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Genetic Diversity of *Jatropha curcas* L. in Veracruz State, Mexico, and its Relationships with the Content of Phorbol Esters

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There is growing interest worldwide for *Jatropha curcas* because of the high oil content in the seeds, which can be used as base material for biodiesel production. This plant is native to Mexico (Mesoamerica) and can be found as toxic and non-toxic. However, there is little information on the genetic diversity of *J. curcas*. The purpose of this study was to analyze the genetic diversity of 64 accessions of *J. curcas* in the state of Veracruz, Mexico, and its relationship with the contents of phorbol esters. We used 12 microsatellites designed previously for this species. Loci were amplified using the polymerase chain reaction and alleles were separated on polyacrylamide gels. From all loci amplified, six were selected according to the clarity of the alleles and the polymorphisms generated. Heterozygous individuals were detected in only two of the six loci used. The accessions collected from northern Veracruz state were clustered together. Phorbol ester analysis confirmed the presence of toxic and non-toxic *J. curcas*. At the genetic level, none of the microsatellites employed were able to distinguish between toxic and non-toxic samples. Toxic plants were found in the central and southern parts of the state with phorbol ester contents ranging from 0.12 to 0.66 mg g^{-1}, while non-toxic plants were distributed throughout the state. Given the importance of *J. curcas* as a biofuel, and the diversity of this plant species in the state of Veracruz, molecular techniques are helpful in locating and selecting individuals for breeding programs.

Keywords: Genetic diversity, Phorbol esters, *Jatropha curcas*, Microsatellites, Veracruz

INTRODUCTION

In many countries, the interest in studying *Jatropha curcas* has increased due to its high seed-oil content as raw material for biodiesel production. *Jatropha curcas* belongs to the family Euphorbiaceae, and in Mexico is commonly known as *piñón* or *piñoncillo* (Rodríguez-Acosta et al. 2009). This plant can grow up to 7 m height, has a lifespan of 50 years (Achten et al. 2008), and although it grows in diverse soil and climate conditions (Herrera et al. 2010), it

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is mainly found in areas of tropical and subtropical climate (Pecina-Quintero et al. 2011). The plant is often used as a living fence (hedgerow) (Reyes and Rosado 2000) and in some rural communities in Veracruz and Puebla states, the seeds are used in cooking local dishes (Makkar et al. 1998; Martínez-Herrera et al. 2006).

The wide distribution of *J. curcas* in Mexico, and the presence of 46 species in the genus (Steinmann 2002), suggests that Mexico is the center of origin from which it was dispersed by Portuguese traders to Thailand and other Asian countries during the last two centuries (Ovando-Medina et al. 2011a; Senthil Kumar et al. 2009; Sirithunya and Ukoskit 2010; Sun et al. 2008; Tatikonda et al. 2009). In the town of Papantla, Veracruz (Totonaca region), there are non-toxic accessions, which contain little or no phorbol esters (Makkar et al. 1998).

The establishment of commercial plantations of *J. curcas* in Mexico and other countries has been limited by problems such as: 1) high variability in yield per plant, 2) asynchrony in fruit ripening, 3) presence of toxic compounds in the oil and by-products, and 4) attacks by pests and diseases. These problems are explained because the plant is still in the process of domestication, thus requiring the selection of individuals with outstanding characteristics for a breeding program (Grativol et al. 2011).

In the search for genetic diversity, the use of DNA molecular markers have successfully been used in many plant species with commercial importance (Botta et al. 1995; Varshney et al. 2005). Basha and Sujatha (2007) reported low levels of genetic diversity among 42 accessions of *J. curcas* from different regions of India. Using 400 RAPD and 100 ISSR primers, they found polymorphisms for 42% and 33.5% of each primer type tested, respectively. In a subsequent study, Ranade et al. (2008), using seven RAPD primers and four DAMD primers, found high levels of genetic variation among 12 *J. curcas* accessions in India, and attributed the increased variability to the inclusion of wild material.

The use of new microsatellite sequences in *J. curcas* (Pamidimarri et al. 2009), and the use of microsatellites from related species such as *Manihot esculena* (Wen et al. 2010) have made it possible to reveal the genetic diversity among accessions collected from different geographic places and to elucidate phylogenetic relationships among some species of *Jatropha* (Sudheer et al. 2011). These developments have also proven be useful in differentiating between toxic and non-toxic accessions.

