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Genetic diversity and structure of *Jatropha curcas* L. in its centre of origin

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Abstract

To investigate the genetic diversity and structure of *Jatropha curcas* L. oilseed plant, in this study, native populations from Chiapas, Mexico, were evaluated, using microsatellite DNA markers. A total of 93 representative samples were selected from seven sites in two regions in the state of Chiapas grouped by geographical proximity, where leaf samples were collected to isolate the genomic DNA. Individual polymerase chain reactions were carried out with ten pairs of specific oligonucleotides for the microsatellites of *J. curcas*, separating the products of amplification by acrylamide electrophoresis. Twenty-seven fragments were detected (77% polymorphic) with which heterozygous individuals were distinguished. The most informative microsatellite was *Jcps20* (nine alleles, polymorphic index content 0.354). The average polymorphism per population was 58%. The Hardy–Weinberg tests revealed a reproductive pattern of non-random mating. The diversity descriptors and the analysis of molecular variance revealed that the populations were structured and moderately differentiated (F_{ST} 0.087) and that this differentiation was not due to isolation by distance, as the Mantel test was not significant ($P = 0.137$), but rather due to allopatry. Bayesian analysis revealed that the accessions belonged to only four genetic groups and confirmed the differentiation between the regions. Because some loci were in Hardy–Weinberg disequilibrium, it is proposed that differentiation is due to the clonal reproduction of *J. curcas* practised by farmers in Chiapas, along with the anthropogenic dispersion at regional levels. The results of this study reveal that *J. curcas* in Chiapas has genetic diversity that is greater than that reported in other parts of the world, which represents a potential germplasm pool for the selection of genotypes.

Keywords: biofuels; diversification; genetic structure; Mesoamerica; Mexico

Introduction

Jatropha curcas L. is a new bioenergy crop in the world, belonging to the family Euphorbiaceae, the centre of origin, diversification and domestication of which is probably the Mesoamerican region (Dehgan and Webster, 1979; Heller, 1996; Carels, 2009; Ovando-Medina *et al.*, 2013). Although this species has been used for millennia as a hedge or ‘living fence’ and some genotypes are edible, many authors consider it to be still in the process of domestication (Carels, 2009; Granados-Galván, 2009; Achten *et al.*, 2010). It has other important uses; however, its current relevance is the possibility of converting the seed oil into biodiesel (Gubitz *et al.*, 1999).

Due to its importance as a newly extensive crop, it is estimated that in a few years *J. curcas* will have been planted in nearly 15 million hectares in Mexico as well as in Asia, Africa and the Americas (Renner and Zelt, 2008). However, there are still many technological challenges for its commercial establishment, including

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the lack of genetically improved varieties of high agronomic performance (Mishra, 2009; Ovando-Medina *et al.*, 2013).

Knowledge of the diversity of *J. curcas* on distinct levels (molecular, morphological, physiological and productive) is of primary importance, as from germplasm characterization genotypes could be selected for extensive planting (Basha and Sujatha, 2007) or genetic improvement could be accelerated (Singh *et al.*, 2010). Molecular studies are particularly important as DNA-based markers are not influenced by environmental conditions (Ganesh *et al.*, 2008) and therefore reflect genetic variation more faithfully. In this respect, the molecular diversity of *J. curcas* has been studied, although mainly germplasm collected in Asia (Abdulla *et al.*, 2009; Yu *et al.*, 2010), Africa (Basha *et al.*, 2009; Ambrosi *et al.*, 2010; Ricci *et al.*, 2012), South America (Rosado *et al.*, 2010) and, to a lesser extent, Mexico and Central America (Van-Loo *et al.*, 2008; Ovando-Medina *et al.*, 2011a; Pecina-Quintero *et al.*, 2011) has been characterized. Additionally, the marker systems used have been mainly dominant, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and inter-simple sequence repeats (ISSRs), among others (Ovando-Medina *et al.*, 2011b).

