Genetic Variation in Mexican *Jatropha curcas* L. Estimated with Seed Oil Fatty Acids

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Abstract: The genetic diversity of Mesoamerican populations of the biofuel plant *Jatropha curcas*, using the fatty acids of the seeds as chemical markers was studied. The oil content of the whole seed in 135 accessions from 38 sites varied between 8.020% and 54.28%. The prevalent fatty acids were oleic acid (18:1) and linoleic acid (18:2), and the proportion of unsaturated fatty acids varied between 74.5% and 83.7%. A study with cloned plants grown in common garden showed that both the content of oil as well as the proportion of fatty acids are highly inheritable, therefore these chemical markers are valid for estimating the genetic diversity of the species. An analysis of principal components showed that the fatty acids that contribute more to the variance are stearic, oleic, linoleic, methylpalmitic, gadoleic and ricinoleic. The populations were classified in ten groups when the data were analyzed for fatty acids by analysis of clusters, showing the elevated genetic variation in natural populations of this native species of Mesoamerica. A discriminant analysis separated the populations in accordance with their geographic origin, which was verified with a Mantel test. Using the Monmonier's algorithm two genetic barriers between the populations were identified. The results are discussed in light of their microevolutionary significance.

Key words: seed oil, *Jatropha*, Mesoamerica, Mexico, genetic diversity

1 INTRODUCTION

Jatropha curcas L. is perhaps currently the plant of greatest importance for the extraction of oil for the fabrication of biodiesel. Various authors have mentioned the advantageous characteristics of this plant from the family Euphorbiaceae, in comparison with other oleaginous plants, highlighting particularly its adaptation to marginal environments¹⁻⁴.

Although there is no consensus about the origin of this species, many researchers point to the Mesoamerican region (Mexico and Central America) as its center of origin⁵⁻⁷, while others mention South America^{8, 9}; but there is no doubt that Mesoamerica is the center of diversification of the genus *Jatropha*¹⁰ and that this region held the source material for establishment of present populations in Africa and Asia. Moreover, only in Mexico do domesticated genotypes exist of low or null toxicity^{11, 12}.

In the last decade, the number of studies and publications on *J. curcas* have grown geometrically, driven by the growing interest of governmental agencies in many parts of the world, but above all in Southeast Asia, in the cultivation

of the plant. Notoriously, the majority of these reports used Asian accessions to study the agronomic performance, the genetic variation through molecular markers, and of course, the content and composition of the seed oil¹³⁻¹⁸. To a lesser extent, accessions from South America^{19, 20} and from Africa²¹ have been studied. When samples from the Mesoamerican region have been used, it has been only for comparison. On the other hand, little attention has been paid to the composition of fatty acids of the seeds as chemical markers for estimating the genetic diversity of this plant; instead, the studies have been focused on determining the proportion of saturated or unsaturated fatty acids due to their importance in the production of biodiesel, as foodstuff and for usage in industrial processes²²⁻²⁵, with the exception of the work of Wang *et al.*²⁶, who studied some accessions of *J. curcas* introduced in China.

In the present study the content of oil in the seeds was investigated along with its composition of fatty acids in Mexican populations of *J. curcas*, with the purpose of estimating the level of chemical variation and relating this with its evolutionary meaning.

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2 EXPERIMENTAL PROCEDURES

2.1 Sample collection

Plants of *J. curcas* were studied in the Mesoamerican region, covering a total of 38 sites which were *a priori* grouped into 6 populations from areas in the south and southeast of Mexico, and in Guatemala (Table 1, Fig. 4). Each population was represented by at least five trees in the stage of seed production (rainy season, August-September of 2008), from which were collected close to 100 g of seed from each plant. Cuttings of 80 cm in length were also collected, which were rooted in a greenhouse and taken in as 135 accessions of the Bank of *Jatropha* in the Center for Biosciences at the Autonomous University of Chiapas (Tapachula, Chiapas, Mexico).

