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High genetic diversity of *Jatropha curcas* assessed by ISSR

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ABSTRACT. *Jatropha curcas* L. is a highly promising oilseed for sustainable production of biofuels and bio-kerosene due to its high oil content and excellent quality. However, it is a perennial and incipiently domesticated species with none stable cultivar created until now despite genetic breeding programs in progress in several countries. Knowledge of the genetic structure and diversity of the species is a necessary step for breeding programs. The molecular marker can be used as a tool for speed up the process. This study was carried out to assess genetic diversity of a germplasm bank represented by *J. curcas* accessions from different provenance beside interspecific hybrid and backcrosses generated by IAC breeding programs using inter-simple sequence repeat markers. The molecular study revealed 271 bands of which 98.9% were polymorphic with an average of 22.7 polymorphic bands per primer. Genetic diversity of the germplasm evaluated was slightly higher than other germplasm around the world and ranged from 0.55 to 0.86 with an average of 0.59 (Jaccard index). Cluster analysis (UPGMA) revealed no clear grouping as to the geographical origin of accessions, consistent

with genetic structure analysis using the Structure software. For diversity analysis between groups, accessions were divided into eight groups by origin. Nei's genetic distance between groups was 0.14. The results showed the importance of Mexican accessions, congeneric wild species, and interspecific hybrids for conservation and development of new genotypes in breeding programs.

Key words: *Jatropha curcas*; *Jatropha integerrima*; Interspecific hybrids; Physic nut; Molecular marker; Plant breeding

INTRODUCTION

Jatropha curcas L. (Euphorbiaceae) is a perennial tree, monoecious with unisexual flowers (Montes and Melchinger, 2016). Probably native to Mexico (Pecina-Quintero et al., 2014) this species occurs in tropical and subtropical regions of the world, and it is considered one of the most promising oilseeds for biodiesel production due to the high oil content of oilseeds, reaching 40 to 50% (Subramanian et al., 2005). The yield ranges from 0.3 to 3000 kg seeds/ha. It is adapted to different environmental conditions, including low rainfall and low-fertility soil, and does not compete with food crops (Montes and Melchinger, 2016).

Despite the promise, *J. curcas* is an incipiently domesticated species with no availability of stable and commercial cultivars with high oil content and tolerant to pests and diseases that can meet the needs of farmers and processors of the feedstock produced. Therefore, the establishment of *J. curcas* as a commercially viable culture requires the development of a suitable genetic breeding program (Argollo Marques et al., 2013).

J. curcas is a xenogamic plant, highly heterozygous for most characteristics, which implies a high degree of segregation. Consequently, breeding programs require several years to obtain a cultivar. Biotechnological techniques such as tissue culture, genetic engineering, and molecular genetic analysis can be integrating into breeding programs to shorten this time (Argollo Marques et al., 2013).

Genetic diversity is defined as the variety of alleles or genotypes in a given population and may be reflected in morphological, physiological, and behavioral differences among individuals and populations. The establishment of a germplasm bank representing the genetic variability of the species (core collection) is essential to the success of the breeding program.

Assessment of genetic diversity in *J. curcas* germplasm has been carried out by morphological markers (Montes Osorio et al., 2014; Pazeto et al., 2015). Other authors have found large variations in chemical and morphological traits including oil content (27.8-39.0%) and seed weight (44-79 g) in Indian accessions from different geographical origins (Rao et al., 2008). Brazilian accessions have shown variability in plant size, fruits per bunch, the content of oil and phorbol esters, and fatty acid composition (Argollo Marques et al., 2013). Nevertheless, environmental effects can influence the expression of the traits, altering the efficiency of such markers. In turn, molecular markers (specific DNA segments that are representative of differences at genomic level) allow the exploration of genetic polymorphism between individuals, whether or not correlated with phenotypic expression, because they are not affected by the environment or by pleiotropic effects or epistasis (Mondini et al., 2009).