On the other hand, microsatellite markers (simple sequence repeats (SSRs)) have the advantage of detecting heterozygous individuals, so they are considered to be co-dominant, as well as of having a greater number of polymorphisms than dominant markers (Kalia *et al.*, 2011). However, in the case of *J. curcas*, SSR-based studies have generally detected less diversity in Asian and South American germplasm (Basha *et al.*, 2009; Rosado *et al.*, 2010; Ricci *et al.*, 2012) compared with those found with dominant markers. AFLP-based studies conducted with germplasm from the state of Chiapas, Mexico, and from Guatemala have detected the highest genetic diversity of this species, although questions remain about the structure and variation that can be detected with co-dominant markers. For that reason, this study was carried out with the main aim of analysing, using SSR markers, the genetic structure and diversity of populations of *J. curcas* located in the centre of diversification of this species.

Materials and methods

Plant material

Plants from the Biosciences Centre (CenBio, initials in Spanish) Germplasm Bank of the Autonomous University of Chiapas (Mexico) were used and samples were obtained from cuttings. We selected 93 representative samples from seven sites of two regions in the state of Chiapas (Table 1) grouped by geographical proximity.

DNA extraction

Samples used were fresh foliar tissues, which were transferred on ice to the laboratory, where they were washed three times with sterile distilled water and 70% ethyl alcohol and maintained at −30°C until processing. The genomic DNA was isolated and purified by the method described by Doyle and Doyle (1990) modified for processing samples of 200 mg (Ovando-Medina *et al.*, 2011a). In brief, 200 mg of healthy leaves and 60 μg of polyvinylpyrrolidone were ground with liquid nitrogen and then extracted with 1 ml of CTAB buffer (0.1% w/v hexadecyltrimethylammonium bromide, 5 mM EDTA, 1.5 M NaCl, 50 mM Trizma base and 0.1% v/v β-mercaptoethanol, pH 8.0). Extractions were carried out with chloroform–isoamyl alcohol and precipitation with isopropanol. The extracted DNA was purified by re-extraction with a mixture of phenol–chloroform–isoamyl alcohol (25:24:1). The integrity of DNA, dissolved in 60 μl of Milli-Q® water (Sigma-Aldrich®, St. Louis, MO, USA), was verified by electrophoresis on 1% agarose gel and quantified spectrophotometrically at 260 nm (Genova Nano®; Jenway®, Staffordshire, UK).

Microsatellite DNA study

Ten pairs of specific oligonucleotides were used for regions of microsatellite DNA of the *J. curcas* genome reported by Pamidimarri *et al.* (2009a). The descriptive data of the oligonucleotides are given in Table S1 (available online).

Individual amplifications were carried out by polymerase chain reaction (PCR) following the protocol reported by Pamidimarri *et al.* (2009a), as modified with the following reaction mixture: 25 ng of DNA, 50 pmol of each oligonucleotide, 4 μl of Taq® 10 × buffer, 2 μl of $MgCl_2$ (25 mM), 0.5 μl of dNTPs (10 mM) and 1 U of Taq polymerase (GoTaq® Flexi DNA Polymerase; Promega®, Madison, WI, USA); the final volume was 25 μl. The thermal profile consisted of an initial denaturation step at 94°C for 4 min and 40 amplification cycles of three steps each: 30 s of denaturation at 94°C, 30 s of alignment at the temperature specific for each pair of oligonucleotides (Table S1, available online) and 1 min of polymerization at 72°C. It involved a final extension step at 72°C for 7 min, followed by cooling at 15°C. The PCR products were stored at −30°C until electrophoresis. Negative controls without DNA were used to rule out contamination in the samples.