However, in most studies using microsatellites or other molecular DNA markers, low genetic diversity among *J. curcas* accessions has been reported within countries, and only some genetic variability has been revealed when accessions from different countries were included. In China (Sun et al. 2008), 58 accessions of *J. curcas* were analyzed using microsatellite markers and AFLP 's and from 77 microsatellite loci tested, only one was polymorphic with two alleles, whereas of seven AFLP 's tested, 14.3% were polymorphic. However, only with microsatellites, genetic variability among accessions from China and Malaysia was detected.

In studies that have included Mexican accessions, variability is attributed to the presence of the Mexican accessions, which are generally non-toxic (Na-ek et al. 2011). In the analysis of toxic and non-toxic accessions using microsatellites, special attention has been paid to identify microsatellite markers that help differentiate among the two groups. Basha et al. (2009) tested 17 microsatellites from 72 accessions from 13 countries and found that the microsatellite jcSSR26 had four alleles of which one was specific to non-toxic accessions. Sudheer et al. (2009) found a homozygous allele for microsatellite jcms21 among six divergent toxic accessions, which included a non-toxic accession from Mexico. Tanya *et al*. (2011) reported that five microsatellites (MPN006, MPN007, MPN008, MPN016, MPN046), out of 49 designed by them, showed different banding pattern among 26 non-toxic Mexican accessions and 10 Asian toxic accessions. Although the authors indicated the usefulness of these new microsatellites to differentiate between toxic and non-toxic forms of the accessions, it was unclear whether the genetic variability reported was related to the origin of the accessions or to its toxicity.

In Mexico, the information available on the genetic diversity of *J. curcas* is still limited. Studies from the state of Chiapas, Mexico, have suggested high genetic diversity. Pecina et al. (2011), using six AFLP markers from 88 accessions reported high levels of polymorphism (90%) and genetic diversity (60%). Similar results were obtained by Ovando et al. (2011b) who analyzed AFLP markers from five populations of *J. curcas* located along living fences (hedgerows). The polymorphism level obtained was 81.18%, and according to the Analysis of Molecular Variance (AMOVA), most variation was found within the populations (87.8%) rather than among different populations (7.88%).

The objectives of this study were to use microsatellite markers to determine the genetic diversity available in the germplasm of *J. curcas* from the state of Veracruz, Mexico, to look for a correlation between genetic diversity and phorbol ester content, and to identify individuals with high genetic diversity and low toxicity for potential integration into breeding programs.

MATERIALS AND METHODS

Plant material

During September and October, *J. curcas* from different parts of the state of Veracruz were located (Figure 1). The collection of accessions was made in different types of