2.2 Extraction of oil and preparation of FAMES

The seeds from each site were dried at room temperature for at least 30 days, until they possessed less than 2% moisture. The whole seeds (including seed coat, endosperm and embryo) were crushed in a mortar washed with hexane, homogenized, and sampled in triplicate 10 g of the resulting paste for determining the content of oil. The method utilized was Soxhlet 920.39 of the Association of Official Analytical Chemists²⁷⁾. To prepare Fatty Acid Methyl Esters (FAMES), the method of Ichihara *et al.*²⁸⁾ was followed with modifications. In a screw-top test tube previously washed with hexane and dried for 2 h at 300°C, 2 mL of n-hexane were introduced and 40 mg of crude oil were added. The sealed test tubes were brought to 40°C in a bath of water for 1 min and 200 µL of a solution of methanolic NaOH 2.0 M were added. These were agitated in vortex for 2 min, returned to heat for 1 min and centrifuged at 5000 rpm for 10 minutes. The upper hexane phase was separated containing the prepared FAMES.

2.3 Determination of the composition of fatty acids by gas chromatography

The fatty acids were analyzed by gas chromatography/mass spectrometry (Chromatograph Agilent 6890), injecting 0.5 µL of the FAMES from each accession into a column of intermediate polarity (Equity-1, Supelco 28046-U, 30 m × 250 µm × 0.30 µm), with the following program of temperatures: 100°C, 25°C min⁻¹ to 200°C, 2.5°C min⁻¹ to 230°C per one minute, 10°C min⁻¹ to 250°C). The chromatograph was operated using helium as carrier gas (7.48 psi). The injector was programmed in mode Split 60:1 to a temperature of 280°C. A selective mass detector was used (Agilent 5973 Network) operated at a voltage of ionization of 70 eV, with a temperature interface of 280°C, Scan mode and mass rank of 50 to 550 m/z. The compounds from each one of the samples were identified by comparing their mass spectra with those of Library of the National Institute of Standards and Technology (NIST98). The concentration of each compound was obtained comparing its abundance

with that of the internal standard, methyl ester of nonadecanoic acid, and considering the total concentration of the fatty acids as 100%. The relative proportions for each compound were reported in percentage.

2.4 Study in common garden with cloned plants

To study if the composition and proportion of fatty acids in the oil of *J. curcas* of Mesoamerica are fixed characters, 15 accessions clonally reproduced and situated in the Bank of *Jatropha* were evaluated under similar conditions (cultivated during a year in rain-fed andosol approximately 2500 mm rainfall in the 2008-2009 cycle-, average annual temperature of 30°C, without agronomic management). The plants initiated their flowering in May 2009 and the production of seeds in July, so that 100 g of seeds were collected from three clones per accession during August-September of 2009. The preparation of samples, extraction of oil, obtaining of FAMES and chromatographic analysis were made under the conditions described above.

2.5 Statistical analysis

The data for oil content were processed through an analysis of variance. The proportion of saturated/unsaturated fatty acids was compared between sites, and an exploration was made for possible Pearson correlations (α 0.05) between the altitude and latitude of the collection sites and the content and composition of the oils. For this, the software XLStat© 2010.3.03 (AddinSoft, USA) was utilized.

With the data derived from plants cloned and grown in common garden the broad sense heritability was studied for the fatty acids and the oil content, utilizing the online software PBStat© 1.02²⁹⁾. With the data of proportions for fatty acids in seeds collected from the sites, analyses of principal components, discriminant and clusters (utilizing Euclidean distances and grouping by average linkage) were made. These analyses were done with XLStat© 2010.3.03.

Isolation by distance was tested through the correlation of matrices of Euclidean and geographic distances using the Mantel test with 10000 permutations. The geographic distances were obtained by means of latitude/longitude of the sites, using GenAlEx 6.0³⁰⁾. To test the existence of possible genetic barriers between populations the software Barrier 2.2³¹⁾ was utilized, correlating a Fisher distance matrix, as generated in a discriminant analysis, with a matrix of average decimal geographic distances of each population.