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Table 1. Samples of *Jatropha curcas* (Euphorbiaceae) from the CenBio Germplasm Bank of the Autonomous University of Chiapas collected in Southern Mexico

Collection sites	Code	Number of accessions	Geographical data	*A priori* population
Puerto Chiapas	PC	8	14.47226°N/92.27851°W	Soconusco
Suchiate	SCH	4	14.39234°N/92.13000°W	
Cacahoatán	CAC	3	14.57985°N/92.09047°W	
Huixtla	HUX	2	15.05368°N/92.28908°W	
Mapastepec	MAP	5	15.25132°N/92.53940°W	Mapastepec
Arriaga	ARR	9	16.11944°N/93.53615°W	Isthmus
Jiquipilas	JIQ	7	16.39879°N/93.38902°W	Riviera
Cintalapa	CIN	2	16.41105°N/93.42659°W	
Berriozábal	BERR	3	16.47353°N/93.16 274°W	Centre
Ocozocuautla	OCZ	3	16.45974°N/93.22694°W	
Tuxtla	TUX	2	16.42383°N/93.06688°W	
Simojovel	SIM	1	17.08312°N/92.42438°W	
Ixtapa	IXT	1	16.47221°N/92.54399°W	
Villa Comaltitlán	VCO	3	16.11038°N/93.15774°W	Frailesca
Pujiltic	PUJ	8	16.17112°N/92.26478°W	
La Concordia	CCR	5	16.06436°N/92.41483°W	
Villa Flores	VIF	3	16.14063°N/93.15310°W	
Venustiano Carranza	VCR	1	16.18311°N/92.33464°W	
Revolución Mexicana	RV	3	16.09292°N/93.04303°W	
Jaltenango	JAL	2	15.52722°N/92.43270°W	
Villa de las Rosas	VLR	1	16.19243°N/92.20578°W	
Comalapa	COM	4	15.38752°N/92.07857°W	Border
Ciudad Cuauhtémoc	CDCU	5	15.40559°N/92.00520°W	
Rizo de Oro	RIZ	4	15.57323°N/92.28489°W	
Lagos de Colón	LACO	3	15.50308°N/91.54230°W	
Chicomuselo	CHIC	1	15.44623°N/92.16722°W	

The amplification products were separated by electrophoresis on 12% acrylamide gels in TAE buffer (48.4 g Trizma base, 10.9 g acetic acid, 2.92 g EDTA and 1 litre H_2O) at 80 V for 250 min and revealed with ethidium bromide (Sambrook *et al.*, 1989). The size of the bands was estimated using the molecular marker 25 bp DNA Step Ladder (Promega®, Madison, WI, USA). Image analysis was carried out using the Universal Hood (ChemiDoc®; Model 170-8126 Bio-Rad®, USA) equipment coupled to the Quantity One© software (Bio-Rad®, Hercules, CA, USA).

Population analysis

The locus informative descriptors were calculated using the program PicCal© 1.0 (Vekemans *et al.*, 2002) and those of diversity of populations using the program GenAlEx© version 6.5 (Peakall and Smouse, 2012), specifically the percentage of polymorphism (P%), the effective number of alleles (N_e), Shannon's informative index (I), observed heterozygosity (H_o) and expected heterozygosity (H_e), and fixation index (F). To test the hypothesis of isolation by distance, a Mantel correlation test was conducted between Nei's genetic distance and geographical distance, which was performed with 10,000 permutations using the GenAlEx© program, version 6.5. The degree of differentiation within and among the populations and regions was determined by analysis of molecular variance (AMOVA), while estimating F statistics, including the F_{ST}. The AMOVA was conducted with 100,000 permutations using the Arlequin© software version 3.5.1.3 (Excoffier and Lischer, 2010). To show the existence of evolutionary forces acting on individuals and populations, a series of tests of Hardy–Weinberg equilibrium (HWE) (for each locus) was conducted with the same software. The genetic population structure was studied with Bayesian statistics using the Structure© program, version 2.3.2. (Pritchard *et al.*, 2000), with 100,000 burn-in iterations and 1,000,000 iterations after burn-in or Markov Chain Monte Carlo simulation. We used a model of admixtured ancestry, with 20 repetitions of each set of genetic populations (K1–K20). The value of K was estimated following the procedure of Structure Harvester© program version 0.6.93 (Earl and Vonholdt, 2012), which uses the procedure of Evanno *et al.* (2005) for estimating the actual number of genetic groups. For comparison purposes, a database of AFLP markers was used for the same populations and individuals, created by Sánchez-Gutiérrez (2010) and Ovando-Medina *et al.* (2011a), from which diversity descriptors for dominant markers were determined.