Molecular markers like RAPD (randomly amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), SSR microsatellites (simple sequence repeat), ISSR

(inter-simple sequence repeat), among others have been employed to assess genetic diversity in *J. curcas* germplasm from different parts of the world (Basha and Sujatha, 2007; Ganesh-Ram et al., 2008; Grativol et al., 2011; Tanya et al., 2011; Sudheer et al., 2011; Montes et al., 2014; Pecina-Quintero et al., 2014). Most of these studies have reported narrow diversity in germplasm (Basha and Sujatha, 2007; Sun et al., 2008; Shen et al., 2010), independent from the marker system used, which indicate the need to broaden the genetic base in these countries. Assessing genetic variation by RAPD, AFLP, and combinatorial tubulin-based polymorphism (cTBP) in 38 *J. curcas* accessions from 13 countries on 3 continents revealed narrow genetic diversity. However, 6 different species of *Jatropha* from India exhibited pronounced genetic diversity indicating possibilities of improving *J. curcas* by interspecific breeding (Popluechai et al., 2009). On the other hand, wide genetic diversity has been observed in materials from Mexico and other countries of Central America (Pamidimarri and Muppala, 2014; Pecina-Quintero et al., 2014; Santos et al., 2016).

In Brazil, some studies using ISSR markers (Grativol et al., 2011) and cTBP markers (Breviario et al., 2007) revealed reduced genetic diversity within and between Brazilian accessions from different origins. In contrast, Pioto et al. (2015) and Rosado et al. (2010) using AFLP and RAPD markers, respectively, reported low genetic diversity on accessions from São Paulo and other Brazilian states.

The lack of genetic diversity in most of the countries where *Jatropha* is distributed is implicated in the slowing of progress in developing new *Jatropha* cultivars with improved yield and quality potential. To broad the *Jatropha* genetic base, this may be accomplished by a collection of available germplasm or by the introgression of new genetic materials (genes) through inter- and intra-specific hybridization that will increase the genetic base and allow the development of new cultivars (Argollo Marques et al., 2013). The present study aimed to assess genetic diversity of elite *J. curcas* germplasm for breeding purposes using molecular markers. The results will allow understanding the genetic relationships among elite accessions from IAC (Agronomic Institute of São Paulo State) germplasm bank for guiding crossing and aid the creation of new genotypes. Besides, the evaluation of the system markers would help in future studies to rapidly evaluate the breeding material.

MATERIAL AND METHODS

We analyzed 66 genotypes comprising 56 accessions of *J. curcas* previously shown to be phenotypically diverse, one F1 interspecific hybrid (*J. curcas* x *J. integerrima*) and 8 backcrosses of *J. curcas/J. curcas/J. integerrima*, and the congeneric species *J. integerrima.* All accessions belong to physic nut germplasm of IAC located at Campinas, SP (Table 1).

DNA extraction

Genomic DNA was isolated from young leaves pulverized in N2 liquid using the CTAB protocol described by Doyle and Doyle (1990). Approximately 100 mg plant tissue was suspended in 800 µL extraction buffer (1.4 M NaCl, 20 mM EDTA, pH 8.0, 100 mM Tris-HCl, pH 8.0, 1% Polyvinylpyrrolidone MW 10,000, 2% CTAB and 0.2% β-mercaptoethanol) and incubated at 65°C for 60 min. Then, we added 800 µL chloroform/isoamyl alcohol 24:1 (v/v) and centrifuged at 10,000 *g* for 10 min. This step was performed twice. DNA was precipitated with 70% (v/v) ice-cold isopropanol and centrifuged for 10 min at 10,000 *g*. The pellet was washed with 1 mL 70% ethanol and dried at room temperature. DNA was suspended in 50 µL

TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA). The integrity of the DNA extracted was evaluated on 1% agarose gel and the quantity determined by NanoVue spectrophotometer (GE Healthcare).

Table 1. *Jatropha* spp accessions analyzed in this study and their origins.

