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Genetic diversity in *Jatropha* species from different regions of Brazil based on morphological characters and inters-simple sequence repeat (ISSR) molecular markers

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Jatropha **belonging to Euphorbiaceae family has around 170 species distributed throughout tropical semi-arid regions of Africa and the Americas. Some of its species include *Jatropha curcas* L. (Physic Nut), *Jatropha pohliana* Müll.Arg. (Brazilian Purging Nut) and *Jatropha gossypiifolia* L. (Black Physic Nut). Phenotypic and genetic studies of a population are important for plant improvements, helping in the characterization of accesses, as well as facilitating selection of parental for directed crossings. Thus, the present study aimed to evaluate accesses of three *Jatropha* species from different regions of Brazil, through morphological characters and ISSR molecular markers, in order to group them according to the existing degree of divergence. A higher interspecific variability, rather than intraspecific, was observed among accesses, for morphological as well as molecular characters. Qualitative and quantitative characters showed variability between accesses and may serve as reference for future genetic studies. There was no relation between similarity patterns and geographical origin of accesses in the group analysis. Average percentage of polymorphism found for inter-simple sequence repeat (ISSR) markers between the studied accesses was 40.6%. A higher genetic variability interspecific was observed than intraspecific, suggesting the search for genetic variability among species *Jatropha* through interspecific crosses.**

Key words: Germplasm, *Jatropha curcas*, *Jatropha gossypiifolia*, *Jatropha pohliana*, variability.

INTRODUCTION

Jatropha (Euphorbiaceae) is represented by around 170 species, distributed throughout tropical semi-arid regions of Africa and the Americas. Dehgan and Webster (1979) recognized two subgenres (*Jatropha* and *Curcas*) and 10

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sections and subsections based on their morphological and intergeneric relations. Some of the genre's species are *Jatropha curcas* L. (Barbados Nut), *Jatropha pohliana* Müll.Arg. (Brazilian Purging Nut), and *Jatropha gossypiifolia* L. (Black Physic Nut). These species, classified as autogamous, have been studied due to the high oil content of their seeds, which represent an important source for plant biofuel production. *J. curcas* L. and *J. pohliana* Müll.Arg., seeds may contain an average of 33 to 38% (Nunes et al., 2008), and 25% (Targino and Silva, 2013) oil, respectively. While, *J. gossypiifolia* L. contains about 23% (Oliveira et al., 2006). Biofuel is a generic name for fuels and additives derived from renewable sources. Compared to petroleum-derived diesel, biofuel may reduce up to 78% CO_2 emission, considering reabsorption by plants (Accarini, 2006). Furthermore, it decreases smoke emission by 90% and practically eliminates sulfur dioxide emission (Sousa, 2006). Nowadays, little is known about genetic divergence (or genetic dissimilarity) between these species genotypes, which would be of great use to the selection of parentals for the creation of segregant populations, as well as for the maintenance of genetic diversity in improvement programs and germplasm banks. These estimates reveal availability of alternative alleles for characters of interest, which are the base for long-term selection gain. Crossings between genetic divergent parentals provide a much higher genetic variability between progenies than that obtained from the crossing of plants more closely related and, thus, increase opportunities of breeders obtaining superior progenies (Messmer et al., 1993). To this end, the creation of a germplasm bank which includes promising accesses that are mutually divergent is capital, warranting the need of further knowledge about the genetic diversity found in natural populations (Cruz et al., 2012).

Molecular markers constitute an important technological instrument in processes for the selection and increase of genetic variability, chiefly when associated with the phenotypic analysis of populations. Among those available and widely used for characterization of genetic diversity in a population, we have inter simple sequence repeat (ISSR), which are dominant markers, meaning they do not allow differentiation between homozygotes and heterozygotes. However, they are excellent tools to be used in initial studies on the genetic diversity due to its nonspecificity. Studies of this nature with *Jatropha* species are lacking in literature. Divakara et al. (2010), in a revision of biological aspects and improvement of *J curcas*, presents nine references of studies that used molecular markers to characterize accesses of *J. curcas* L., among those, Basha and Sujatha (2007) evaluated 42 *J. curcas* L. accesses from different regions in India. These authors used random amplified polymorphic DNA (RAPD) and ISSR markers to determine genetic diversity and reported on the immediate need to increase the genetic base of *J. curcas* L. in India, due to the low genetic diversity found between accesses. Góis et al. (2006) evaluated eight accesses in Minas Gerais, four in Goiás, one in Espírito Santo, and one in Sergipe, through the isoenzyme technique, finding highly similar accesses and others quite divergent. Therefore, the aim of this study was to evaluate genetic diversity in three species of *Jatropha* genre from different regions of Brazil, using morphological characters and ISSR molecular markers.

MATERIALS AND METHODS

Plant material

A total of 84 accesses were used, 76 *J. curcas* (Barbados Nut), seven *J. pohliana* (Brazilian Purging Nut) and one *J. gossypiifolia* (Black Physic Nut), for the most part belonging to the Banco Ativo de Germoplasma (Active Germplasm Bank) from Embrapa Algodão (Embrapa Cotton), Campina Grande – PB, and also spontaneous plants collected in the Northeast and Southeast regions of Brazil (Table 1). Accesses were cultivated in an experimental area of the Department of Plant Production at UNESP, in the Jaboticabal Campus.

Field evaluation

Jatropha spp. accesses were organized according to the coordinates 21° 15' 29" S and 48° 16' 47" W at an average altitude of 614 m. The spacing used was 3 x 2 m, respectively, between lines and plants. Local weather is Cwa, humid temperate climate with dry and hot summer winter, according to the Köppen Climate Classification System; mean annual rainfall averages of 1425 mm, with mean temperature of 22.2°C and relative air humidity of 75% (annual). Seventeen morphological characters were analyzed, having as criteria descriptors used for castor bean (*Ricinus communis* L.), adapted for colorations in the studied species (Embrapa Cotton, 2008). The qualitative descriptors used were: 1) leaf pilosity: present and absent; 2) stem waxiness: present and absent; 3) color of young leaves, observing coloration between 80 to 120 days of planting: green, red and purple; 4) color of adult leaves, observing the second adult leaf below primary raceme, taking an average of ten random leaves: dark green and light green; 5) color of foliar veins, noting the adaxial side of three mature leaves selected randomly from ten plants: green, purple and red; 6) petiole leaf position: opposed, alternate and mixed; 7) type of plant ramification, during maturation of raceme: trifurcate, when there are two lateral branches, and from these, two more, giving the appearance of a trident; bifurcate, while from the stem there's one lateral branch, having the appearance of a bipartite stem; cup-like, when branches emerge from the stem forming a structure similar to a wine glass, and universal, when branches outweigh the primary raceme in height; 8) seed pattern: single color, painted, spotted and stripped; 9) shape of seeds: elongated, rounded, and flattened; 10) caruncle type: protuberant and non-protuberant; 11) fruit dehiscence: dehiscent, semi-dehiscent and indehiscent; and the quantitative descriptors were: 12) number of leaf lobes, taking an average of 10 adult leaves from the middle third of plants; 13) plant height in centimeters; 14) stalk diameter in centimeters; 15) maturation cycle, which corresponds to the number of days between germination and physiological maturity, considering: precocious (up to 275 days), average (from 276 to 306 days) and late (over 306 days); 16) length of seeds in centimeters, and 17) width of seeds in centimeters.

Table 1. Identification of the 76 *Jatropha curcas* accesses, seven *Jatropha pohliana* accesses and one *Jatropha gossypiifolia* access evaluated.