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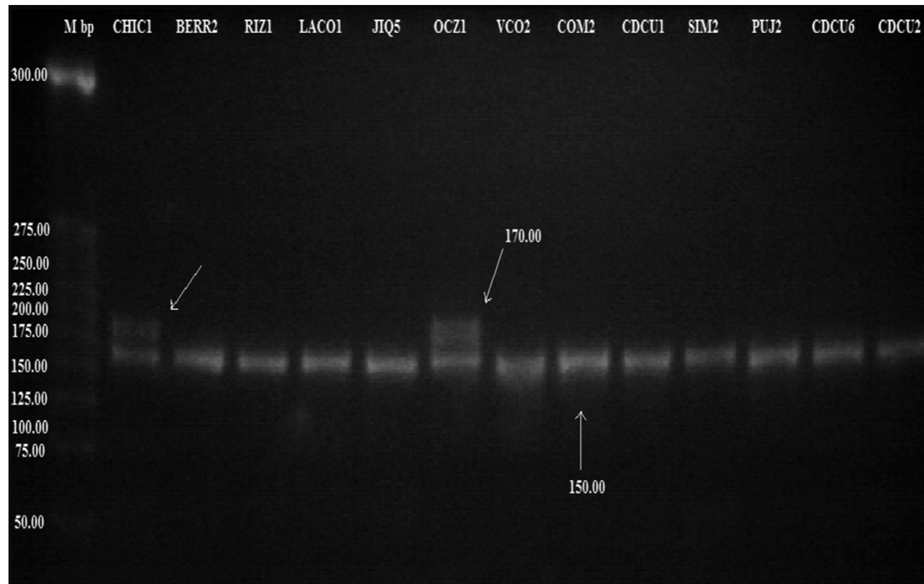

Fig. 1. Acrylamide gel showing alleles of 150 and 170 bp of the microsatellite *Jcds24* in 13 accessions of *Jatropha curcas* of Chiapas, Mexico. Accessions CHIC1 and OCZ1 are heterozygous for this locus. M: Molecular marker 25 bp DNA Step Ladder (Promega®, Madison, WI, USA).

Results

Marker informativeness

The ten pairs of oligonucleotides allowed for the detection of 27 amplified fragments, of which 77.78% were polymorphic, and it was possible to distinguish homozygous individuals from heterozygous ones (Fig. 1). The microsatellite regions *Jcds58* and *Jcps6* were found to be monomorphic, as they only exhibited alleles of 108 and 110 bp, respectively, in all the individuals studied. The average number of alleles (N_a) per locus was 2.286, and the most informative microsatellite was *Jcps20*, which exhibited nine alleles (158, 196, 200, 208, 214, 224, 230, 234 and 244 bp), with a polymorphic index content (PIC) value of 0.784. Only two alleles were private: 158 bp from the locus *Jcps20* for the population Soconusco and 114 bp from the locus *Jcds10* for the population Riviera. Table 2 lists the alleles and PIC data of all the studied microsatellites.

Genetic diversity

The polymorphism per population ranged from 37.5% (Mapastepec) to 87.5% (Centre), with an average of

Table 2. Informativeness of microsatellite loci for the populations of *Jatropha curcas* from Chiapas, Mexico[a]