3 RESULTS

3.1 Content and composition of the seed oil

The quantity of oil in the seed of Mesoamerican *J. curcas* by individual varied between 8.02 and 54.28%, while the variation per site was between 12.09 and 44.27% (Ta-

Table 1 Location data, oil content seed and proportion of unsaturated fatty acids in accessions of *Jatropha curcas* L. from 38 sites in the Mesoamerican region.

Population	Site	Number of accessions	Latitude / Longitude	Altitude (masl)	Seed Oil Content (%)	Unsaturated Fatty Acids (%)
Coast of Chiapas	ACA	5	15.1040/-92.3511	51.0	37.22	78.96
Coast of Chiapas	CAC	2	14.5901/-92.5941	472.0	25.13	82.29
Coast of Chiapas	HUX	5	15.0402/-92.2922	23.0	37.11	74.52
Coast of Chiapas	MAP	5	15.2540/-92.5384	34.8	39.88	79.62
Coast of Chiapas	PC	5	14.4371/-92.2578	5.4	33.59	81.05
Coast of Chiapas	SCH	5	14.3957/-92.1032	24.3	34.65	78.31
Coast of Chiapas	TAP	5	14.5453/-92.1925	74.4	32.23	78.91
Coast of Chiapas	TON	1	16.0343/-93.5078	12.0	39.29	83.42
Coast of Chiapas	ARR	5	16.1141/-93.6359	53.0	40.25	82.50
Coast of Chiapas	PIJ	5	15.5552/-92.5956	79.0	33.80	79.99
Center of Chiapas	BERR	4	16.4760/-93.1625	895.8	35.76	82.85
Center of Chiapas	CIN	4	16.4113/-93.4265	574.8	17.51	80.44
Center of Chiapas	JIQ	4	16.4004/-93.3922	533.3	40.69	79.69
Center of Chiapas	OCZ	3	16.4623/-93.2372	803.3	19.99	82.10
Center of Chiapas	TYL	1	16.2269/-93.4918	689.0	14.18	77.84
Center of Chiapas	TUX	1	16.4233/-93.0670	894.0	12.20	78.72
Center of Chiapas	CCR	4	16.0664/-92.4104	537.5	12.09	83.02
Center of Chiapas	PUJ	3	16.1569/-92.1747	511.0	37.71	78.22
Center of Chiapas	RV	3	16.0993/-93.0453	560.7	43.12	81.02
Center of Chiapas	VCR	1	16.1831/-93.0454	830.0	18.90	77.69
Center of Chiapas	VCO	1	16.1169/-93.1563	579.0	26.21	77.72
Center of Chiapas	VIF	1	16.1482/-93.1578	564.0	31.16	78.43
Center of Chiapas	VLR	1	16.1924/-92.2057	1304.0	21.14	83.78
Center of Chiapas	CHIC	5	15.4465/-92.1672	587.8	14.52	83.44
Center of Chiapas	CDCU	5	15.4050/-92.0012	700.8	26.76	82.71
Center of Chiapas	COM	5	15.3918/-92.0801	677.4	27.12	83.20
Center of Chiapas	LACO	2	15.5029/-91.5422	635.0	44.27	79.25
Center of Chiapas	LAGOS	5	15.5034/-91.5437	631.0	30.04	80.26
Center of Chiapas	RIZ	3	15.5794/-92.2884	561.0	27.90	81.77
Center of Chiapas	IXT	4	16.4722/-92.5463	813.0	32.17	77.31
Guatemala	GUA	5	14.3627/-90.2908	1245.6	31.88	80.97
Oaxaca	ECO	4	15.4339/-96.3386	61.8	28.41	80.44
Oaxaca	ZIM	1	15.5056/-96.0015	66.0	22.39	79.55
Oaxaca	PA	5	16.1125/-97.4476	37.8	32.00	80.71
Oaxaca	PIN	3	16.1931/-97.5355	263.7	25.24	81.96
Guerrero	COP	4	16.3747/-99.0074	8.5	24.77	79.46
Guerrero	ACAP	5	16.4996/-99.4688	19.2	24.73	80.49
Michoacán	APA	5	19.0424/-102.2210	312.4	29.33	77.97

ble 1), which was significant ($F = 8.205$, $P < F 0.0001$). It was found that the oil content is negatively correlated with the altitude at which the plants were growing (Fig. 1a). There was no significant correlation with the latitudes of collection sites.

