0.158 (CMN 7/7 and CMN 208) to 0.731 (CMN 5 and CMN 4). Group III encompassed populations from two distinct mesoregions (namely, center-north and south-west) of Piauí state, confirming the existence of genetic diversity in this area from Brazil.

Although the value of G_{ST} (0.6306) showed genetic differentiation among the populations, AMOVA indicated that intra-population variability was the most striking feature. Similar results were obtained in the study by Sirithunya and Ukoskit (2010) in which ISSR markers detected 83.31% of genetic differentiation within the *J. curcas* populations. Additionally, Gupta et al. (2008) reported that the use of AFLP markers detected 87.8% variability within *J. curcas* populations. These two investigations demonstrate the high efficiency of ISSR and AFLP markers.

Evaluation of population structure using the Bayesian approach through the delta K method and Structure software confirmed that the 97 accessions formed four distinct groups ($K = 4$) in accordance with cluster analysis. This result demonstrated the consistency and

reliability of the analyses performed in our study. Structure software has been employed by Gupta et al. (2012) in a previous study involving *J. curcas*, in which it was possible to identify a close relationship between native and exotic accessions from India, Africa and North and South America.

Allogamous species typically exhibit considerable intra-population genetic variability, although the divergence of populations may be reduced by increased gene flow (Galetti et al 2012). In addition, perennial plants with a long life cycle and broad geographic distribution normally present large intra-population diversity (Hamrick and Godt 1989). In our study, we found that the differences among the *J. curcas* populations maintained at Embrapa Mid-North and Embrapa Cotton were significant according to the analysis of pair-wise Φ_{ST} values, and that the selected ISSR markers were very efficient in revealing the variability and structure of these populations.

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