Loci	Average number of alleles per population	Detected alleles (bp)	Reported alleles (bp)[b]	Calculated PIC	Reported PIC[b]
Jcds10	1.143	108/114	108/112/122	0.021	0.470
Jcds24	2.000	204/240	204/210/216/246	0.284	0.230
Jcds41	1.286	102/138	102/114	0.021	–
Jcds66	1.571	228/250	216/228	0.087	0.240
Jcms21	3.286	75/66/81/103	75/78/81/89/90/92	0.544	0.388–0.390
Jcps9	1.286	150/170	132/140/145	0.076	0.170
Jcps20	6.286	158/196/200/208/214/224/230/234/244	224/260/271/278	0.784	0.030
Jcps21	1.429	202/250	189/200/208	0.092	0.230
Jcds58	Monomorphic	102	104/112	–	–
Jcps6	Monomorphic	270	288/305/380	–	0.220

PIC, polymorphic information content.
[a] The data were obtained by amplification of 93 samples from seven populations. [b] Data from Pamidimarri *et al.* (2009a, b, 2010), Ricci *et al.* (2012); and Vischi *et al.* (2013).

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Table 3. Diversity descriptors from seven populations of *Jatropha curcas* from Chiapas, Mexico

Populations	Region	*n*	N_a	N_e	*I*	H_o	H_e	*F*
Soconusco	Chiapas Coast	17	2.375	1.686	0.372	0.169	0.186	0.068
Mapastepec	Chiapas Coast	5	1.750	1.530	0.324	0.175	0.183	0.056
Isthmus	Chiapas Coast	9	2.125	1.820	0.421	0.181	0.220	0.289
Riviera	Central Chiapas	9	2.375	1.796	0.474	0.314	0.254	−0.190
Centre	Central Chiapas	10	2.500	1.671	0.538	0.313	0.301	−0.031
Frailesca	Central Chiapas	26	2.500	1.640	0.447	0.247	0.239	−0.009
Border	Central Chiapas	17	2.375	1.565	0.412	0.243	0.225	−0.074
Total		93	2.286	1.673	0.427	0.234	0.230	0.001
Standard error		–	0.237	0.155	0.073	0.043	0.037	0.042

n, Number of individuals; N_a, number of alleles; N_e, effective number of alleles; *I*, Shannon's information index; H_o, observed heterozygosity; H_e, expected heterozygosity; *F*, fixation index.

58.9%. There was a marked difference between the populations from the Chiapas Coast, which had 50% or less polymorphism, and those from Central Chiapas, with values >62.5%. Table 3 summarizes the diversity descriptors as found, indicating that in all the populations the observed N_a was greater than the N_e.

The *I* value ranged from 0.324 in the least diverse population (Mapastepec) to 0.538 in the most diverse one (Centre), with an overall average of 0.427. Populations from the coastal region of Chiapas exhibited heterozygote deficiency, as their values of H_e were higher than those of H_o and therefore their *F* were positive. On the other hand, populations from the central region of Chiapas had negative values of *F*, indicating excess of heterozygotes (Table 3).

Reproductive pattern

The HWE tests revealed that the majority of loci were in equilibrium; that is, the genotype frequencies observed for each locus in each population were consistent with expectations. However, the loci *Jcps20*, *Jcds66* and *Jcms21* departed significantly from HWE, and in particular, the locus *Jcps20* was in disequilibrium in all the populations (Table S2, available online), indicating a reproductive pattern of non-random mating.

Genetic structure of populations

The AMOVA (Table 4) revealed that the populations were structured and moderately differentiated. As expected, the largest proportion of variation was found within the populations; however, the F_{ST} differentiation index had a value of 0.087 ($P < 0.05$), indicating that there are important genetic differences between the regions ($F_{RT} = 0.056$; $P < 0.000$) and, to a lesser extent, among the populations within each region ($F_{SR} = 0.033$; $P < 0.000$). The differentiation found among the populations was not due to isolation by distance, as the Mantel test was not significant ($r(x,y) = 0.513$, $P = 0.137$, $r^2 = 0.263$). Evidence was found that differentiation might be due to allopatry (i.e. the speciation process that occurs when populations become isolated from each other preventing the genetic interchange), with the mountain range Sierra Madre de Chiapas being the main geographical barrier between the coastal and central regions. Bayesian analysis revealed that accessions belonged to only four genetic clusters ($\Delta K = 4$) and confirmed the differentiation between the regions. In Fig. 2, the proportion of alleles in each genetic group (four colours) for each individual (upper vertical bar) for each geographical population is shown. Pie charts for each geographical population are also shown in the Chiapas satellite image in the figure. In each population, there