ID	Genotype	Location	Group	ID	Genotype	Location	Group
1	*J. integerrima*	Unknown	Wild	34	L12P8	Minas Gerais	Brazil southeast
2	L6P7	Mato Grosso do Sul	Brazil midwest	35	L12P9	Minas Gerais	Brazil southeast
3	L6P25	Mato Grosso do Sul	Brazil midwest	36	L4P44	São Paulo	Brazil southeast
4	L6P31	Mato Grosso do Sul	Brazil midwest	37	L4P50	São Paulo	Brazil southeast
5	L12P41	Tocantins	Brazil midwest	38	L13P48	Sao Paulo	Brazil southeast
6	L13P30	Pernambuco	Brazil northeast	39	L3P29	São Paulo	Brazil southeast
7	PARÁ6	Pará	Brazil northeast	40	L2P21	São Paulo	Brazil southeast
8	L8P54	Bahia	Brazil northeast	41	L3P27	São Paulo	Brazil southeast
9	PARÁ5	Pará	Brazil northeast	42	L5P48	São Paulo	Brazil southeast
10	L13P20	Pernambuco	Brazil northeast	43	L11P7	São Paulo	Brazil southeast
11	L13P25	Pernambuco	Brazil northeast	44	L12P23	Minas Gerais	Brazil southeast
12	L12P51	Pernambuco	Brazil northeast	45	L12P29	Minas Gerais	Brazil southeast
13	L13P4	Pernambuco	Brazil northeast	46	L13P55	São Paulo	Brazil southeast
14	L9P1	Bahia	Brazil northeast	47	L17U8(3)	São Paulo	Brazil southeast
15	L9P32	Bahia	Brazil northeast	48	L17U5(2)	São Paulo	Brazil southeast
16	L8P29	Bahia	Brazil northeast	49	L14P1	São Paulo	Brazil southeast
17	L2P39-A	Fortaleza	Brazil northeast	50	L9P51	Minas Gerais	Brazil southeast
18	L12P57	Fortaleza	Brazil northeast	51	L2P7-A	China	China
19	L13P9	Pernambuco	Brazil northeast	52	L1P40-A	China	China
20	L13P46	Pernambuco	Brazil northeast	53	MÉXICO-A4	Mexico	Mexico
21	L9P44	Minas Gerais	Brazil southeast	54	MÉXICO-A3	Mexico	Mexico
22	L9P49	São Paulo	Brazil southeast	55	MÉXICO-A20	Mexico	Mexico
23	L6P11	São Paulo	Brazil southeast	56	MÉXICO-A10	Mexico	Mexico
24	L12P52	São Paulo	Brazil southeast	57	MÉXICO-A5	Mexico	Mexico
25	L4P49	São Paulo	Brazil southeast	58	RC1F1-1	Backcross	Breeding program
26	L4P19	São Paulo	Brazil southeast	59	RC1F1-91	Backcross	Breeding program
27	L2P23	São Paulo	Brazil southeast	60	RC1F1-74	Backcross	Breeding program
28	L2P19	São Paulo	Brazil southeast	61	RC1F1-25	Backcross	Breeding program
29	L1P7	São Paulo	Brazil southeast	62	RC1F1-9	Backcross	Breeding program
30	L10P5	Minas Gerais	Brazil southeast	63	RC1F1-64	Backcross	Breeding program
31	L12P4	São Paulo	Brazil southeast	64	RC1F1-37	Backcross	Breeding program
32	L12P2	São Paulo	Brazil southeast	65	RC1F1-100	Backcross	Breeding program
33	L11P5	São Paulo	Brazil southeast	66	Hybrid F1	*J. curcas/J. intergerrima*	Breeding program

ISSR analysis

Ten ISSR primers previously shown to be polymorphic for *J. curcas* were used for the genotyping (Table 2). PCRs were performed in a final volume of 20 µL containing 40 ng DNA, 1X buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl), 0.25 mM dNTPs, 2.5 mM $MgCl_2$, 0.25 mM of each primer, and 1 U Taq polymerase (Fermentas, MBI). PCRs were run with initial denaturation at 94°C for 1 min, followed by 35 cycles of denaturation at 94°C for 45 s, 45 s annealing at 48°-56.5°C depending on the primer, a 1 min extension at 72°C, and finally 72°C for 5 min. Amplified products were analyzed by electrophoresis on 1.5% agarose gel in 0.5X TBE buffer, pH 8.3. Gene Ruler Mix of 1000 bp (Fermentas, USA) was used as a molecular weight marker. DNA fragments were visualized under UV light on an AlphaImager photodocumentation system (AlphaImager System for Cell HP® Biosciences).