Identification	Code	Origin	Latitude	Longitude	Species
Acess 1	CNPA PM IV P1	Tocantinópolis-TO	06°19'S	47°24'W	*J. curcas*
Acess 2	CNPA PM IV P2	Tocantinópolis-TO	06°19'S	47°24'W	*J. curcas*
Acess 3	CNPA PM II P1	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 4	CNPA PM II P2	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 5	CNPA PM II P3	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 6	CNPA PM II P4	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 7	CNPA PM II P5	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 8	CNPA PM II P6	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 9	CNPA PM II P7	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 10	CNPA PM II P8	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 11	CNPA PM II P9	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 12	CNPA PM II P12	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 13	CNPA PM II P16	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 14	CNPA PM II P17	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 15	CNPA PM II P19	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 16	CNPA PM II P20	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 17	CNPA PM II P21	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 18	CNPA PM II P22	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 19	CNPA PM II P24	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 20	CNPA PM II P25	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 21	CNPA PM II P26	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 22	CNPA PM II P30	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 23	CNPA PM II P31	Garanhuns-PE	08°53'S	36°30'W	*J. curcas*
Acess 24	CNPA PM XI P1	Mundo Novo-PE	07°35'S	37°11'W	*J. curcas*
Acess 25	CNPA PM XI P2	Mundo Novo-PE	07°35'S	37°11'W	*J. curcas*
Acess 26	CNPA PM XI P3	Mundo Novo-PE	07°35'S	37°11'W	*J. curcas*
Acess 27	CNPA PM XI P4	Mundo Novo-PE	07°35'S	37°11'W	*J. curcas*
Acess 28	CNPA PM VII P1	Mundo Novo-PE	07°35'S	37°11'W	*J. curcas*
Acess 29	CNPA PM VII P2	Mundo Novo-PE	07°35'S	37°11'W	*J. curcas*
Acess 30	CNPA PM VII P6	Mundo Novo-PE	07°35'S	37°11'W	*J. curcas*
Acess 31	CNPA PM VII P7	Mundo Novo-PE	07°35'S	37°11'W	*J. curcas*
Acess 32	CNPA PM IX P1	Pugmil- TO	06°24'S	37°48'W	*J. curcas*
Acess 33	CNPA PM IX P2	Pugmil- TO	06°24'S	37°48'W	*J. curcas*
Acess 34	CNPA PM IX P3	Pugmil- TO	06°24'S	37°48'W	*J. curcas*
Acess 35	CNPA PM IX P4	Pugmil- TO	06°24'S	37°48'W	*J. curcas*
Acess 36	CNPA PM IX P5	Pugmil- TO	06°24'S	37°48'W	*J. curcas*
Acess 37	CNPA PM IX P6	Pugmil- TO	06°24'S	37°48'W	*J. curcas*
Acess 38	CNPA PM IX P7	Pugmil- TO	06°24'S	37°48'W	*J. curcas*
Acess 39	CNPA PM IX P8	Pugmil- TO	06°24'S	37°48'W	*J. curcas*
Acess 40	CNPA PM VIII P1	Alagoinha-PB	06°57'S	35°32'W	*J. curcas*
Acess 41	CNPA PM VIII P2	Alagoinha-PB	06°57'S	35°32'W	*J. curcas*
Acess 42	CNPA PM VIII P4	Alagoinha-PB	06°57'S	35°32'W	*J. curcas*
Acess 43	CNPA PM VIII P5	Alagoinha-PB	06°57'S	35°32'W	*J. curcas*
Acess 44	CNPA PM VI P1	Tocantinópolis-TO	10°42'S	48°54'W	*J. curcas*
Acess 45	CNPA PM VI P2	Tocantinópolis-TO	10°42'S	48°54'W	*J. curcas*
Acess 46	CNPA PM VI P3	Tocantinópolis-TO	10°42'S	48°54'W	*J. curcas*
Acess 47	CNPA PM VI P5	Tocantinópolis-TO	10°42'S	48°54'W	*J. curcas*
Acess 48	CNPA PM VI P6	Tocantinópolis-TO	10°42'S	48°54'W	*J. curcas*
Acess 49	CNPA PM VI P7	Tocantinópolis-TO	10°42'S	48°54'W	*J. curcas*
Acess 50	CNPA PM VI P8	Tocantinópolis-TO	10°42'S	48°54'W	*J. curcas*

Table 1. Contd

Acess 51	CNPA PM X P1	Marizópolis- PB	09°47'S	49°39'W	*J. curcas*
Acess 52	CNPA PM X P2	Marizópolis- PB	09°47'S	49°39'W	*J. curcas*
Acess 53	CNPA PM X P3	Marizópolis- PB	09°47'S	49°39'W	*J. curcas*
Acess 54	CNPA PM X P4	Marizópolis- PB	09°47'S	49°39'W	*J. curcas*
Acess 55	IT1	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 56	IT2	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 57	IT3	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 58	IT4	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 59	IT5	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 60	IT6	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 61	IT7	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 62	IT8	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 63	IT9	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 64	IT10	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 65	IT11	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 66	IT12	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 67	IT13	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 68	IT14	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 69	IT15	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 70	IT16	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 71	IT17	Ituverava- SP	20°20'S	47°46'W	*J. curcas*
Acess 72	JB1	Jaboticabal- SP	21°15'S	48°17'W	*J. curcas*
Acess 73	CG1	Campina Grande- PB	07°13'S	35°52'W	*J. curcas*
Acess 74	CG2	Campina Grande- PB	07°13'S	35°52'W	*J. curcas*
Acess 75	CG3	Campina Grande- PB	07°13'S	35°52'W	*J. curcas*
Acess 76	RB1	Riachão do Bacamarte- PB	07°14'S	35°39'W	*J. curcas*
Acess 77	GT1	Galante- PB	07°17'S	35°45'W	*J. pohliana*
Acess 78	GT3	Galante- PB	07°17'S	35°45'W	*J. pohliana*
Acess 79	GT4	Galante- PB	07°17'S	35°45'W	*J. pohliana*
Acess 80	GT5	Galante- PB	07°17'S	35°45'W	*J. pohliana*
Acess 81	GT6	Galante- PB	07°17'S	35°45'W	*J. pohliana*
Acess 82	GT7	Galante- PB	07°17'S	35°45'W	*J. pohliana*
Acess 83	RB2	Riachão do Bacamarte- PB	07°14'S	35°39'W	*J. pohliana*
Acess 84	GT2	Galante- PB	07°17'S	35°45'W	*J. gossypiifolia*

DNA extraction and amplification

The genomic DNA was extracted from leaves of each access and the methodology used varied according to the species. For *J. curcas* L. the protocol proposed by Doyle and Doyle (1990) was used with a few modifications described by Ferreira and Grattapaglia (1998). Due to singularities in *J. gossypiifolia* L. and *J. pohliana* Müll.Arg. DNA extractions for these species followed the methodology proposed by Elias et al. (2004) with modifications, based on CTAB extraction buffer, method (Dellaporta et al.,1983), in which leaves undergo a period of dehydration in a greenhouse at 60°C for 72 h, and are then macerated in a porcelain mortar, without the use of liquid nitrogen. The temperature and duration of the dehydration process proposed for cassava in the original method by Elias et al. (2004) were modified in order to expedite maceration, due to the fleshy character of *J. gossypiifolia* L. and *J. pohliana* Müll.Arg., leaves. One hundred ISSR primers from collection # 9 synthesized by the University of British Columbia (UBC) Biotechnology Laboratory in Canada, and described by Zietkiewicz et al. (1994) were used. Polymerase Chain Reaction (PCR) was carried out for all 84 *Jatropha* accesses. The reaction was carried out for a final volume of 25 µl containing 0.12 µl of Taq polymerase (5 units μl^{-1}), 2.5 µl 10 x amplification buffer (no Mg^{++}), 2.0 µl $MgCl_2$ (25 mM), 1.0 µl primer (10 µM), 0.5 µl dNTPs (2.5 mM each), 1.25 µl DNA (50 ng μl^{-1}), and 17.63 µl ultrapure water. The reaction was carried out in a Bio rad thermal cycler, according to the following parameters: denaturation step for 4 min at 94°C, followed by 35 denaturation cycles (92°C at 30 s), 1 min for the primer annealing at a specific temperature for each primer used, and extension for 2 min at 72°C. The final extension was extended to 7 min (Soares, 2010). Annealing temperatures used were determined through subjacent temperature gradient tests (Table 4). Subsequently, amplification products were separated by electrophoresis on 3% agarose gel treated with ethidium bromide, at 80 V and 400 mA, for approximately 3 h. Visualization was done on Bio Rad Gel Doc 2000 transilluminator and digitalized by Quantity One® software. The sizes of amplified fragments were estimated by comparison with the 100 pb DNA ladder molecular

Table 2. Description of qualitative characters adopted and frequency of occurrence of the categories in the 84 accesses of *Jatropha* spp. Jaboticabal, SP.