Eleven transesterified fatty acids were found: Decanoic Acid (Capric Acid 10:0), Tetradecanoic Acid (Myristic Acid 14:0), Hexadecanoic acid (Palmitic Acid 16:0), 9-Hexadecenoic Acid (Palmitoleic Acid 16:1), 14-Methyl Hexadecanoic Acid (Methyl Palmitic Acid 17:0), Octadecanoic Acid

(Stearic Acid 18:0), 9-Octadecenoic Acid (Oleic Acid 18:1), 7-Hydroxy,9-Octadecenoic Acid (Ricinoleic Acid 18:1), 9,12-Octadecadienoic Acid, (Linoleic acid 18:2), Eicosanoic Acid (Arachidic Acid 20:0), Eicosenoic Acid (Gadoleic Acid 20:1). In addition, several accessions showed 3-Octyl,Oxirane-Octanoic Acid (16:0) and / or Decanedioic Acid (11:0, Sebacic Acid, Dicarboxylic), which could be intermediary products of synthesis or degradation of other fatty acids.

The most abundant fatty acids were oleic (average 38.7%), linoleic (average 40.7%), palmitic (average 12.8%) and stearic (6.1% in average). The proportion of unsaturated fatty acids varied between 59.14 and 86.76%, considering the individuals, while the variation per site was between 74.5 and 83.8%, which was significant ($F = 1.633$, $P = 0.029$; Table 1). The proportion of unsaturated fatty acids is weakly but positively correlated with the altitude (Fig. 1b). There was no significant correlation with the latitude of the sites. The oil content and the unsaturated fatty acid proportion are negatively correlated (Fig. 1c).

3.2 Most informative and heritability of characters

The principal components analysis (PCA) showed that the variables stearic, oleic and linoleic acids contributed mainly to the first principal component; consequently, those variables can be considered the most informative of the variation detected (Table 2). The study with cloned plants showed that almost all fatty acids had very high broad sense heritabilities (around 90%), except for the case of palmitoleic acid, of which it was not possible to estimate the heritability, since statistically significant differences between genotypes were not detected; whereas the

Fig. 1 Significant correlations between parameters of the seed oil of Mesoamerican *Jatropha curcas*; a) Seed oil content vs. altitude, b) Unsaturated fatty acids vs altitude, c) Unsaturated fatty acid vs seed oil content.

Table 2 Contributions of the variables (%) to the first two principal components (F1 and F2).

Variable	F1	F2
Myristic Acid (14:0)	0.033	0.463
Palmitic Acid (16:0)	6.767	10.802
Palmitoleic Acid (16:1)	0.504	17.875
Methyl-Palmitic Acid (17:0)	1.288	17.468
Stearic Acid (18:0)	18.666	0.493
Oleic Acid (18:1)	24.353	6.621
Linoleic Acid (18:2)	31.988	3.672
Arachidic Acid (20:0)	4.827	0.452
Gadoleic Acid (20:1)	2.690	23.049
Capric Acid (10:0)	0.874	0.595
Ricinoleic Acid (18:1)	1.777	18.332
Seed Oil Content (%)	6.234	0.176

Bold values correspond, for each variable, to the factor for which the squared cosine is the largest.

Table 3 Estimation of the broad sense heritability of the characters studied.