Data analysis

The profiles of ISSR markers were scored for band presence (1) or absence (0) to create a binary matrix used to calculate the Jaccard similarity coefficient. Dendrograms

were constructed using the UPGMA method (unweighted pair group method with arithmetic average) in NTSYS-pc 2.2 (Rohlf, 1989).

Genetic diversity measures were calculated among genotypes and between groups (populations) formed according to accession origin (Table 1). Genetic diversity was calculated using the Nei's index (*H*) and Shannon index (*I*), and obtained values of total heterozygosity (H_T), mean heterozygosity within populations (H_S), diversity among populations (D_{ST}), coefficient of population differentiation (G_{ST}), and gene flow (*N*m), using the POPGENE software 1.3.2 (Yeh et al., 1999).

The STRUCTURE software 2.3 (Pritchard et al., 2000) was used to define the most likely number of groups (K) in the samples. The determination of the K number was based on 50,000 Markov Chain Monte Carlo simulations; with burn-in of 500,000 and 20 interactions for each group (K), ranging from one to ten. The most apparent determination with the proposed ones was performed using ΔK, according to Evanno et al. (2005), using the Structure Harvester tool (Earl and VonHoldt, 2012).

RESULTS

ISSR polymorphism analysis

Ten ISSR combinations produced 271 polymorphic bands, with 98% of them polymorphic. The number of polymorphic bands per primer ranged from 11 (MANNY) to 37 (ISSR1 and ISSR3), with an average of 22.7 (Table 2), with amplification products ranging from 100 to 2500 bp. An example of the ISSR profile is shown in Figure 1.

Table 2. Primer sequence, annealing temperature, and polymorphic bands of ISSR primers used to assess the genetic diversity of *Jatropha* spp.

ISSR primer	Primer sequence (5'-3')	Annealing temperature (°C)	Polymorphic bands (PB)	% PB
ISSR 1	AGAGAGAGAGAGAGAGT	53.0	3	97.3
ISSR 2	AGAGAGAGAGAGAGAGYT	48.0	23	100
ISSR 3	AGAGAGAGAGAGAGAGYA	53.0	37	97.3
ISSR 5	ACACACACACACACACYA	56.5	32	100
ISSR 6	GACAGACAGACAGACA	53.6	18	100
ISSR 7	DBDACACACACACACACAC	48.2	17	100
ISSR 898	CACACACACACARY	52.0	17	100
OMAR	GAGGAGGAGGAGRC	52.0	16	94.2
MANNY	CACCACCACCACRC	52.0	11	100
TERRY	GTGGTGGTGGTGRC	52.0	19	100

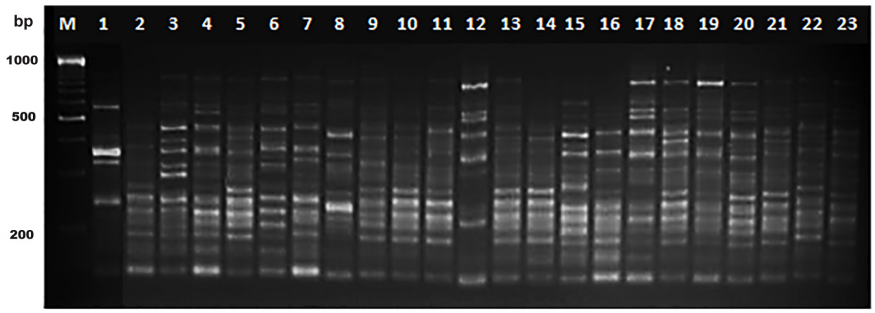

Figure 1. ISSR profile of the 23 accessions of *Jatropha* spp (*lanes 1-23*) shown in Table 1 and amplified by ISSR1 primers as in Table 2. *Lane M* = marker.