Descriptors	Categories	Relative frequency (%)	Descriptors	Categories	Relative frequency (%)
Leaf pilosity	Present	8	Type of plant ramification	Trifurcate	14
	Absent	92		Bifurcate	4
				Cup-like	79
Stem waxiness	Present	99		Universal	4
	Absent	1			
Color of young leaves	Green	7	Seed pattern	Single color	90
	Red	5		Painted	10
	Purple	88		Spotted	0
				Stripped	0
Color of adult leaves	Dark green	89	Shape of seeds	Elongated,	92
	Light green	11		Flattened	8
				Rounded	0
Color of foliar veins	Green	93	Caruncle type		
	Red	1		Protuberant	10
	Purple	6		Non-protuberant	90
Petiole leaf position	Opposed	0	Fruit dehiscence	Dehiscent	92
	Alternate	100		Semi-dehiscent	8
	Mixed	0		Indehiscent	0

weight marker.

Statistical analysis

Morphological and phenotypic analysis

Variables were initially standardized, preceding the multivariate statistical analysis for quantitative and qualitative characters. All analyzes were carried out using the Genes Software (Universidade Federal de Viçosa, Viçosa, MG, Brazil) (Cruz, 2013). Dissimilarity estimates were acquired by the Euclidean Distance function for quantitative characters, while morphological qualitative characters were obtained from Cole-Rodgers et al. (1997), where the traits, which cannot normally be ordered, can be analyzed as discrete quantitative traits (Cruz and Carneiro, 2006). With this index, a determined value expresses the percentage of coincidences considering the various characters being analyzed for each binary and multicategorical trait, contemplating concordance and discordance information from each class. Both clusters were done by the UPGMA (Unweighted Pair-Group Method Arithmetic Average) hierarchical clustering method.

Molecular evaluations

Polymorphism indicative binary matrices were formed from the results observed on the gel, based on the presence (1), or absence (0) of marks (bands) for each allele found. The matrix thus found was used for genetic distance calculations, based on dissimilarity measures the formation of clusters by the Unweighted Pair-Group Method Arithmetic Average (UPGMA) method, using the software Genes (Cruz, 2013). The obtained electrophoretic profiles will enable the similarity measures (Cii') to be converted to dissimilarity through the arithmetic complement (D), using the Jaccard Coefficient = a/a + b + c, where a is the number of concordances type 1-1, b is the number of discordances type 1-0 and c is the number of discordances type 0-1.(Cruz and Carneiro, 2006). The use of this coefficient was opted for not only due to its mathematical properties, but also because it enables intraspecific differences to be evinced and will not consider the absence of bands as synonym of genetic similarity.

RESULTS

Morphological and phenotypic data analysis

Observing frequency data on binary and multicategorical qualitative morphological characters evaluated for the 84 *Jatropha* accesses (Table 2), the existence of little phenotypic variability is noted, with many individuals concentrated in a single class. In order to carry out the multivariate analysis, the character for position of the leaves on the branches was removed, since all accesses presented the same pattern, that is, alternate leaves throughout the petiole. Plants presented glabrous leaves, with purple coloration when young and green in adulthood, green foliar veins, waxy stems; a character that possibly helps control excess loss of water under

long periods of drought. Other striking characteristics between accesses, also shown in Table 2, were the plant structure in a glass-like shape, alternate leaves throughout the branches, fruits containing three elongated seeds, black coloring, and non-protuberant caruncle. Most accesses presented dehiscent fruits, which indicate populations that are still wild. The *J. pohliana* access code GT1 (77), from Galante, Paraíba, presented glabrous leaves, differently than other *J. pohliana* accesses from this site, which have hairy adaxial and abaxial leaf surfaces. Similarly, GT1 presented young leaves with purple coloring, while other accesses from Galante had green young leaves. Access GT2, the single *J gossypiifolia* access, possesses morphological structures much different than *J. curcas* and *J. pohliana*, with small, delicate, glabrous, purplish leaves, rather exotic in appearance, with characteristics similar to ornamental plants. Accesses of all three species presented green adult leaves, with the exception of eight accesses from Garunhuns – PE and 1 access from Mundo Novo – PE, which had mostly discolored adult leaves. *J. curcas* accesses presented mostly leaves with green veins, whereas for *J. pohliana* accesses veins were purplish, with the exception of access RB2 (82), which had veins of red coloring. All plants from access GT2 (84), *J. gossypiifolia*, presented purplish veins, with darker, more intense purple veins in young, and lighter in adult leaves. Observing Figure 1 and considering a percentage of dissimilarity close to 34%, the formation of four distinct clusters is noted. Two of these groups were composed of *J. pohliana* accesses, where access 77 was isolated from the other accesses of the same species, 80, 81, 78, 79, 82 and 83, which in their turn composed a second group. The formation of a large group occurred, which encompassed all 76 *J. curcas* accesses. The *J. gossypiifolia* access constitutes the fourth group, isolated from the other *J. curcas* and *J. pohliana* accesses. Observing the group formed by *J. curcas* accesses it is possible to assume that accesses sharing the same origin have had greater proximity within the group. Analyzing maximum, minimum, average and standard deviation values for the five quantitative characters evaluated (Table 3), it is evident that the number of lobes character varied among the studied accesses, oscillating from 3 to 7 lobes. In regards to *J. curcas*, such variation was observed even in a single plant, in which case, the criteria adopted was ten adult leaves from the middle third of the plant. For *J. pohliana* accesses there was no such a variation because all the plants presented serrated, fleshy leaves with 5 lobes (Table 3). The GT2 (84) access, the only *J. gossypiifolia* sample, presented jagged leaves with 3 well defined lobes and it was the only one, among the 84 accesses studied, that did not present visible stem waxiness (Table 3). There was great variability in regards to plant height and diameter of the stem among the 84 accesses studied (Table 3), being that this difference are even greater among interspecific accesses. Plant height and stem diameter were, in general, quite regular between accesses of *J. curcas* with most accesses being over 100 cm in height, reaching up to 230 cm. The average diameter of the stem was around 5 cm (Table 3). Plants derived from Garanhuns – PE became taller than the other accesses of *J. curcas.* Accesses of *J. pohliana* presented an average height superior to all accesses of the other studied species, often reaching 2.00 m in height.

However, the diameter of the stem remained close to the average of the *J. curcas* accesses. *J. gossypiifolia* plants, access GT2 (84), are visibly smaller than the other accesses, yet their stem diameter is close to *J. curcas* and *J. pohliana*. In regards to the maturation cycle, it is possible to recognize a great variability among accesses, with a minimum of 240 and maximum of 342 days (Table 3). As for the seeds, values largely varied among accesses, ranging from 0.82 and 0.11 cm, maximum of 1.93 and 1.24 cm and average 1.76 and 1.08 cm, respectively for length and width (Table 3). Observing the dendrogram formed based on quantitative characters (Figure 2) and taking clustering criteria at the same percentage of genetic dissimilarity of 34%, the formation of 3 distinct groups is noted, where only access 84 (*J. gossypiifolia*) and 83 (*J. pohliana*) formed isolated groups. *J. pohliana,* access 83, originated from the state of Paraíba, in the region of Riachão do Bacamarte, was isolated from other accesses of this species mainly due to the reduced size of its seeds, both in length (average 1.12 cm) and width (average 0.11 cm), values quite similar to those observed for *J. gossypiifolia* seeds, 0.82 cm and 0.50, respectively, for length and width. All other accesses of *J. pohliana* (77, 78, 79, 80, 81, and 82) were allocated along with the 76 *J. curcas* accesses forming a single group. However, it is possible to see that *J. pohliana* accesses remained together in the lower extremity of the dendrogram and the same happened to *J. curcas* accesses which were allocated in the top extremity of the cluster, always mutual neighbors, meaning there was no mixture of accesses of different species within the group. Such results show the low variability found among accesses of the same species and that only accesses of different species had morphological differences.