Variable	V _G	V _P	h ² _{bs} (%)
MYRISTIC	0.0056	0.0063	89.23
PALMITIC	29.8427	30.4945	97.86
PALMITOLEIC	0.0000	0.5984	0.00
MPALMITIC	0.0028	0.0031	92.08
STEARIC	9.7004	10.1895	95.20
OLEIC	24.0714	25.2182	95.45
LINOLEIC	36.0180	37.2547	96.68
ARACHIDIC	0.0509	0.0515	98.82
GADOLEIC	0.0032	0.0037	86.48
RICINOLEIC	0.0089	0.0215	41.35
OILCONTENTS	17.9423	25.5240	70.30

V_G: Genotypic variation; V_P: Phenotypic variation;
h²_{bs}: broad sense heritability, in percentage.

oil content had heritability of 70% (Table 3).

3.3 Description of the variation

Having confirmed the high heritability of fatty acids of the seed, we described the genetic variation using these characters as chemical markers. The analysis of ascending hierarchical classification grouped the 135 accessions into 10 groups, as shown in the dendrogram of Fig. 2, where the dotted line indicates the limit for the formation of groups. In general, it was observed that accessions are grouped according to their geographical origin, but some that belong to a same town or locality are in a different group.

It is noteworthy that the groups I, II and III with one, one and four accessions of the Coast of Chiapas, respectively, are clearly separated from the other accessions, including those of the rest of the mentioned population, i.e. they were outliers (Fig. 2). Groups IV to VIII contained accessions from the Center of Chiapas and populations of Guatemala, Oaxaca, Guerrero and 3 accessions of Michoacán, along with some accessions from the Coast of Chiapas. Groups IX and X contained almost exclusively accessions from the Coast of Chiapas, except ZIM1 (Oaxaca) and APA2 and APA5 (Michoacán).

The genetic variation encountered can be considered high given that the line forming groups stood at a Euclidean distance of 7.5, while the maximum distance between groups was 33. In addition, the variation within classes accounted for only 10.7%, while the variation among classes was 89.3%.

Discriminant analysis, it showed that the first two factors explain 89% of the variation and separated the accessions into three major groups in the bidimensional geometric space: the accessions of Michoacán, the Coast of Chiapas

and Center of Chiapas; accessions of the other populations were mixed with those of the last two populations (Fig. 3a). To confirm the trends in spatial location, the centroids were plotted for each population (Fig. 3b), confirming that the population of Michoacán is isolated and that those of Chiapas are clearly far apart. The centroids of the populations of Guatemala and Guerrero overlap the Center of Chiapas. The Oaxacan population is related to that of Guerrero.

3.4 Isolation of populations

To evaluate the apparent ranking of accessions according to their origin, a Mantel test of association was performed, with significant results ($r(AB) = -0.082$; $P < 0.0001$), which indicates that there was a correlation between genetic distance (Euclidean) and geographic distance in kilometers. Isolation was also shown by the existence of genetic barriers detected with Barrier vers. 2.2. The major barrier (a) separates the population of Central Chiapas of the rest of the populations, and the second barrier (b) isolates the population of Michoacán (Fig. 4).

4 DISCUSSION

The storage of oils in seeds is a generalized feature in higher plants, which serve as a source of energy for the embryo during the heterotrophic stage³². This stage is crucial in the success or failure of the embryo to germinate, emerge and establish itself as a new plant³³, and therefore, the content of the endosperm, at least in part, determines reproductive success or "fitness" of the plant. As a result, the total content and composition of seed oil should be

Fig. 3 Discriminant analysis of 135 accessions of *Jatropha curcas* L. from six Mesoamerican populations, based on the proportion of fatty acids in the seed; a) distribution in the bidimensional geometric space based in factors 1 and 2, b) distribution based on the centroids.

vious reports for this species, either cultivated in Asia or Africa^{5, 34}) or collected in the Mesoamerican region^{11, 12}). Similarly, the fact that there is a pattern of negative correlation with altitude confirm the findings of Pant *et al.*³⁵) who determined the variation in yield and oil content of Indian accessions of *J. curcas* in two soil conditions and three altitudinal ranges, finding that there is a higher content of oil when the plant is found in non-arable soils and low elevation.