Genetic diversity and cluster analysis

Jaccard similarity coefficient from the ISSR marker ranged from 0.55 between genotypes of *J. curcas* MÉXICO-A3 and *J. integerrima* to 0.86 between genotypes L8P29 and L11P7. The average of the 66 accessions was 0.59. Regarding the geographic regions adopted, the similarity coefficient was 0.63 and 0.58 for Brazilian and Mexican accessions, respectively. The clustering dendrogram of Figure 2 separated the genotypes into two main clusters. The congeneric species *J. integerrima* was clearly separately from all *J. curcas* genotypes. A large group includes all *J. curcas* accessions evaluated as also the backcrosses. The F1 hybrid was assigned to a subcluster between *J. integerrima* and *J. curcas*; however, closer to *J. curcas*. In cluster I, the accessions of the different geographical origins did not cluster together. Accessions from Mexico were distributed into several subgroups of dendrograms (Figure 2).

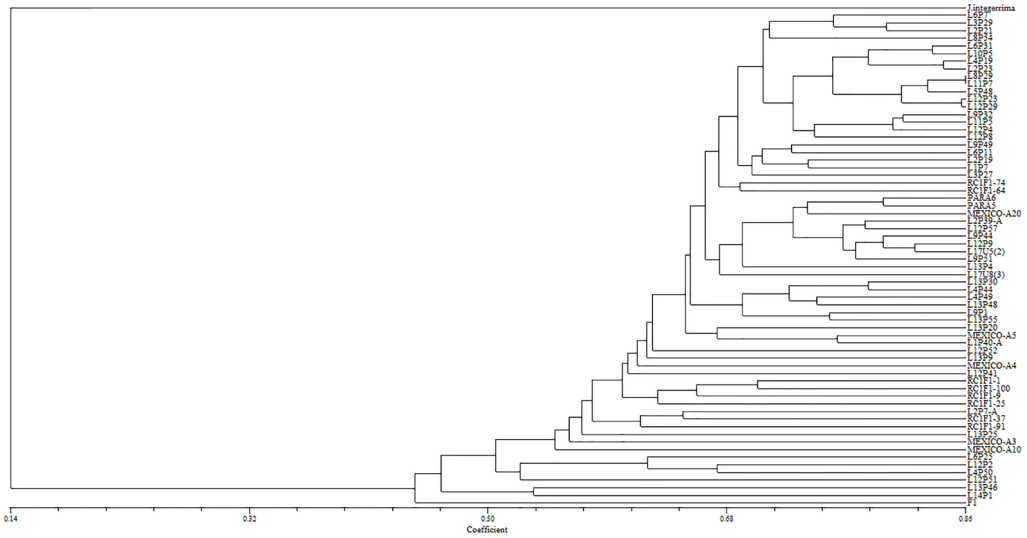

Figure 2. Dendrogram of 66 *Jatropha* accessions obtained by UPGMA based on Jaccard's genetic similarity coefficient using ISSR data.

Genetic diversity among Jatropha groups

At the group level proposed in Table 3 and assuming Hardy-Weinberg equilibrium, the total genetic diversity (Nei, 1972) was 0.14 (Table 3). There was a differentiation between groups (G_{ST} = 0.59) and a high level of gene flow (Nm = 0.34). The genetic structure of the groups (UPGMA) was calculated using the Nei's genetic distance (1972). The dendrogram (Figure 3) shows two main clusters that separate wild species from other genotypes of *J. curcas*. A greater genetic identity was found between the groups from southeastern and northeastern Brazil (0.99). The group of accessions from Mexico was more similar to the Brazilian accessions than to the Chinese group.