Molecular data analysis

Of all 100 ISSR markers used, 43 were selected because they presented quantity, quality and reproducibility of amplified bands. All 43 primers presented polymorphism for the 84 studied accesses. A total of 451 bands were produced (Table 4), of which 185 (39.6%) were polymorphic, revealing an intermediate level of similarity among accesses. The average genetic dissimilarity among accesses of each species and between species was high (Table 5). The *J. gossypiifolia* species,

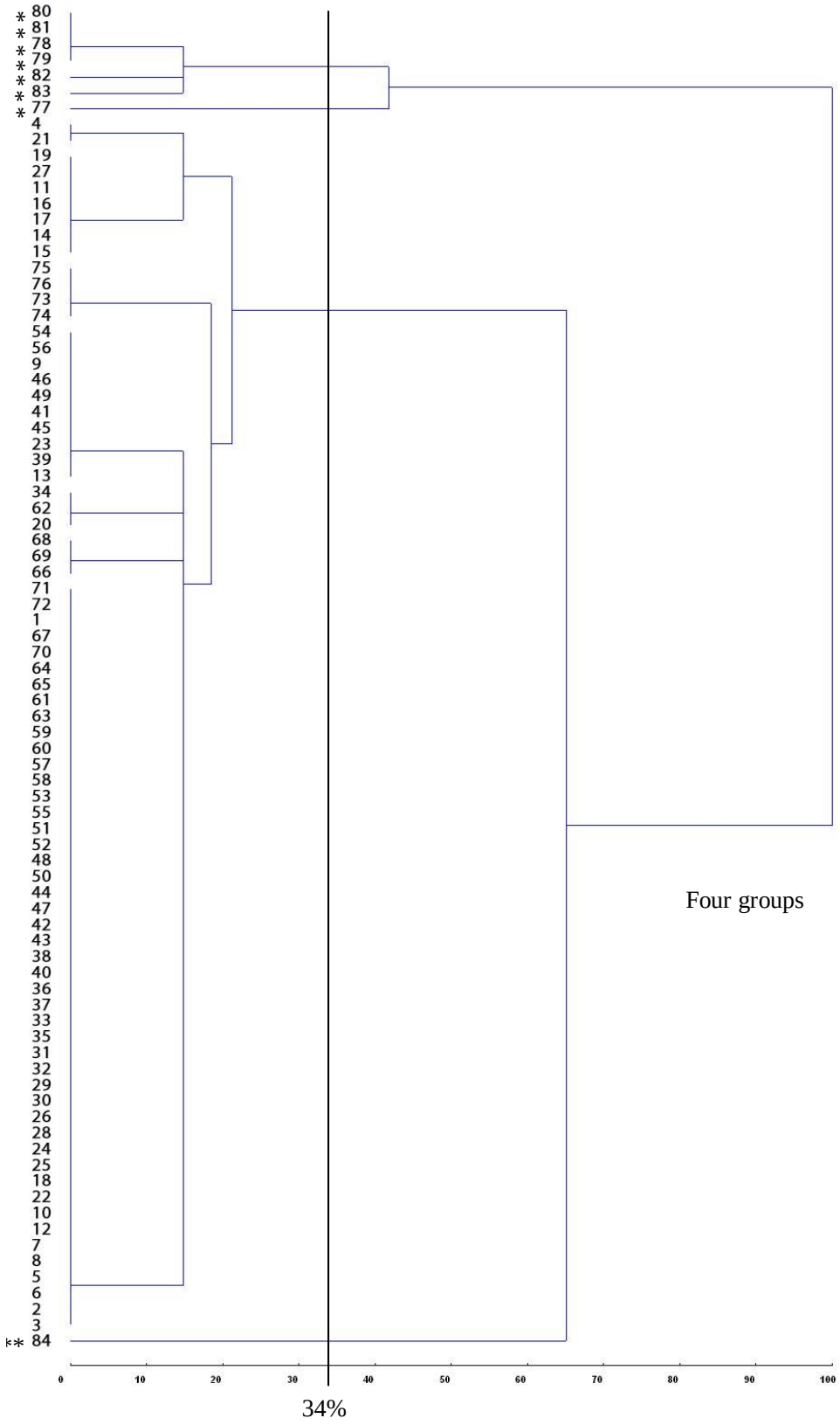

Figure 1. Dendrogram by UPGMA method, originated from the dissimilarities of the 84 accessions of *Jatropha* spp., based on qualitative morphological characters. The numbering of the access matches that specified in Table 1. Unmarked, *J. curcas; *J. pohliana; **J. gossypiifolia*

Table 3. Minimum values, mean, maximum and standard deviations for the quantitative characters used in the evaluation of the 84 accessions of *Jatropha* spp.

Quantitative descriptors	Values			Standard deviations
	Minimum	Mean	Maximum	
Number of leaf lobes	3.00	6.00	7.00	0.75
Plant height (cm)	74.80	117.05	230.00	28.91
Stalk diameter (cm)	4.18	5.31	6.18	0.47
Maturation cycle (days)	240.00	291.00	342.00	22.94
Length of seeds (cm)	0.82	1.76	1.93	0.19
Width of seeds (cm)	0.41	1.08	1.24	0.12

represented only by access 84, presented a null value for intraspecific variation, as expected. The *J. curcas* species, in its turn, despite being represented by more accesses than the others, presented low intraspecific variation. These results can be related to self-reproduction facilitated by the occurrence of individual plants and how this species was introduced in Brazil. The lowest estimated interspecific variation value (10.54) occurred between *J. pohliana* and *J. gossypiifolia* and the highest (12.96) between *J. pohliana* and *J. curcas*, very close to the value found between *J. curcas* and *J. gossypiifolia* (12.68). The number of bands produced by primer varied from five (ISSR 840) to 21 (ISSR 834), with an average of 10.5 bands per primer (Table 4). The level of polymorphism by starter was approximately 4.3 bands, with a variation from 1 (ISSR 840) to 15 polymorphic bands (ISSR 834). The size of the amplified fragments varied from 150 to 1900 pb, approximately. It is important to state that the differences between accesses were determined based on the more frequency of bands than on the presence or absence of specific bands. Nevertheless, it is important to point out the occurrence of specific bands in certain accesses by means of a determined primer, being that these bands are potentially useful for future studies on genetic improvement. The result of the hierarchization of all 84 *Jatropha* accesses is shown in Figure 3. There was a formation of ten distinct groups, generally, *J. curcas* accesses formed five distinct groups and *J. pohliana* and *J. gossypiifolia* accesses composed the five remaining groups. With the exception of the *J. gossypiifolia* access 84, which had been allocated along with a *J. pohliana* access (82), to the *J. pohliana* access (77), allocated to a group composed only of *J. curcas* accesses; as well as *J. curcas* accesses 70, 72, and 76, which had been allocated in distinct groups containing *J. pohliana* accesses (78, 79, and 81, respectively, a greater divergence between interspecific rather than intraspecific accesses was noted. The average genetic dissimilarities between accesses of each species were lower than dissimilarity among species (Table 5). In a study realized by Dhillon et al. (2009), interspecific hybrids of *J. curcas* and *Jatropha intergerrima* were developed to combine desirable agronomic traits and RAPD was used to distinguish the legacy of fragments in the hybrid progeny. The species *J. gossypiifolia*, being represented only by the access 84, presented a null value for intraspecific variation, as expected. The species *J. curcas*, however, despite being represented by more accesses than the others, presented low intraspecific variation. The lowest estimated interspecific variation value (10.54) occurred between *J. pohliana* and *J. gossypiifolia* and the highest (12.96) between *J. pohliana* and *J. curcas*, very close to the value found between *J. curcas* and *J. gossypiifolia* (12.68).