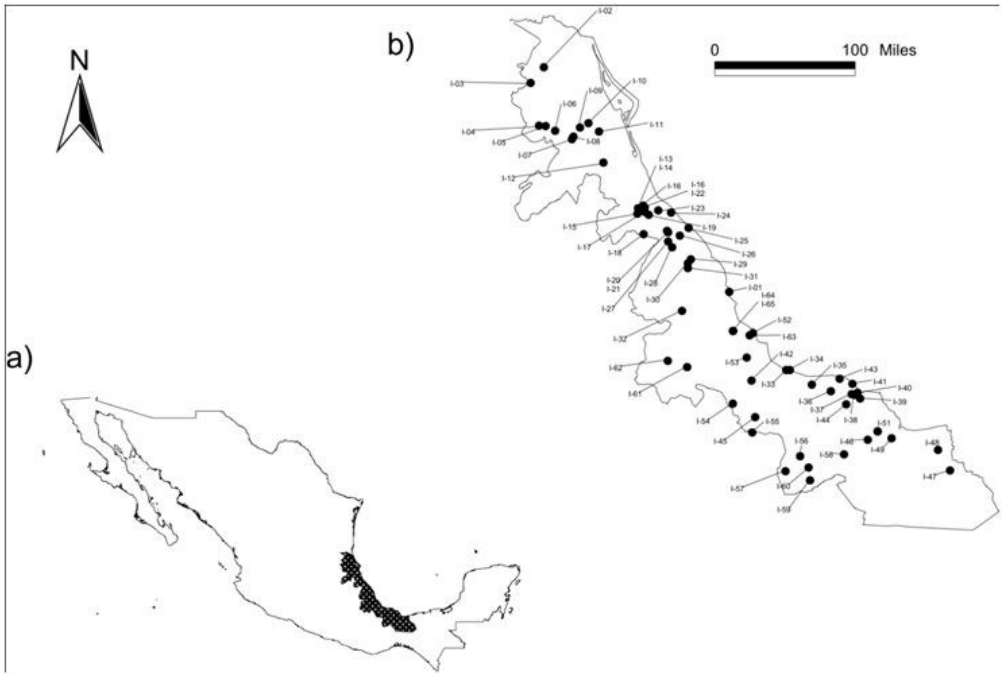

Figure 1 a) Location of Veracruz state in Mexico Map; b) Sites of the accessions of *J. curcas* collected in Veracruz

vegetation, climate and altitude, to obtain wild and cultivated material present in the state. A total of 64 accessions were collected (Table 1), from which young leaves were sampled for DNA extraction. The leaves were disinfected with a 5% bleach solution and stored in plastic bags with silica until being processed in the laboratory. During harvesting, 38 plants in the fruiting stage were included so a sample of 500 g of seed was used to determine phorbol ester content and toxicity.

DNA extraction, amplification and microsatellite analysis

DNA extraction was performed using 200 mg of dried leaf tissue and the *DNeasy Plant Mini Kit* (Qiagen, Germany) following supplier specifications. DNA concentration and quality were determined using 0.8% agarose electrophoresis, and DNA concentration was standardized for all accessions before amplification.

Twelve microsatellite loci previously characterized for *J. curcas* were tested [19], from which six were selected that were polymorphic, being: jcds10, jcds41, jcps6, jcps9, jcps21 and jcms30 (Table 2). Except locus jcds41, annealing temperatures reported for other loci were modified as follows: 59 °C for jcds10, 57 °C for jcds41 and jcps6, 65 °C for jcps9 and jcps21, and 55 °C for jcms30. The temperature changes were made because hybridization in preliminary tests at recommended temperatures yielded byproducts of the amplification reaction in the acrylamide gels, requiring new temperatures be used to prevent their occurrence and to obtain gels with sharp and clear bands.

Microsatellite amplification was performed in a volume of 25 µL which contained: 1X TBE buffer (10 mMTrizma, 8.9 mM boric acid and 2 mM Na_2 EDTA), 1.5 mM magnesium chloride, dNTPs at a concentration of 2 mM each, 10 µM microsatellite loci, 25 ng of DNA template and 1.5 units of Taq polymerase (Invitrogen, California, USA). The amplification reaction was performed in a thermocycler (*Labnet Multigene II*) following Pamidimarri et al. (2009).

PCR products were separated by vertical electrophoresis (Thermo-Scientific Owl P10DS Dual Gel System) using 6% polyacrylamide gels and 1X TBE buffer according to Benbouza et al. (2006). Prior to electrophoresis, the DNA was denatured for two minutes at 92 °C. Two marker sizes were used, one in the first gel row which consisted of 11 fragments ranging from 100 to 1100 base-pairs (bp) in increments of 100 bp (Promega, California, USA), and another in the middle consisting of 12 fragments from 500 to 12,000 bp (Invitrogen, Carlsbad, California, USA). Gels were stained with silver nitrate to reveal the bands. Images were captured using a Kodak Digital Science® camera. To determine allele size (bp), the software 1D *Image Analysis* (version 3.0) was used with reference to the two markers.

Collection and analysis of phorbol esters

Phorbol esters were obtained according to Makkar and Becker (1997) with minor modifications. One gram of *J. curcas* seed endosperm was sonicated for three minutes with 10 mL of absolute methanol and then centrifuged at

Table 1. Accession, municipality, region, geographic coordinates and altitude of the sites where accessions of *J. curcas* were collected in the state of Veracruz, Mexico.