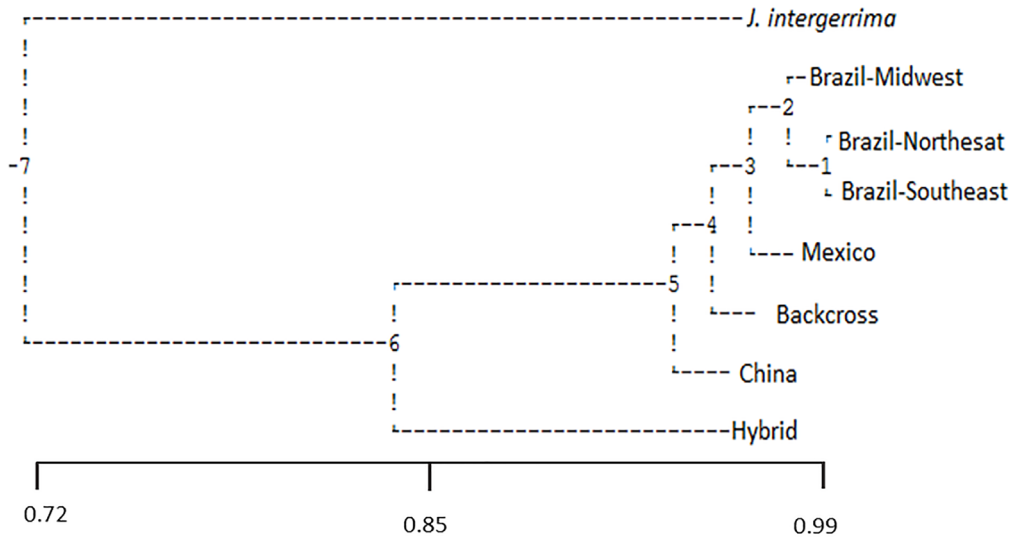

Figure 3. Dendrogram of the *Jatropha* groups based on Nei's similarity coefficient.

Table 3. Genetic parameters of intra- and intergroup genetic diversity among the five *Jatropha* groups by ISSR markers.

Group	ISSRs				
	N	N_A	N_E	*H*	*I*
J. integerrima	1	1.00	1.00	0.00	0.00
Brazil midwest	4	1.23	1.17	0.09	0.14
Brazil northeast	15	1.43	1.18	0.11	0.18
Brazil Southeast	30	1.4	1.15	0.09	0.15
Mexico	5	1.23	1.15	0.09	0.13
China	2	1.09	1.09	0.04	0.06
Backcrosses	8	1.31	1.2	0.11	0.17
Hybrid	1	1.00	1.00	0.00	0.00
Total	66	1.76	1.19	0.12	0.20

N = samples size; N_A = observed number of alleles; N_E = effective number of alleles, Kimura and Crow (1964); *H* = Nei's (1972) gene diversity; *I* = Shannon's information index, Lewontin (1972).

Genetic structure of *Jatropha* spp accessions

The diversity structuring of the 66 genotypes without a previous hierarchization, using Bayesian analysis revealed three genetic groups (K = 3) as shown in Figure 4, where each group was represented by a different color.

Groups I (red) and II (green) showed a partial association of accessions from different geographical regions. The group I was made up of five accessions from three different geographical regions of Brazil, one from Mato Grosso, one from Pernambuco, and tree from São Paulo. The group II was formed by 57 accessions, mainly from Brazil, five accessions from Mexico, two Chinese accessions, and five backcross genotypes. The group III (blue) was clearly distinct by joining the *J. integerrima*, the hybrid F1, one Backcross, and one accession from Pernambuco, Brazil.

The results of the genetic structure showed that there was not a strong relationship between the genetic structure and the geographical origin. This result was found by the UPGMA analysis. A proportion of genetic recombination was found. For example, accessions 12 (L12P51) and 49 (L14P1), showed a mixture of the three groups, while 38 (L13P48), 46 (L13P55), 36 (L4P44), and 6 (L13P30) exhibited a mixture of groups I and II, in approximately equal proportions and the backcrosses; 58 (RC1F1-1), 59 (RC1F1-91), 61 (RC1F1-25), 62 (RC1F1-9), 64 (RC1F1-37), and 65 (RC1F1-100) were admixed but with a stronger adherence to the *J. curcas* group.