Table 4. Analysis of molecular variance of 93 accessions of *Jatropha curcas*, belonging to seven populations and two regions of Chiapas, Mexico

Sources of variation[a]	Degrees of freedom	Sum of squares	Mean squares	Estimated variance	Variation (%)
Among regions (Central *vs.* Chiapas Coast)	1	6.858	6.858	0.059	6
Among populations	5	8.950	1.790	0.033	3
Within populations	179	174.284	0.974	0.974	91
Total	185	190.091	–	1.066	100

[a] Obtained using data from eight microsatellite loci.

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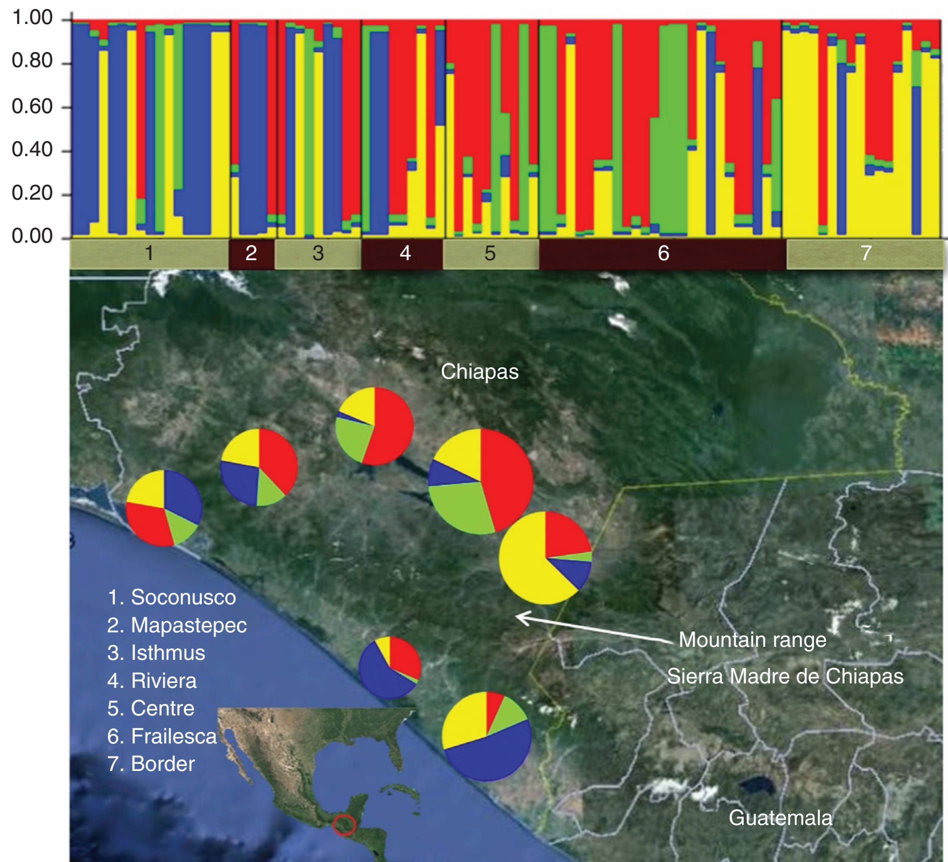

Fig. 2. Genetic structure of seven populations of *Jatropha curcas* in Chiapas, Mexico. Upper part scale shows individuals (vertical bars) and their ancestry proportion. This was obtained using the Structure© 2.3.2. software (Pritchard *et al.*, 2000) with 100,000 burn-in, 1,000,000 repetitions after burn-in and 20 runs of each K (1–20). Ancestry model: admixtured. The K value was 4 (Evanno *et al.*, 2005).

are a relatively large fraction of alleles (colours) from other populations, showing mixed ancestry.