DISCUSSION

Very similar qualitative characters were observed in all the accesses, even among different species, for instance alternate and spiral leaves along the petiole (Table 2). Vasconcelos et al. (2010) while studying phytoalexin of *J. curcas* accesses from the states of Bahia and Paraíba found similar results to those observed in the present study. The same authors characterized *J. curcas* leaves as being greenish and glabrous when fully formed; however, the leaves when young had a vinaceous color, indicating photosynthetic inactivity, which was also observed in *J. curcas* accesses in this study (Table 2). Melo et al. (2007), on the other hand, described leaves with pilosity on the adaxial surface, indicating intraspecific variability in *J. curcas.* Regarding the ramification structure, a predominance of the goblet-shape was observed, 79% of the *Jatropha* spp accesses, which corresponds to the monopodial stem type, present in the 57 *J. curcas* accesses studied by Vasconcelos et al. (2010). Previous works corroborate for the classification found for coloration of leaf veins. Melo et al. (2007) when morphologically characterizing accesses of *J. curcas* from the active germplasm bank of the Federal University of Sergipe, verified that of the 15 accesses studied, all had greenish veins. Nonetheless, both Vasconcelos et al. (2010) and Avelar et al. (2008) observed leaf nerves with whitish protrusions on the abaxial surface of *J. curcas* accesses. Many of the characteristics presented by *J.*

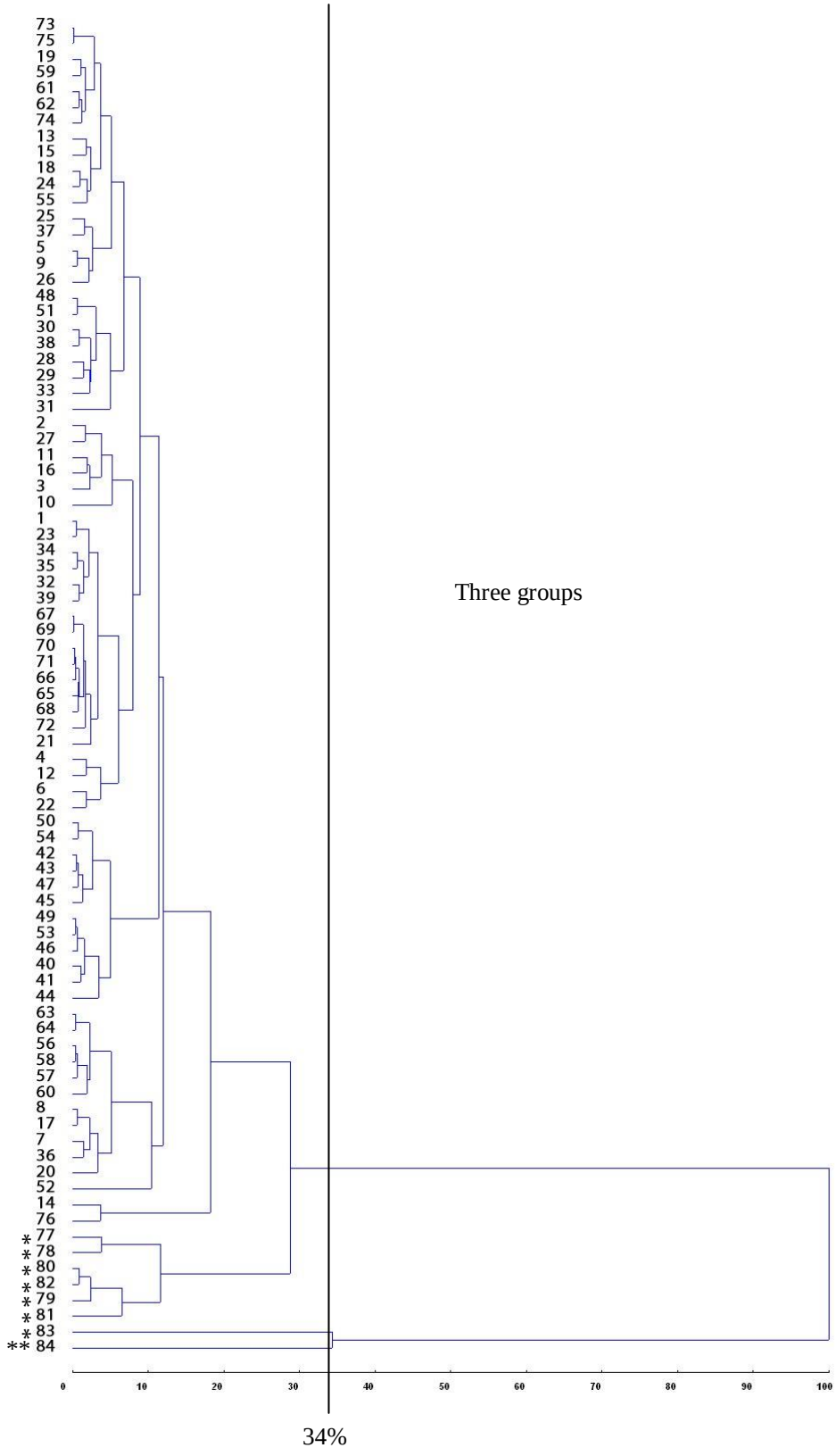

Figure 2 Dendrogram by UPGMA method that originated from the dissimilarities of the 84 accessions of *Jatropha* spp., based on quantitative morphological characters. The numbering of the access matches that specified in Table 1. Unmarked, *J. curcas; *J. pohliana; **J. gossypiifolia*

Table 4. Sequence of nitrogenous bases, annealing temperature (Ta), total number of fragments (TNF), number of polymorphic fragments (NPF) and percentage of polymorphism (P%) obtained in the analyzes of the 43 ISSR markers in 84 accessions of *Jatropha* spp.

Primer	Sequence (5' 3') (1)	Ta (°C)	TNF	NPP	P%
807	AGA GAG AGA GAG AGA GT	49.8	9	4	44.4
808	AGA GAG AGA GAG AGA GC	56.0	9	4	44.4
809	AGA GAG AGA GAG AGA GG	51.5	11	5	45.5
810	GAG AGA GAG AGA GAG AT	55.0	17	9	52.9
811	GAG AGA GAG AGA GAG AC	55.0	17	8	47.1
812	GAG AGA GAG AGA GAG AA	50.0	13	7	53.8
813	CTC TCT CTC TCT CTC TT	55.0	7	4	57.1
815	CTC TCT CTC TCT CTC TG	55.0	9	3	33.3
817	CAC ACA CAC ACA CAC AA	55.0	15	5	33.3
818	CAC ACA CAC ACA CAC AG	55.0	14	2	14.3
819	GTG TGT GTG TGT GTG TA	55.0	10	6	60.0
821	GTG TGT GTG TGT GTG TT	55.0	11	7	63.6
822	TCT CTC TCT CTC TCT CA	55.0	8	4	50.0
823	TCT CTC TCT CTC TCT CC	55.0	14	8	57.1
824	TCT CTC TCT CTC TCT CG	55.0	12	9	75.0
825	ACA CAC ACA CAC ACA CT	55.0	12	4	33.3
826	ACA CAC ACA CAC ACA CC	55.0	15	7	46.7
827	ACA CAC ACA CAC ACA CG	52.0	17	7	41.2
828	TGT GTG TGT GTG TGT GA	55.0	8	4	50.0
829	TGT GTG TGT GTG TGT GC	50.0	10	6	60.0
830	TGT GTG TGT GTG TGT GG	52.0	9	6	66.7
834	AGA GAG AGA GAG AGA GYT	52.5	21	15	71.4
835	AGA GAG AGA GAG AGA GYC	53.9	9	4	44.4
836	AGA GAG AGA GAG AGA GYA	53.7	9	3	33.3
840	GAG AGA GAG AGA GAG AYT	54.2	5	1	20.0
841	GAG AGA GAG AGA GAG AYC	55.0	8	4	50.0
842	GAG AGA GAG AGA GAG AYG	55.0	8	1	12.5
847	CAC ACA CAC ACA CAC ARC	53.3	10	4	40.0
850	GTG TGT GTG TGT GTG TYC	55.0	6	2	33.3
853	TCT CTC TCT CTC TCT CRT	55.0	7	1	14.3
858	TGT GTG TGT GTG TGT GRT	55.0	9	4	44.4
860	TGT GTG TGT GTG TGT GRA	55.0	10	4	40,0
863	AGT AGT AGT AGT AGT AGT	55.0	8	1	12.5
864	ATG ATG ATG ATG ATG ATG	55.0	10	2	20.0
866	CTC CTC CTC CTC CTC CTC	55.0	13	1	7.7
867	GGC GGC GGC GGC GGC GGC	50.0	13	2	15.4
876	GAT AGA TAG ACA GAC A	55.0	9	2	22.2
880	GGA GAG GAG AGG AGA	55.0	9	1	11.1
881	GGG TGG GGT GGG GTG	55.0	7	3	42.9
884	HBH AGA GAG AGA GAG AG	55.0	7	3	42.9
887	DVD TCT CTC TCT CTC TC	55.0	7	2	28.6
889	DBD ACA CAC ACA CAC AC	53.6	11	3	27.3
891	HVH TGT GTG TGT GTG TG	50.4	8	3	37.5
Total			451	185	
Averages		54.0	10.5	4.3	39.6