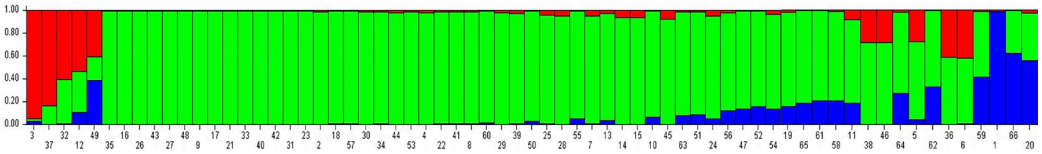

Figure 4. Bayesian admixture proportion of individual accessions of *Jatropha* spp for a K = 3 population model. The groups are indicated by different colors.

DISCUSSION

The characterization of the genetic diversity of the *Jatropha* germplasm is critical to establish strategies for conservation and genetic breeding. Molecular markers based on differences in nucleotide sequence are widely applied in studies of germplasm characterization, which allows eliminating the drawbacks of a selection based solely on the phenotypic analysis (Mondini et al., 2009) and have been employed to assess genetic diversity in several studies with *Jatropha* (Montes et al., 2014; Pecina-Quintero et al., 2014; Pioto et al., 2015; Mavuso et al., 2016).

In this study, ISSR markers were used to characterize the genetic structure and diversity of the germplasm content in *J. curcas* genotypes from different Brazilian states and countries as well hybrids between *J. curcas* and *J. integerrima* species (F1 and backcross generations).

The ISSR marker was adopted because it can produce multilocus profiles widely spanning the genome even in the absence of prior genetic information (Eguiarte et al., 2007). In the present study, the ISSR markers used detected high levels of locus polymorphism (98.9%) with an average of 22.7% per primer. The high degrees of polymorphism found are comparable with those registered in other studies on *Jatropha* using ISSR markers (Murty et al., 2013; Mavuso et al., 2016). Besides, the ISSR marker showed to be capable of differentiating the accessions, where the average similarity coefficient was 0.59. Since ISSR generates high levels of polymorphism, it could be a method of choice for discriminating genotypes closely related and with a narrow genetic base as in the case of *J. curcas*.

The genetic diversity found in the present study was greater than that reported in studies conducted in China (Shen et al., 2010), Malaysia (Sun et al., 2008), India (Mastan et al., 2012), Taiwan (Mavuso et al., 2016), Thailand (Sirisak et al., 2015), and even studies conducted in other germplasm banks in Brazil (Rosado et al., 2010; Grativol et al., 2011; Lira-Medeiro et al., 2013; Alves et al., 2013). The genetic diversity among groups analyzed by the Nei's index (1987) was significantly higher (0.12) than that found among Chinese accessions (0.19) as reported by Cai et al. (2010).

Genetic relationships among accessions are important for selection activities, the formation of work collection, and to direct crosses between accessions with complementary agronomic traits. Dendrogram obtained by the UPGMA method (Figure 2) revealed two major groups at *Jatropha* germplasm: one made up of wild accession and another group with accessions of *J. curcas* from Brazil, Mexico, China, and the improved Backcross genotypes. Similar results were reported by Ganesh-Ram et al. (2008). Our dendrogram revealed that Mexican genotypes were not grouped in a separately and single cluster. Instead, they were distributed into different subgroups together with the accessions from different Brazilian states and the two Chinese genotypes, sharing a genetic identity. Although underrepresented in this study, Mexican accessions dispersed in the dendrogram indicate the existence of a wide genetic variability, suggesting that more intensive utilization of Mexican germplasm could allow broadening of diversity in *J. curcas* breeding programs.

The dendrogram showed no clear grouping of the accessions according to geographical origins. Similar results were found by Pazeto et al. (2015); Rosado et al. (2010), and Shen et al. (2010). A lack of correspondence between the molecular classification and geographic origin of the accessions could be due to the possible common origin of accessions. This result was corroborated by the Bayesian analysis, which grouped all the accessions into three major groups with some genetic recombination among groups. Although the large geographical distance between the Brazilian regions, *J. curcas* accessions representing these regions were gathered in groups I and II (Figure 4) indicating the possibility of allele exchange between accessions of these groups.