Discussion

The microsatellite loci used in this study were selected for being among the most informative from previous studies (for a review, see Achten *et al.*, 2010); however, two of them were monomorphic and a further five had PIC values <0.100 (Table 2), showing that such markers are scarcely informative for at least the populations studied. Nevertheless, the eight polymorphic loci did allow for estimating population diversity, because they all had the capacity for detecting heterozygotes as three of them had high PIC values, especially *Jcps20* (PIC $= 0.784$). The last *locus* exhibited nine alleles, while only four had been reported (Pamidimarri *et al.*, 2009a). In general, the alleles detected were within the range of those that had been detected in the Asian accessions of *J. curcas* (Pamidimarri *et al.*, 2009b; Ricci *et al.*, 2012), although there were 20 alleles that had not been found in this species. Also, the degree of polymorphism (eight of ten microsatellites) was similar to that reported by Pamidimarri *et al.* (2009b), who found that seven of the 12 microsatellites studied were polymorphic. These findings let us infer that further research is required for more hypervariable co-dominant markers, as those available might not detect variation in populations of *J. curcas* with low diversity, such as Asian (Sun *et al.*, 2008; Wen *et al.*, 2010; Sato *et al.*, 2011; Yadav *et al.*, 2011) and South American (Rosado *et al.*, 2010). On the other hand, and in case of confirmation of absence in other populations in a larger study, the two specific or private bands could be isolated, sequenced and converted to robust sequence characterized amplified region (SCAR)-type markers. The availability of SCAR markers would facilitate the detection of migrant genotypes in other populations, whether by natural causes or human dispersal.

Recent years have seen an exponential increase in the number of studies carried out on the genetic diversity of *J. curcas*; however, most of the research has been based on dominant molecular markers (for a review, see

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Ovando-Medina *et al.*, 2011b). The dominant marker systems, such as AFLP, RAPD, and ISSR, among others, have the advantage of generating a large number of DNA bands, but have the disadvantage of not revealing the proportion of homozygous and heterozygous individuals. Among the several co-dominant markers, the SSRs or microsatellite DNAs have several advantages due to their co-dominant nature, are abundant in the genome, are highly reproducible, possess hyperpolymorphism, are rapidly evolving, and can be applied both on an infraspecific level and between related species (Yadav *et al.*, 2011). The type of marker used can skew the values of diversity indices; for example, the use of SSR markers entails the risk of overestimating the diversity indices, especially when using a less number of loci, owing to the high allelic variability in SSR sequences (Xiang *et al.*, 2007). Nevertheless, studies using SSR markers have found low-to-moderate genetic diversity in the germplasm of *J. curcas* in Asia and South America (Sun *et al.*, 2008; Rosado *et al.*, 2010; Wen *et al.*, 2010; Sato *et al.*, 2011; Yadav *et al.*, 2011). The present study has demonstrated a low informative level of microsatellites reported for *J. curcas* (see Achten *et al.*, 2010, for a review of the SSR loci discovered in this species); however, this is not the cause for the low polymorphism detected in the accessions of Old World and South America, but rather its low diversity, as high diversity was found in the accessions of Chiapas at the same loci. These results confirm the results reported by Ambrosi *et al.* (2010), who studied microsatellite allelic variation in *J. curcas* from around the world and included nine individuals of Jalisco, Mexico, finding that the greatest genetic diversity was in the Mexican germplasm.

In general, the genetic diversity of *J. curcas* in Mexico estimated with SSR markers is similar to that estimated using dominant markers such as AFLP (Ovando-Medina *et al.*, 2011a). In the present study, a comparison of the diversity detected with AFLP and SSR markers (see the Materials and methods section) was made, from which it may be concluded that although global values of H_e (0.230) and I (0.427) obtained using SSR markers are higher than those obtained using AFLP markers ($H_e = 0.190$, $I = 0.294$), the diversity pattern is the same; that is, populations from Central Chiapas were more diverse ($I = 0.340$) than those from the Chiapas Coast ($I = 0232$). In another study of *Jatropha* populations of Chiapas using fatty acids as chemical markers, a genetic barrier was detected, isolating populations from Central Chiapas with respect to those from Guatemala and the Chiapas Coast (Ovando-Medina *et al.*, 2011c). This confirms the differentiation between *J. curcas* populations at the regional level.