(1)B, D, H, R, V and Y mean degenerate oligonucleotide: B = (T, C, G); D = (A, G, T); H = (A, T, C); R = (A, G); V = (A, C, G) and Y = (C, T).

Table 5. Averages of genetic dissimilarities (%) estimated by molecular ISSR primers, adopting the arithmetic complement of Jaccard coefficient for three species of *Jatropha*. Jaboticabal, SP.

	J. curcas	***J. pohliana***	***J. gossypiifolia***
J. curcas	2.09	12.96	12.68
J. pohliana	-	2.56	10.54
J. gossypiifolia	-	-	0.00

curcas, *J. pohliana* and *J. gossypiifolia* fruits in this study were also verified in *Jatropha elliptica* fruits (Añez et al., 2005), another species of the genre, with the same explosive propulsion mechanism of seeds. The dehiscence observed in *J. curcas*, *J. pohliana* and *J. gossypiifolia* fruits (Table 2) is also present in fruits of other genres of Euphorbiaceae. Barroso et al. (1999) pointed out that all of these species are related to one another and must have originated from a common ancestor. In the present study, the height of *J. curcas* and *J. pohliana* plants, in general among accesses, was very high (Table 2). In this case, the selection of smaller genotypes in order to facilitate harvest operations is optimal (Dias et al., 2007). A study conducted with 57 *J. curcas* accesses from Petrolina - BA and Columbia-PB showed an average height of 164.00 cm in accesses from the first group and 190.00 cm in the second group (Vasconcelos et al., 2010), only confirming the results observed here. Studies on the contribution of characters for diversity are important in order to select those characters which best differentiate accesses and exclude those that do not effectively contribute to genotype discrimination (Cruz et al., 2012). There was great variability among accesses regarding trait for number of days for fruit maturation (Table 4), which may be justified by the fact that those are allogamous species, dependant on pollinator insect, which in their turn can be insufficiently or irregularly distributed in the environment. Such differences may be still explained by genetic divergences that naturally occur among accesses of species of a determined kind and origin.

Also, heterogeneous fructification was observed within a plant, which probably occurred for the reasons previously cited. Table 2 also presents seed length and width data for the 84 accesses studied. Lower values than those found in the present study have been observed by Añez et al. (2005) for *J. elliptica* seeds, having an average of 0.82 cm length and 0.43 cm width. However, *J. gossypiifolia* seeds were the smallest among the studied species, with average values of 0.82 and 0.50 cm, respectively, for length and width, much closed values to those found by Añez et al. (2005) for *J. elliptica*. The high variability observed between accesses for the number of lobes, height of plants, stem diameter, maturation cycle, and length and width of seeds traits can, and certainly will serve as base for future studies. As for the other characters studied, namely, the qualitative characters, there was also variability, which enabled the separation of accesses into a number of different groups, superior even to the quantitative characters. However, by observing Table 2, it is possible to observe many accesses placed within the same classification category, for instance, 92% of accesses presented glabrous leaves, and only 8% hairy leaves, however when using a larger number of qualitative characters, as in the case in this study, the chances of obtaining variability between accesses were increased. By observing the results for each qualitative character individually (Table 2), low variation between accesses is noted, which may be explained by the narrow genetic base of the studied plants from the germplasm bank, or by the monogenic nature of these characters. There was congruency between some of the groups formed in the clustering analysis for qualitative and quantitative characters (Figures 1 and 2). In both cases, the totality of *J. curcas* accesses was arranged in one large group. *J. pohliana* accesses, in general, were allocated in the same group and access 84, the only *J. pohliana* sample, formed and isolated group in the two analysis carried out. Such observations show low intraspecific variation of these *Jatropha* species, as opposed to the interspecific diversity found.

The molecular analysis indicated that the investigated species shared 260 alleles (57.6%), with a polymorphism rate of 42.3%, which considered a relatively high number. Cai et al. (2010) evaluated a set of 224 *J. curcas* accessions including 219 from all the adaptation areas in China and five from Myanmar, 15 UBC primers provided among the 169 amplified bands, 127 (75.15%) were polymorphic which meant that Chinese *Jatropha* had high genetic diversity. Souza et al. (2009), however, analyzing 11 ISSR primers, obtained a low polymorphism rate (23.09%). Few accesses were distinguished by their diversity, the interspecific variability (between accesses of different species) higher than the intraspecific variability (occurring within a species) (Table 5), which is justified by the higher degree of relationship existing between accesses of the same species as well as by the narrow genetic base of the studied species. Ram et al. (2008) found 80.2% polymorphism when studying genetic diversity of eight species of *Jatropha* genre, including *J. curcas* and *J. gossypiifolia*, and indicative of the existence of high interspecific variability within *Jatropha* genre. The authors also observed, through UPGMA, the