Mexico is considered the center of origin of *J. curcas*, which has spread to other continents mainly by the Portuguese during the formation of their colonies (Pamidimarri and Muppala, 2014; Guo et al., 2016). As the center of origin of the species, the germplasm around the world is derived from Mexico, and a high similarity among them is expected. Moreover, the occurrence of migration or exchange of genetic material by human activities, favoring introgression of genes between accessions and the sharing of alleles could explain the lack of a strong genetic structure among the accessions even from different countries.

Genetic diversity among groups estimated by the Nei's index (1987) was higher (0.12) when compared to the values found for Chinese accessions (0.19) reported by Cai et al. (2010). Even, the values were higher (0.28) than those observed by Grativol et al. (2011) for Brazilian accessions. The genetic differentiation coefficient (G_{ST} = 0.59) indicating that the total variation is due mostly to the distinction between groups rather than among groups. Biabani et al. (2012) verified lower values of differentiation using ISSR and AFLP markers in populations of *J. curcas* from Asia. Gene flow (0.72) estimated was considered low, which favored the group differentiation. These results could be attributed to low levels of cross-pollination among groups.

The closest genetic relationships among accessions from the Northeast and Southeast regions of Brazil can be due to the founder effect; a few genotypes gave rise to materials that have spread across the country, mainly through vegetative propagation and consequently fixation of genotypes. Meanwhile, the low diversity among these accessions may also be a result of the selection practice of farmers to obtain plants with higher yield, uniformity, oil content, and other traits of agronomic interest, which associated with vegetative propagation, cause narrowing of the genetic base. The low genetic differentiation among groups represented by genotypes from different regions of Brazil and the group of accessions from Mexico may indicate that Brazilian accessions have originated from the Mexican genetic background, as

mentioned before and supporter by Pamidimarri and Muppala (2014); Pazeto et al. (2015) and Guo et al. (2016) .

The challenge of all plant genetic breeding programs is to maintain the genetic diversity within the target species while improving desired traits that enable plant materials to perform well. To broaden the genetic diversity of *J. curcas,* interspecific hybridization is presented as a promising method to transfer the diversity conserved in the related species with introgression of desirable traits. This methodology can ensure the success of future breeding improvement programs.

Sudheer et al. (2011) and Sirisak et al. (2015) have identified *J. integerrima* and *J. gossypiifolia* as the most genetically close accessions of *J. curcas*.

One of the approaches for *J. curcas* genetic improvement used by our team was the introgression of genes from related wild species into the genetic background of target *J. curcas* seeking to improve agronomic traits such as biotic and abiotic tolerance. *J. integerrima*, highly tolerant to pests and disease, was used as male parental genotypes in the crossing aiming the transfer this trait *J. curcas* using backcrosses. The UPGMA (Figure 2) among accessions and groups showed that interspecific hybrid F1 of the study is closely related to *J. curcas* than *J. integerrima* indicating that the maternal species is the probable donor of the major part of the genome. However, it was separated from the rest of the accessions of *J. curcas* showing genetic differentiation.

Backcrossing is a breeding strategy to transfer a characteristic from a donor into the genomic background of a recurrent parent. The proportion of genome from the donor parent tends to zero as generations accumulate, except for the part hosting the characteristic of interest. Based on the UPGMA and Structure results, the backcrosses evaluated were genetically closer to the recurrent genitor (*J. curcas*) as desired to the *J. curcas* plant breeding.

CONCLUSIONS

The success on breeding programs is largely dependent on understanding the genetic diversity of the germplasm. Various studies using molecular markers had been approaching showing low to moderate genetic diversity in *Jatropha* germplasm around the world. Our results evidence high genetic diversity in the germplasm evaluated compared whit the germplasm from other countries. The Mexican germplasm and the wild related *Jatropha* species represented a large and important source to broaden the genetic diversity. Also, the use of *J. curcas* x *J. integerrima* interspecific hybrid was demonstrated by the ISSR marker as a way to broaden the genetic diversity of *J. curcas*.

Clearly, these findings improved our knowledge about the situation of *J. curcas* diversity in Brazil and led to appropriate information, which is useful for the successful management of germplasm on the breeding programs.

Overall, the present results confirm the usefulness of ISSR markers for characterization and genetic diversity analysis of *J. curcas* accessions.

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