The results of this study support the hypothesis of Mesoamerica being the centre of diversification of the genus *Jatropha*, and *J. curcas*, in particular, although other studies have obtained results that do not support this idea. For example, Vischi *et al.* (2013) analysed 26 loci, but only seven exhibited polymorphism (16 alleles) in 29 *J. curcas* accessions from six countries, including Mexico. Most of the genotypes that they studied were completely or highly homozygous and the genetic variability was very low, even in accessions from Mexico. These authors attribute their results to the sampling method, because they collected isolated plants instead of populations. We hypothesize that the other possibility is that sampling was biased towards non-toxic genotypes, whereas in Mexico most of the *J. curcas* genotypes are toxic.

Regarding the genetic structure, the AMOVA revealed that the largest proportion of variation was within the populations; however, the differentiation index F_{ST} (0.087) indicated that the populations were moderately differentiated, which was mainly due to the contrast between the regions ($F_{RT} = 0.056$). In the same vein, it was found that the values of fixation index (F), which indicates the rate of inbreeding in a population, were negative in populations from Central Chiapas, indicating excess of heterozygotes, while populations from the Chiapas Coast had heterozygote deficiency (F positives). In the same vein, Bayesian analysis revealed that the seven populations declared *a priori* had alleles of only four genetic groups, indicating genetic migration (mixed ancestry) among the populations within regions. However, there exists a clear differentiation between the central and coastal regions of Chiapas, especially between Soconusco and Border (Fig. 2), demonstrating that Sierra Madre de Chiapas is an effective geographical barrier that has prevented gene flow between them. The mountain range called Sierra Madre de Chiapas arose about the middle Miocene–early Pliocene (Burkart, 1978; Aguayo and Trápaga, 1996), probably before the *Jatropha* colonization of Chiapas (after the closure of the Isthmus of Panama, about three million years ago; Carels, 2009; Ovando-Medina *et al.*, 2013), possibly indicating two routes of *Jatropha* dispersion: throughout the coastal zone and to the North of the mountain. Furthermore, differentiation was not due to isolation by distance (Mantel $P = 0.137$), but probably due to allopatry. It is known that genetic differentiation among populations depends on gene flow through pollen and seed dispersal (Loveless and Hamrick, 1984) and in the case of cultivated plants, such as *J. curcas*, by human dispersion. The latter, together with the fact that the plant is mainly propagated by cloning, may explain the differentiation pattern found, as farmers tend to exchange germplasm on a regional level and less likely among the regions. The HWE tests revealed that the loci

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Jcps20, *Jcds66* and *Jcms21* departed from the equilibrium and especially the locus *Jcps20* was in disequilibrium in all the populations, indicating a reproductive pattern of non-random mating, and possibly that evolutionary forces that act upon some individuals of the populations exist (Hedrick, 2011). Such forces include migration (caused by the germplasm interchange by farmers) and asexual reproduction, due to the propagation technique used by Mesoamerican *Jatropha* farmers. Other researchers raised similar ideas to explain the Hardy–Weinberg disequilibrium exhibited by *J. curcas* populations. For example, five microsatellite loci studied in the Asian accessions of *J. curcas* exhibited equilibrium deviations, due to the presence of null alleles or as a result of natural dispersal disturbances due to human activity (Basha and Sujatha, 2007; Sudheer *et al.*, 2008; Pamidimarri *et al.*, 2009a).

The results of this study reveal that *J. curcas* in Chiapas has genetic diversity that is greater than that reported in other parts of the world, which represents a potential germplasm pool for the improvement of the plant or for the selection of genotypes for field planting.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262114000550

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