It has been documented that there is considerable variation in oil content of this species that can be generated by genetic and environmental factors, including rainfall and soil fertility^{36, 37}). Heller⁵) reported that variability in this characteristic might be influenced by the origin of the accessions studied and genotype-environment interactions. Contrary to this, Kaushik *et al.*³⁸) reported that the oil content of twenty-four accessions of Indian *J. curcas* had a broad sense heritability of 99%, implying a limited influence of the environment. However, they also reported a variation of only 10% among accessions and very low coefficients of phenotypic and genotypic variation, which con-

Fig. 4 Map showing the sites of collection of *Jatropha curcas* evaluated in their composition of oil from Southern Mexico and from Guatemala, and the two main genetic barriers (lines "a" and "b" in yellow) found by the algorithm of Monmonier (Barrier vers. 2.2), as based on Fisher distances of fatty acid composition of the seed. Map kindly prepared by Francisco Javier Pérez Racancoj.

firms the narrow genetic basis of *J. curcas* in Asia. In the same way, Gohil and Pandya³⁹) reported 89.7% of broad sense heritability for this character.

Unlike the explanation of that phenomenon for Asian accessions, where *J. curcas* was introduced a few centuries ago and where the variation might be due to strong environmental effects by having a narrow genetic base⁴⁰), in Mesoamerican populations the reason could be the selection of genotypes adapted to adverse conditions (higher temperature) with increased production of energy substances for the embryo during germination. This increases seedling vigor and thus, the fitness of the species.

It is noteworthy that the oil content is a characteristic that had a high heritability (70%), while it had a high coefficient of genotypic variation and 32% of variation among accessions (Tables 1 and 3), which shows that populations in Mesoamerica, where most likely *J. curcas* is a native of, are more variable than in other parts of the world.

Although there have been determinations of the oil content in higher plants for a long time, perhaps the first study to look for relationships between ecological parameters and accumulation of oil was that of Levin⁴¹), who studied the relationship between oil content and the habit and habitat of over a thousand species of angiosperms, finding that the oil content, evolutionarily, has increased with the development of woody stems and shade tolerance, and there is no relation to the latitudinal origin. In the case of the tropical semi-domesticated plant *J. curcas*, in this study there was no significant correlation with latitude of

collection sites. In a thorough review, no reports were found which examine this relationship, possibly conforming to that postulated by Levin. The high oil content is also consistent with the hypothesis of Levin because *J. curcas* can be considered a woody tree shrub. To test the hypothesis of variation according to the habits of growth, it would be of great scientific interest to know the correlation between the accumulation of seed oil and the degree of stem lignification of the 175 species of genus *Jatropha*.

4.2 Pattern in the fatty acid composition

Except for ricinoleic acid, which is characteristic of *Ricinus communis* L., all other fatty acids have been reported for *J. curcas*; although this fatty acid has been reported recently in the species *J. gossypifolia* and other Euphorbiaceae⁴²⁾. Either way, it was only found in seven of the 135 accessions studied and its heritability was low (Table 3); however, the PCA revealed that it is a relatively informative variable by having the highest percentage of variability in the second principal component (Table 2).

The fatty acid profile is consistent with that reported in other studies, which report the same four main fatty acids^{5, 11, 12, 43, 44)}. Behenic (22:0) and linolenic (18:3) fatty acids, which have been reported for *J. curcas*⁴⁵⁾ were not found in the evaluated accessions. The proportion of unsaturated fatty acids is an important variable in the biodiesel manufacturing, since quality standards indicate it must be minimized⁴⁶⁾. Interestingly, this study found that oil content and the proportion of unsaturated fatty acids are negatively correlated, which, if confirmed in a larger study of genetic association with outstanding genotypes, will permit genetic improvement for increased oil yield while decreasing unsaturated fatty acids.