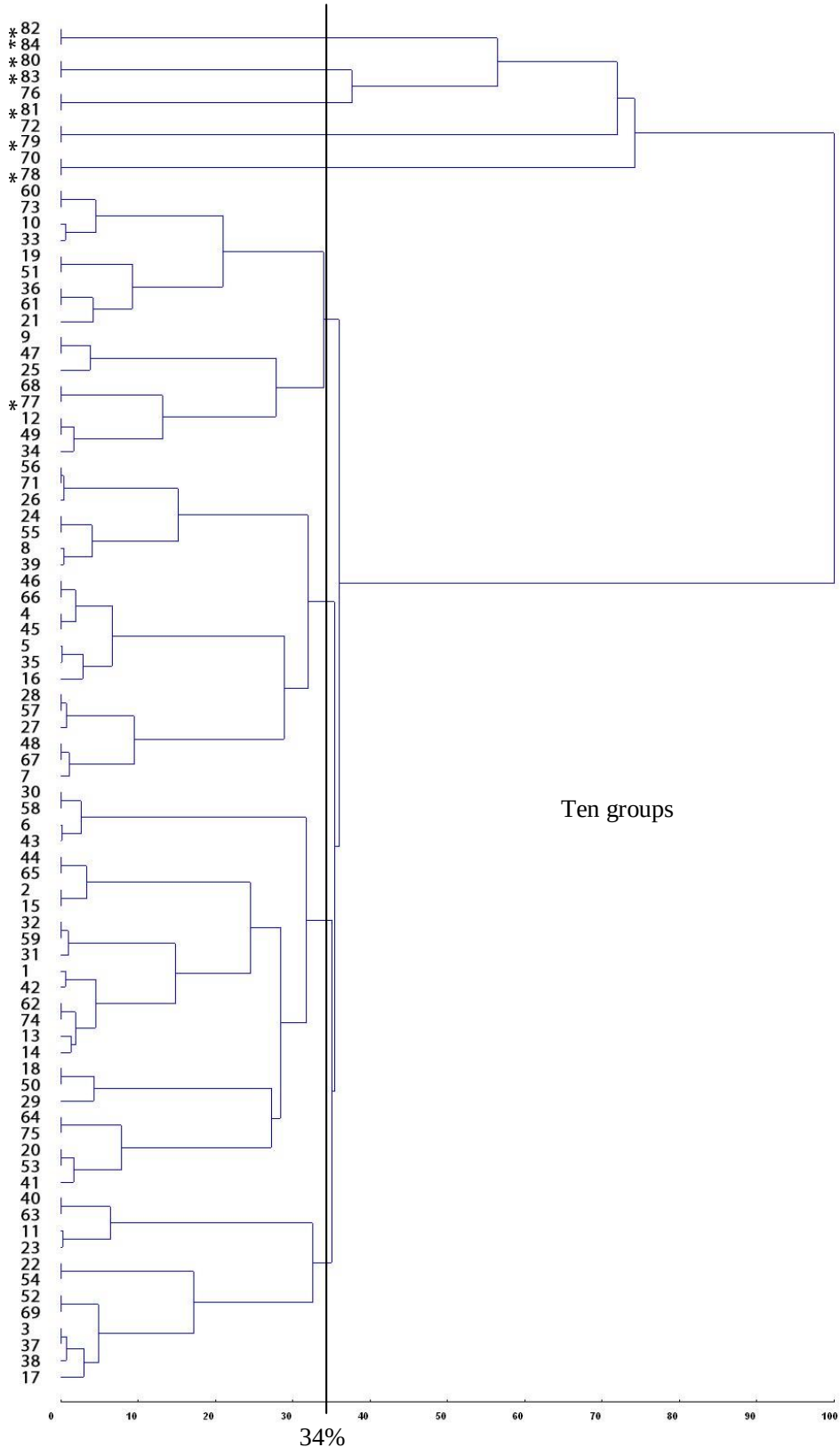

Figure 3. Dendrogram by UPGMA method, originated from arithmetic complement of Jaccard Coefficient between 84 accessions of *Jatropha* spp., based on ISSR molecular markers. The numbering of the access matches that specified in Table 1 Unmarked, *J. curcas; *J. pohliana; **J. gossypiifolia*

formation of three distinct groups, one of them formed exclusively of *J. curcas* accesses. In this study, the dendrogram formed by the UPGMA method based on molecular data (Figure 3), created ten distinct groups, where it was possible to observe *J. curcas* accesses in distinct, but very close groups. It is still possible to observe that *J. curcas* accesses 70, 72, and 76 were allocated into groups with *J. pohliana* accesses in the top extremity of the dendrogram. *J. pohliana* access 77 was allocated in a group composed only by *J. curcas* accesses, and the *J. gossypiifolia* access 84 was in a group with *J. pohliana* access 82, also from Galante – PB. Therefore, there was interspecific mixing, when 34% dissimilarity was used, however it is possible to observe that this mixing kept intraspecific accesses in close groups, in other words, with a high degree of genetic similarity.

Dehgan and Webster (1979), in their study on the classic taxonomy of *Jatropha* genre, concluded that *J. curcas* is a primitive ancestor species of this genre, due to its morphological differentiation, and that species from other sections evolved from it and other ancestor forms. By observing Figure 3, where *J. curcas* accesses distinguish themselves molecularly from the other two species, this hypothesis is once again reinforced. Past studies with *Jatropha*-related species using RAPD and ISSR markers reported the *J. curcas* accessions in a distinct group (Ganesh Ram et al., 2008; Senthil Kumar et al., 2009, respectively). Sujatha et al. (2005), studying the genetic diversity between toxic and non-toxic *J. curcas* using RAPD markers, found a percentage of 96.3% genetic similarity. In another study, Sudheer et al. (2009) reported 84.9 and 83.6% between toxic and nontoxic *J. curcas* plants analyzed by RAPD and AFLP, respectively, and identified specific RAPD and AFLP markers for both varieties. A inter and intrapopulation study conducted with RAPD and ISSR markers on 42 *J. curcas* accesses from different regions in India, and one non-toxic genotype from Mexico presented 42.0 and 37.4% polymorphism through RAPD and ISSR, respectively (Basha and Sujatha, 2007). The same authors in further studies, working with 200 RAPD, 100 ISSR and 50 organelle specific microsatellite primers for *Jatropha* species from India, observed 98.5% polymorphism, with high interspecific genetic variation (Basha and Sujatha, 2009). Furthermore, ISSR primers have also been successfully used to estimate the degree of intra and interspecific genetic diversity in other species, including rice (Joshi et al., 2000), wheat (Nagaoka and Ogihara, 1997), Eleusine coracana (Salimath et al., 1995), Vigna (Ajibade et al., 2000), sweet potato (Huang and Sun, 2000), and Plantago (Wolff and Morgan-Richards, 1998). The formation of main groups containing the majority of *J. curcas* accesses are a clear indication of the low genetic diversity between individuals of different origins and reinforces the idea that *J. curcas* genotypes from different origins share a common ancestry, excluding the genetic concept of diversity by origin.

Oliveira (2007), working with RAPD markers, verified the formation of four distinct groups independent of the origin of the genotypes. The lack of relationship between the similarity pattern found and the geographical origin on the accesses has been reported in other studies (Basha and Sujatha, 2007). Additional efforts to cover the lack of knowledge on the genetic diversity of the *Jatropha* genre using molecular markers and morphological characters are obviously needed. Finally, the ISSR markers and morphological characters used allowed us to distinguish the species and accesses of the *Jatropha* genre, detecting low variation between intraspecific, and high variation between interspecific accesses, and may be very useful in monitoring genetic variability in germplasm banks when nuclear collection are eventually established.

Conflict of interests

The author(s) did not declare any conflict of interest.

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REFERENCES

Accarini JH (2006). Biodiesel no Brasil: estágio atual e perspectivas. Revista Bahia, Análise & Dados 16(1):51-63.

Ajibade SR, Weeden NF, Chite SM (2000). Inter-simple sequence repeat analysis of genetic relationships in the genus *Vigna*. Euphytica 111(1):47–55.

Añez, LMM, Coelho MFB, Albuquerque MCF, Dombroski JLD (2005). Caracterização morfológica dos frutos, das sementes e do desenvolvimento das plântulas de *Jatropha elliptica* Müll.Arg.. Arg. (Euphorbiaceae). Rev. Bras. Bot. 28(3):563-568.

Avelar RC, Silva FM, Castro Neto P, Fraga AC (2008). Avaliação do desenvolvimento de pinhão- manso (*Jatropha curcas* L.) do banco de germoplasma da UFLA. In: Anais do Congresso Brasileiro de Plantas Oleaginosas, Óleos, Gorduras e Biodiesel, Lavras (CD Room).

Barroso MB, Morin NP, Peixoto AL, Ichaso CLF (1999). Frutos e sementes. Morfologia Aplicada à Sistemática de Dicotiledôneas. 2ª edição. Editora UFV, Viçosa, 444 pp.

Basha SD, 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(3):375-386.

Basha SD, Sujatha M (2009). Genetic analysis of *Jatropha* species and interspecific hybrids of *Jatropha curcas* using nuclear and organelle specific markers. Euphytica 168(2):197–214.

Cai Y, Suna D, Wud G (2010). ISSR-based genetic diversity of *Jatropha curcas* germplasm in China. Biomass Bioenergy 34(12):1739-1750.

Cole-Rodgers P, Smith DW, Bosland PW (1997). A novel statistical approach to analyze genetic resource evaluations using Capsicum as an example. Crop Sci. 37(3):1000-1002.