Accession	Municipality	Region	Latitude (N)	Longitude (W)	Altitude (masl)
I-01	Actopan	Capital	19°35'	96°23'	4
I-02	Panuco	Huasteca Alta	21°54'	98°17'	30
I-03	El Higo	Huasteca Alta	21°45'	98°25'	20
I-04	Tantoyuca	Huasteca Alta	21°18'	98°20'	130
I-05	Tantoyuca	Huasteca Alta	21°18'	98°16'	94
I-06	Tantoyuca	Huasteca Alta	21°15'	98°10'	113
I-07	Ixcatepec	Huasteca Baja	21°10'	98°00'	258
I-08	Ixcatepex	Huasteca Baja	21°11'	97°59'	228
I-09	Chontla	Huasteca Baja	21°17'	97°55'	281
I-10	Tantima	Huasteca Baja	21°20'	97°50'	184
I-11	Tancoco	Huasteca Baja	21°14'	97°43'	136
I-12	Temapache	Huasteca Baja	20°55'	97°40'	31
I-13	Papantla	Totonaca	20°27'	97°19'	173
I-14	Papantla	Totonaca	20°27'	97°19'	170
I-15	Papantla	Totonaca	20°24'	97°19'	98
I-16	Papantla	Totonaca	20°19'	97°16'	31
I-17	Papantla	Totonaca	20°15'	97°15'	77
I-18	Papantla	Totonaca	20°11'	97°15'	119
I-19	Papantla	Totonaca	20°23'	97°12'	64
I-20	San Rafael	Nautla	20°13'	97°01'	35
I-21	San Rafael	Nautla	20°12'	97°00'	96
I-22	Papantla	Totonaca	20°28'	97°15'	43
I-23	Gutiérrez Zamora	Totonaca	20°26'	97°06'	10
I-24	Tecolutla	Totonaca	20°24'	96°59'	5
I-25	Tecolutla	Totonaca	20°15'	96°48'	5
I-26	San Rafael	Nautla	20°10'	96°53'	9
I-27	Martínez de la Torre	Nautla	20°06'	96°00'	70
I-28	Misantla	Nautla	20°03'	96°58'	54
I-29	Misantla	Nautla	19°55'	96°46'	366
I-30	Misantla	Nautla	19°53'	96°48'	631
I-31	Yecuatla	Nautla	19°50'	96°48'	1054
I-32	Coatepec	Capital	19°24'	96°52'	892
I-33	Alvarado	Papaloapan	18°47'	95°47'	21
I-34	Alvarado	Papaloapan	18°47'	95°45'	22
I-35	Lerdo de Tejada	Papaloapan	18°38'	95°31'	5
I-36	Ángel R. Cabada	Papaloapan	18°34'	95°20'	160
I-37	Catemaco	Tuxtlas	18°32'	95°07'	783
I-38	Catemaco	Tuxtlas	18°31'	95°05'	520
I-39	Catemaco	Tuxtlas	18°29'	95°02'	67
I-40	Catemaco	Tuxtlas	18°33'	95°03'	24
I-41	San Andrés Tuxtla	Tuxtlas	18°38'	95°06'	8

Table 1. Continue

I-42	San Andrés Tuxtla	Tuxtlas	18°40'	96°09'	30
I-43	San Andrés Tuxtla	Tuxtlas	18°41'	95°14'	16
I-44	San Andrés Tuxtla	Tuxtlas	18°26'	95°10'	258
I-45	Hueyapan de Ocampo	Tuxtlas	18°18'	96°06'	490
I-46	Acayucan	Olmeca	18°04'	94°57'	143
I-47	Las Choapas	Olmeca	17°45'	94°06'	50
I-48	Moloacán	Olmeca	17°57'	94°13'	42
I-49	Chinameca	Olmeca	18°05'	94°42'	17
I-51	San Pedro Soteapan	Olmeca	18°09'	94°51'	186
I-52	Veracruz	Sotavento	19°10'	96°8'	10
I-53	Cotaxtla	Sotavento	18°55'	96°12'	12
I-54	Tierra Blanca	Papaloapan	18°26'	96°20'	55
I-55	Cosamaloapan	Papaloapan	18°08'	96°08'	43
I-56	Playa Vicente	Papaloapan	17°54'	95°39'	69
I-57	Playa Vicente	Papaloapan	17°44'	95°48'	77
I-58	Playa Vicente	Papaloapan	17°55'	95°12'	135
I-59	Xochiapa	Papaloapan	17°39'	95°33'	117
I-60	Playa Vicente	Papaloapan	17°47'	95°34'	157
I-61	Yanga	Montañas	18°49'	96°49'	523
I-62	Ixtaczoquitlan	Montañas	18°53'	97°01'	1006
I-63	Veracruz	Sotavento	19°8'	96°10'	12
I-64	Manlio Fabio Altamirano	Sotavento	19°11'	96°20'	16
I-65	Manlio Fabio Altamirano	Sotavento	19°11'	96°20'	15