To date great progress has been made in molecular understanding of the origin of the fatty acids in seeds and their diversification mechanisms^{47, 48)}. However, it is surprising how little attention has been given to the selection factors that drive the evolution of the fatty acid composition of seeds. In this regard, Linder⁴⁹⁾ suggested that the temperature of germination is an important selective agent that causes the seed oils of plants native to high latitudes (or altitudes) to have a higher proportion of unsaturated fatty acids than plants in lower latitudes/altitudes. The explanation is that in cooler environments (high latitudes and altitudes) the catabolism of unsaturated fatty acids is more feasible compared to saturated, thus the seeds with more unsaturated fatty acids germinate and grow faster at lower temperatures, increasing their fitness, even at the expense of having less total energy available. On the other hand, in warm environments, such as the tropical, seeds with more saturated fatty acids are selectively favored because they have more energy and they do not need to germinate quickly, since the conditions in the tropics are more or less stable throughout the year.

However, although the proportion of unsaturated fatty acids was positively correlated with the altitude of the sites, the fatty acid composition of *J. curcas* seems to be in disagreement with the hypothesis described, because, being a tropical species, selection has favored a greater proportion of unsaturated fatty acids.

The same author mentions that the lack of association between germination temperature and the proportion of unsaturated fatty acids in one species could be due to the lack of genetic variation in oil composition, which, as described in this paper, is not the case of *J. curcas*.

Based on the foregoing, it is hypothesized that, in the case of *J. curcas*, soil moisture has exerted selection pressure to select a higher proportion of unsaturated fatty acids. It is well documented that this species is tolerant to drought, although adapting to different environments, from humid to semi-arid; however, the plant is susceptible to flooding, for example, according to Dehgan & Schutzman⁵⁰⁾, *J. curcas* is found in South America in seasonally dry tropical areas, but is completely absent in the always wet Amazon region. In the Mesoamerican tropical region, the onset of flowering and seed production coincides with the onset of the rainy season, so seeds, which do not exhibit dormancy, should germinate quickly, usually within five days (for which they require higher proportion of unsaturated fatty acids) and establish themselves as seedlings before soil moisture levels increase to waterlogging.

4.3 Patterns of diversity of Mexican *J. curcas*

The analysis of clusters showed that genetic diversity of Mesoamerican *J. curcas* is high, as shown by the 7.5 units of Euclidean distance that marked the line of formation of the 10 clusters (Fig. 2); unfortunately, there are no reports of similar works with which to compare the variation using fatty acids as markers. Except for the work of Wang *et al.*²⁶⁾, who compared the oil content and fatty acid composition in samples of *J. curcas* collected from three regions of China and India, finding 12 fatty acids and reporting differences between accessions. They concluded that attention should be given to these chemical markers to introduce new Chinese germplasm and for the genetic improvement of the plant.

Discriminant analysis separated the populations more or less according to their geographical origin (Fig. 3); this was checked with the Mantel test, which indicated a correlation between chemical and geographical distance. In contrast to these results, Kaushik *et al.*³⁸⁾ evaluated 24 accessions of *J. curcas* from India to establish the genetic variability, where the pattern of clustering in the Euclidean cluster analysis revealed that geographic diversity does not necessarily represent the genetic diversity.

In our study two genetic barriers could be identified (Fig. 4) that separated populations of the Center of Chiapas and Michoacán. Possible explanations are related to the exis-

tence of the mountain chain "Sierra Madre del Sur" which functions as a geographic barrier; the population of Center of Chiapas is the only one that is to the North of this mountain chain. Other possible reason is the geographical remoteness of the population in Michoacán from the rest of the populations. Studies with molecular markers based on DNA may provide information about the rate of gene flow between populations and confirm or reject the existence of the genetic barriers mentioned in Mesoamerican *J. curcas*.

5 CONCLUSION

The results of the work showed that the variation in the oil content and eleven fatty acid was very high with heritabilities of about 90%, which demonstrate that they are useful markers in estimating the genetic diversity of *J. curcas*. The populations of *J. curcas* from the Mesoamerican region represent valuable genetic resources for the future establishment of extensive plantations of this oil producing plant.

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