Cruz CD (2013). GENES - a software package for analysis in experimental statistics and quantitative genetics. Acta Sci. 35(3):271-

276.
Cruz CD, Carneiro PCS (2006). Modelos Biométricos Aplicados ao Melhoramento Genético – Volume 2. 2ª edição. Editora UFV, Viçosa, 585 pp.
Cruz CD, Regazzi AJ, Carneiro PCS (2012). Modelos Biométricos Aplicados ao Melhoramento Genético- Volume 1. 4ª edição. EditoraUFV, Viçosa, 514 pp.
Dehgan B, Webster GL (1979). Morphology and intergeneric relationship of the genus *Jatropha*. Univ. Calif. Publ. Bot. 74:1–73.
Dellaporta SL, Wood J, Hick JB (1983). A plant DNA minipreparation. Plant. Mol. Biol. Rep. 1(4):19-21.
Dhillon RS, Hooda MS, Jattan M, Chawla V, Bharadwaj M, Goyal SC (2009). Development and molecular characterization of interspecific hybrids of Jatropha curcas X Jatropha integerrima. Indian J. Biotechnol. 8:384-390.
Dias LAS, Leme LP, Laviola BG, Pallini Filho A, Pereira OL, Carvalho M, Manfio CE, Santos AS, Sousa LCA, Oliveira TS, Dias DCFS (2007). Cultivo de Pinhão-manso (*Jatropha curcas* L) para Produção de Óleo Combustível. 1ª edição. Editora UFV, Viçosa, 40 pp.
Divakara BN, Upadhyaya HD, Wani SP, Gowda CLL (2010). Biology and genetic improvement of *Jatropha curcas* L.: a review. Appl. Energy 87(3):732-742.
Doyle JJ, Doyle JL (1990). Isolation of plant DNA from fresh tissue. Focus 12:13-15.
Elias M, Mühlen GS, McKey D, Roa AC, Tohme J (2004). Genetic diversity of traditional South American landraces of cassava (*Manihot esculenta* Crantz): an analysis using microsatellites. Econ. Bot. 58(2):242-256.
Embrapa Algodão (2008). Descritores de Mamona Utilizados pela Embrapa Algodão. Campina Grande, Embrapa Algodão Campina Grande (ed), documento 192, Campina Grande, 39pp.
Ferreira ME, Grattapaglia D (1998). Introdução ao Uso de Marcadores Moleculares em Análise Genética. 3ª edição. Embrapa Recurso Genéticos Vegetais Brasília (ed), Brasília, 220 pp.
Ganesh Ram S, Parthiban KT, Senthil Kumar R, Thiruvengadam V, Paramathma M (2008). Genetic diversity among Jatropha species as revealed by RAPD markers. Genet. Resour. Crop Evol. 55(6):803–809.
Góis IB, Silva R, Boari A de J, Oliveira A dos S, Fraga AC (2006). Caracterização isoenzimática de acessos de pinhão- manso (*Jatropha curcas* L.). In: Embrapa Algodão Campina Grande (ed). Proceedings of the 2º Congresso Brasileiro de Mamona, Aracaju, http://www.cnpa.embrapa.br/produtos/mamona/publicacoes/trabalhos_cbm2/2006.pdf (September 2, 2011)
Huang J, Sun SM (2000). Genetic diversity and relationships of sweet potato and its wild relatives in *Ipomoea* series Batatas (Convolvulaceae) as revealed by inter-simple sequence repeat (ISSR) and restriction analysis of chloroplast DNA. Theor. Appl. Genet. 100(7):1050-1060.
Joshi SP, Grupta VS, Aggarwal RK, Ranjekar PK, Brar DS (2000). Genetic diversity and phylogenetic relationship as revealed by inter-simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. Theor. Appl. Genet. 100(8):1311-1320.
Melo MFV, Silva-Mann R, Santos HO, Souza EM (2007). Caracterização morfológica de acessos de *Jatropha curcas* L. do banco de germoplasma da Universidade Federal de Sergipe. In: Anais do Congresso da Rede Brasileira de Tecnologia de Biodiesel, Brasília (CD Room).
Messmer MM, Melchinger AE, Herrmann RG, Jurgen B (1993). Relationships among early European maize inbreds: II. Comparison of pedigree and RFLP data. Crop Sci. 33(5):944-950.
Nagaoka T, Ogihara Y (1997). Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Apppl. Genet. 94(5):597–602.
Nunes CF, Pasqual M, Santos DN dos, Custódio TN, Araújo AG de (2008). Diferentes suplementos no cultivo in vitro de embriões de pinhão manso. Pesq. Agropec. Bras. 43(1):9-14.
Oliveira AS (2007). Diversidade genética entre acessos de *Jatropha* sp. In: Anais do 2º Congresso da Rede Brasileira de Tecnologia de Biodiesel. MCT/ABIPTI, Brasília, 451 pp.
Oliveira JS, Leite PM, Souza LB de, Mello VM, Silva EC, Rubim JC, Meneghetti SMP, Suarez PAZ (2006). Avaliação Preliminar de parâmetros físico-químicos do óleo e biodiesel de Pinhão Roxo (*Jatropha gossypiifolia* L.). In: Anais da 29ª Reunião Anual da Sociedade Brasileira de Química, Águas de Lindóia, https://sec.sbq.org.br/cd29ra/resumos/T0777-1.pdf (November 19, 2011).
Ram GS, Parthiban KT, Senthil KR, Thiruvengadam V, Paramathma M (2008). Genetic diversity among *Jatropha* species as reveled by RAPD markers. Genet. Resour. Crop Evol. 55(6):803–809.
Salimath SS, Oliveira AC, Godwin ID, Bennetzen JL (1995). Assessment of genome origins and genetic diversity in the genus *Eleusine* with DNA markers. Genome 38(4):757–763.
Senthil Kumar R, Parthiban KT, Govinda Rao M (2009). Molecular characterization of *Jatropha* genetic resources through intersimple sequence repeat (ISSR) markers. Mol. Biol. Rep. 36(7):1951–1956.
Soares BO (2010). Diversidade genética de genótipos de pinhão-manso por meio de RAPD e ISSR. Dissertação (Mestrado)– Universidade Estadual de Montes Claros, Jarnaúba, 54 pp.
Sousa MTB (2006). Análise da utilização do biodiesel como alternativa para o desenvolvimento sustentável. In: Anais do 1º Congresso de Pesquisa e Inovação da Rede Norte Nordeste de Educação Tecnológica, Natal. Natal: CONNEPI.
Souza DA, Moreira GBR, Guimarães JFR, Librelon SS, Fernandes TP, Nietsche S, Costa MR (2009). Análise da diversidade genética intrapopulacional de pinhão-manso (*Jatropha curcas* L.) com marcadores ISSR. In: Anais do Congresso Brasileiro de Plantas Oleaginosas, Óleos, Gorduras e Biodiesel. Montes Claros, pp.2158-2164.
Sudheer PDVN, Singh S, Mastan SG, Patel J, Reddy MP (2009). Molecular characterization and identification of markers for toxic and non-toxic varieties of *Jatropha curcas* L. using RAPD, AFLP and SSR markers. Mol. Bio. Rep. 36(6):1357-1364.
Sujatha M, Makkar HPS, Becker K (2005). Shoot bud proliferation from axillary nodes and leaf sections of non-toxic *Jatropha curcas* L. Plant Growth Regul. 47(1):83–90.
Targino KC de F, Silva FFM da (2013). Rendimento e análise físico-química do óleo extraído das sementes do pinhão-bravo (*Jatropha mollissima* Müell.Arg.). In: Anais do IX Congresso de Iniciação Científica do IFRN, Natal, pp.1326-1334.
Vasconcelos GCL, Arriel NHC, Medeiros KAAL, Medeiros OS, Melo JIM, Lucena AMA, Freire MAO (2010). Caracterização botânica de acessos de pinhão manso do banco de germoplasma da Embrapa Algodão. In: Embrapa Algodão Campina Grande (ed), Anais do Congresso Brasileiro de Mamona & Simpósio Internacional de Oleaginosas Energéticas, João Pessoa, pp.1606-1611.
Wolff K, Morgan-Richards M (1998). PCR markers distinguish *Plantago major* subspecies. Theor. Appl. Genet. 96(2):282-286.
Zietkiewicz E, Rafalki A, Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20(2):176-183.