3,000 g for 8 min. The supernatant was collected and the extraction operation repeated two more times. The recovered supernatant was dried with a rotary evaporator at 40 °C and then redissolved in 1 mL of methanol and transferred to an HPLC vial for analysis. Quantification of phorbol esters was performed using an Agilent 1200 HPLC and an RP18 column (Phenomenex) of 150 x 4.6 mm and 5 m. For the mobile phase, gradients of water and acetonitrile were used. The resulting peaks were integrated and the results expressed as equivalents of phorbol-12-myristate-13-acetate.

Analysis of genetic diversity

Genetic diversity was characterized in terms of the number of alleles (Na), observed and expected levels of homozygosity (H_0) and heterozygosity (H_E) and the Nei diversity index (h) using the program Popgene (Yeh et al. 2000), assuming Hardy-Weinberg equilibrium. The polymorphism information content (PIC) for each locus was calculated using Excel MICROSAT v. 2007, using the formula PIC = 1 - Σ*P*i, where *P*i is the frequency of allele i in the genotypes examined (Weir and Cockerham 1984). The presence of null alleles was calculated using the maximum likelihood estimation (ML) in the EM algorithm (*Expectation-Maximization*) following Dempster et al. (1977) in the program GenePop 4.0 (Rousset 2008). The fixation index (F) was calculated as a measure of excess or deficiency of heterozygosity within the species using the program FSTAT version 2.9.3.2 (Goudet 2001). We built a binary matrix of presence (1) or absence (0) of alleles and calculated the Jaccard Similarity Coefficient. The similarities among the accessions were grouped using the *Unweighted Pair Group Method with Averages* (UPGMA).

RESULTS

Six microsatellite loci were used to characterize the genetic diversity of *J. curcas* in the state of Veracruz, Mexico. Allele sizes ranged from 124 for jcms30 to 806 bp for jcps6. In total, there were 18 alleles with an average of three alleles per locus; jcds41 was the locus having the highest number of alleles (5) (Table 3).

Observed heterozygosity (H_O) ranged between 0 and 0.25, while expected heterozygosity (H_E) ranged between

Table 2. Characteristics of the six microsatellites (Pamidimarri et al. 2009).

Gen Bankmarker	Primer sequence (5'-3')	T_a (ºC)	A	Repeated sequence	Allele size (bp)
jcds10 (EU586340)	F: CATCAAATGCTAATGAAAGTACA R: CACACCTAGCAAACTACTTGCA	46.5	4	(TG)6CACGCA(TG)4	108-122
jcds41 (EU586341)	F: AACACACCATGGGCCACAGGT R:TGCATGTGTGCGGGTTTGATTAC	56.5	5	(CA)6(TA)2	102-114
jcps6 (EU586346)	F: CCAGAAGTAGAATTATAAATTAAA R: AGCGGCTCTGACATTATGTAC	44.0	4	(AT)3G(TA)3... (CT)3 ...(GT)5CT(GT)3	288-305
jcps9 (EU586347)	F: GTACTTAGATCTCTTGTAACTAACAG R: TATCTCTTGTTCAGAAATGGAT	48.0	4	(GT)3GC(TG)2A(GT) 3	140-165
jcps21 (EU586349)	F: CCTGCTGACAGGCCATGATT R: TTTCACTGCAGAGGTAGCTTGTATA	54.8	3	(CA)2...(CA)4	189-200
jcms30 (EU586351)	F: GGGAAAGAGGCTCTTTGC R: ATGAGTTCACATAAAATCATGCA	48.5	2	(GT)5T(TG)2	135-144

T_a (ºC)=annealing temperature; A=alleles

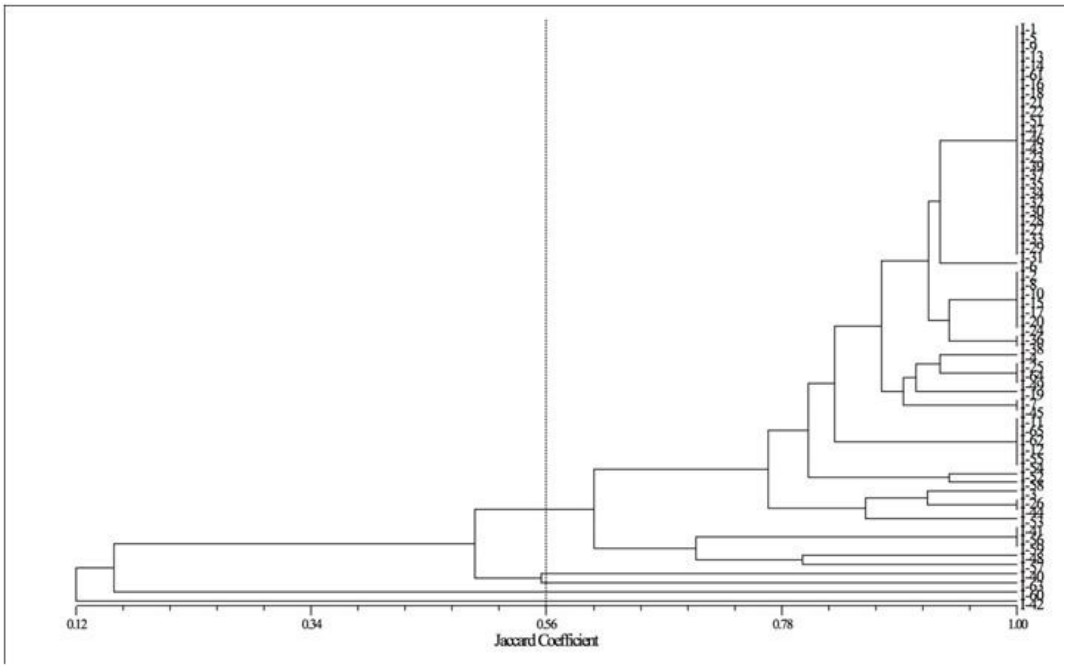

Figure 2 Dendrogram of the 64 accessions de *J. curcas* grouped according to Jaccard's Similarity Coefficient

0.6667 and 0.8929 for the loci jcds10 and jcds41, respectively. The mean H_O and H_E were 0.125 and 0.764, respectively. PIC values varied between 0.375 and 0.746 with a mean of 0.522. According to the fixation index (F), there was an excess of homozygotes at loci jcds10, jcps6, jcps9 and jcps21. The frequency of null alleles ranged from 0.3333 for locus jcms30 to 0.7071 for locus jcds10, jcps9 and jcps21.

The dendrogram shows that similarity values separate the accessions into eight groups (Figure 2). Group I contains accessions collected from the northern and central areas of the state, including those collected from the regions of Huastecabaja (6), Totonaca (11), Capital (1) and Las Montañas (2). Group II contained accessions I-03, I-26, I-44 and I-53, which correspond to the regions Huasteca alta, Nautla, Tuxtlas and Sotavento, respectively. Group III and the remaining groups were formed by the accessions collected from central and southern areas of the state: Sotavento, Papaloapan, Tuxtlas and Olmeca.

Table 4. Phorbol ester content among accessions of *J. curcas* collected from different regions of Veracruz state.

Accession	Region	Phorbolesters (mg g^{-1})
I-01	Capital	0.43
I-02	Huasteca alta	ND
I-04	Huasteca alta	ND
I-05	Huasteca alta	ND
I-08	Huasteca baja	ND
I-11	Huasteca baja	ND
I-13	Totonaca	ND
I-14	Totonaca	ND
I-16	Totonaca	ND
I-18	Totonaca	ND
I-22	Totonaca	ND
I-25	Totonaca	ND
I-26	Nautla	ND
I-27	Nautla	ND
I-29	Nautla	ND
I-30	Nautla	ND
I-31	Nautla	ND
I-32	Capital	ND
I-33	Papaloapan	0.42
I-34	Papaloapan	ND
I-35	Papaloapan	0.45
I-36	Papaloapan	0.66
I-38	Tuxtlas	0.12
I-41	Tuxtlas	ND
I-42	Tuxtlas	0.28
I-43	Tuxtlas	0.44
I-44	Tuxtlas	0.43
I-45	Tuxtlas	0.48
I-47	Olmeca	ND
I-48	Olmeca	ND
I-52	Sotavento	0.23
I-53	Sotavento	0.16
I-57	Papaloapan	0.23
I-59	Papaloapan	0.38
I-61	Montañas	0.65
I-62	Montañas	ND
I-64	Sotavento	ND
I-65	Sotavento	ND

ND: Not Detected

Accessions I-40, I-63, I-60 and I-42 were the most dissimilar and were individually separated from the rest.

Phorbol ester analyses tested negative for 63% of the accessions. Remaining accessions were considered toxic or of low toxicity with phorbol ester contents within a range of 0.12 to 0.66 mg g^{-1} (Table 4). Phorbol esters were not detected in accessions collected from the northern region of the state. However, these compounds were present in samples collected from the central and southern regions (Figure 3).

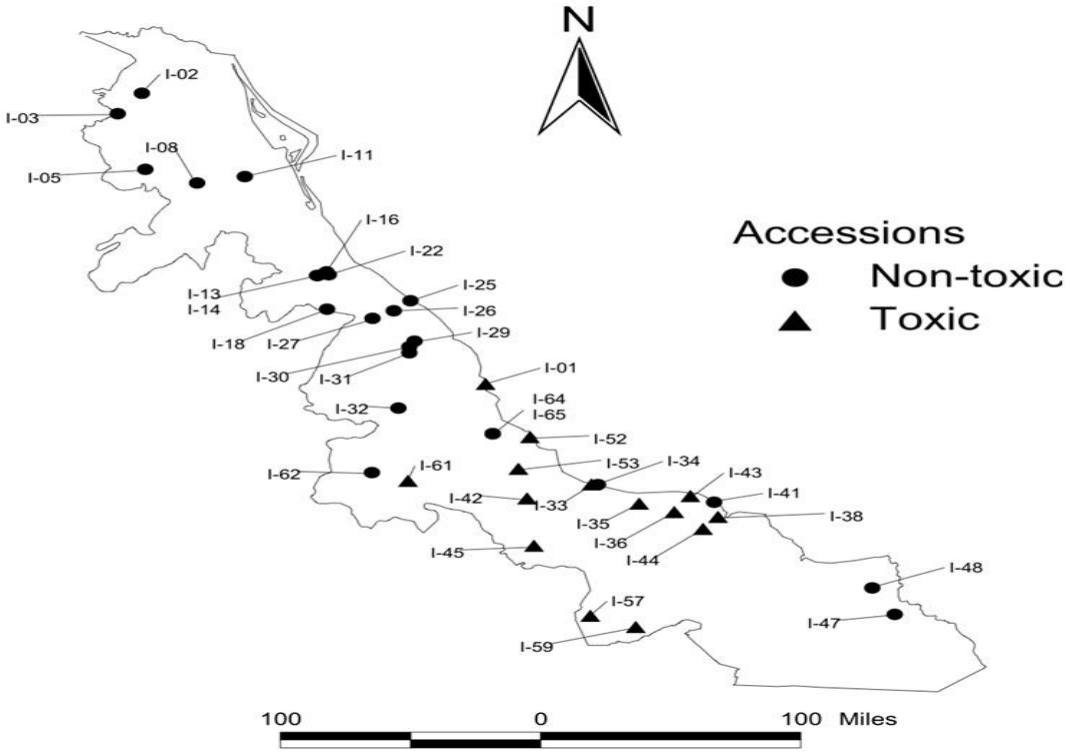

Figure 3 Distribution of toxic and non-toxic *J. curcas* accessions among collection sites in the state of Veracruz

DISCUSSION

Studies of *J. curcas* germplasm genetic diversity in countries like India (Gupta et al. 2008; Pamidimarri et al. 2010; Tatikonda et al. 2009), China (Shen et al. 2010; Sun et al. 2008) and Brazil (Grativol et al. 2011), have reported low variability independently of the molecular marker used. However, in studies examining the genetic variability of *J. curcas* from different countries that have included accessions from Mexico, has been found a clear separation of the Mexican accessions (Basha et al. 2009; Basha and Sujatha 2007; Na-ek et al. 2011; Tanya et al. 2011). Therefore, the genetic diversity of *J. curcas* in Mexico has a particular interest. Our study only considered the genetic analysis of accessions collected in the state of Veracruz, thus we also observed low genetic variability.

The six microsatellites used showed differences in the number and size of the alleles compared to the original work by Pamidimarri et al. (2009). In loci jcds10, jcps9 and jcps21, the number of alleles was reduced, while in locus jcms30 one more allele was observed, and in loci jcps6 and jcds41 the number of alleles was the same. With the exception of locus jcms30, the size of the alleles increased for all loci. The variation in the number and size of the alleles recorded shows differences between Mexican germplasm and that from other geographical areas, as has been widely reported (Na-ek et al. 2011; Ovando-Medina et al. 2011a).

Yet, among the samples studied, low genetic variability was observed, as evidenced by a heterozygote deficit and high fixation index values. Inbreeding may be contributing greatly to this situation, and in *J. curcas* inbreeding can be influenced by several factors. One potential influence is floral and reproductive biology, such as male and female flowers on the same inflorescence. Although both forms open asynchronously to avoid self-pollination (Luo et al. 2007), they frequently open simultaneously. Bressan et al. (2013) found that *J. curcas* produced seeds apomictically, as well as through a combination of self-pollination and cross-pollination. It is also common to multiply the plant through cuttings (Basha et al. 2009), a method commonly used in the state of Veracruz, a practice that increases the probability of crossing between related organisms.

The presence of null alleles can also lead to underestimates of heterozygosity (Espinoza et al. 2007). The loci jcds10, jcps9, jcps21 and jcps6 showed a high frequency of null alleles, a condition more evident with accessions I-40, I- 63, I-60 and I-42. DNA from these accessions, amplified with at least one of the loci tested, was discarded because their occurrence may have been

due to poor quality DNA. These accessions showed no phenotypic difference that could differentiate them from others, although in the dendrogram they were grouped independently. These results were agree with those of Pamidimarri et al. (2009) in relation to loci jcds58, jcds66, jcps1, jcps6 and jcms30 and did not meet expectations for the Hardy-Weinberg equilibrium. These deviations could be due to the presence of null alleles or disturbances in the natural dispersion of the plant due to anthropogenic management of the species.

In the dendrogram, accessions collected from the northern part of the state were grouped together, while accessions from the central and southern parts of the state were distributed among the different groups formed. This distribution can be related to the different uses given to the plant in the different regions of Veracruz, where they were collected. In the south, the plant is preferably used as a living fence (hedgerow) (Reyes and Rosado 2000), while in the north (Totonacapan region) the seeds, after toasting, are used for food preparation (Herrera et al. 2010). Unpublished data suggest that due to the high esteem people associated with seed consumption, plants were moved from the north to other parts of the state.

The results support the idea that phorbol ester content can be determined using the plant and its distribution. The presence of phorbol esters in accessions from the central and southern regions of the state, and their absence in accessions from the north suggests an anthropogenic origin for the selection, domestication and consumption of the species by the ancient Totonacs.

Phorbol esters are a family of chemical compounds found in many species of plants in the families Euphorbiaceae and Thymelaeceae (Haas and Mittelbach 2000). There is great interest in finding markers that can differentiate toxic and nontoxic forms of *J. curcas*, which would allow for further development of new varieties. According to the dendrogram, none of the six microsatellites used in the present study separated toxic from non-toxic accessions, results that are similar to those reported by Na-ek et al. (2011) who used different microsatellites. However, Sudheer et al. (2009) reported the identification of RAPD, AFLP and microsatellite markers useful for differentiating toxic and non-toxic accessions. The microsatellite locus jcms21 displayed a homozygous allele for the toxic variety. Moreover, Tanya et al. (2011) discovered 49 microsatellite loci for which five showed different banding patterns between 26 non-toxic Mexican accessions and 10 toxic accessions from Asia.

Since *J. curcas* can cross-pollinate (Ganesh Ram et al. 2008), there is a risk for toxic genotypes occurring in areas that normally contain non-toxic seeds for human consumption. Microsatellite molecular markers are very useful for analyzing the diversity of *J. curcas* in the state of Veracruz, and although they were grouped by accessions according to collection site, they were not able to differentiate between toxic and non-toxic genotypes. The presence of both genotypes in the state of Veracruz indicates that further research is warranted on the evolutionary, genetic, climatic and physiological factors that influence the establishment of non-toxic accessions almost exclusively in the state of Veracruz, Mexico.

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