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GENETIC DIVERSITY OF *Moringa oleifera* Lam. IN SOUTHEASTERN MEXICO

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ABSTRACT

Moringa oleifera Lam. is a plant with nutritional and nutraceutical properties. It was introduced from India to Mexico, and some of its ecotypes are cultivated for industrial use. These ecotypes show morphological variations, indicating genetic variation. Therefore, the purpose of this study was to evaluate eight simple sequence repeat (SSR) loci to assess the genetic diversity of 10 cultivated *M. oleifera* populations collected from the southeast of Mexico. All markers generated a total of 127 alleles, with 15.8 alleles per locus. The polymorphic information content of markers was 0.869, with an average heterozygosity of 0.194. At the population level, the Nei´s gene diversity was found to be 0.66, with 4.9 different alleles per locus and 5.2 genotypes on average per accession. The distribution of genetic variation was 20 % among populations, 58 % between individuals, and 22 % within individuals. The principal coordinate analysis (PcoA) plot and unweighted pair group method with arithmetic mean (UPGMA) linkage revealed three groups, corresponding to the three original genetic populations identified through structure analysis (Delta K = 3). Results showed significant differences in genetic diversity in *M. oleifera* populations stablished in Southeastern Mexico, which can be leveraged to increase the use of this germplasm.

Keywords: germplasm, SSR, diversity, genetic structure.

INTRODUCTION

Moringa (*Moringa oleifera* Lam.) is native to Northwestern India. It was introduced to Mexico in 1920 (Olson and Fahey, 2011). Today, it is cultivated in 14 states of Mexico (Olson and Alvarado-Cárdenas, 2016). It is a diploid plant (2n = 28) with annual and perennial varieties (Rajalakshmi *et al.*, 2017), which can be propagated both sexually

and asexually. It is cultivated as a crop in live fence and agroforestry systems (Huque *et al.*, 2016). Moringa is a drought-tolerant plant that can survive in regions with 250 mm annual rainfall, clay-loamy soils, temperatures up to 48 °C, and elevations of 2000 m (Velázquez-Zavala *et al.*, 2016), which makes it an important source of food during drought periods, mainly in the tropics and subtropics (Thurber and Fahey, 2009).

Moringa can thrive as a crop in arid and semi-arid regions, highlighting its importance for food security since these types of environments represent 37 % of Earth's surface (Tian *et al*., 2015). All parts of the moringa plant are used for different purposes. Its leaves contain essential amino acids, vitamins, minerals, fatty acids, glucosinolates, and phenolic compounds (Leone *et al*., 2015). These attributes have led to the distribution of outstanding moringa varieties or ecotypes from India to other countries (Amoatey *et al*., 2012). In Southeastern Mexico, the introduced germplasm shows morphological variation, suggesting genetic variation. Therefore, it is important to know the extent of genetic variation within and among cultivated populations of this species to preserve and expand the use of this germplasm.

The degree of genetic variation of a species can be assessed through the analysis of diversity and the genetic and geographic relationships of populations. There are different sets of morphological, physiological, and DNA attributes that can be used to measure this variation (Storz *et al*., 2015). It is well known that, at the DNA level, the imprecision derived from morphological and physiological studies is reduced by the use of molecular markers, which can identify duplicate germplasm as well as to assist strategic programs (Popoola *et al*., 2017).

Different types of DNA markers can be used for exploring diversity and genetic relationships of natural or cultivated populations (Hassanein and Al-Soqeer, 2018). These include microsatellites, or simple sequence repeat (SSR) markers, which are co-dominant, neutral, multi-allelic, repeatable, and easy to interpret (Nadeem *et al*., 2018). Few genetic characterization studies of moringa using SSR have been reported worldwide (Amao *et al*., 2017; Popoola *et al*., 2017; Rajalakshmi *et al*., 2017). In Mexico, particularly in moringa crops from the southeast, there is no research on genetic diversity at the molecular level. Therefore, the aim of this study was to assess the genetic diversity of 10 cultivated populations of *M. oleifera* from the southeast of Mexico, under the hypothesis that there must be genetic variability among cultivars.

MATERIALS AND METHODS

Sample collection

Seeds were collected in April and May 2018 from five states located in Southeastern Mexico that have commercial moringa plantations. These states are Veracruz, Oaxaca, Guerrero, Chiapas, and Yucatán (Table 1). The crops sampled had different edaphoclimatic conditions (Table 2, Figure 1). Half a kilogram of seeds was selected from each population, and 30 seeds were selected at random. Their length (11.91 ±

Table 1. Geographical locations of the *Moringa oleifera* Lam. populations selected for study in Southeastern Mexico.

Population	State	Municipality	Locality	Longitude (°)	Latitude (°)
MoV1	Veracruz	Soledad de Doblado	El Progreso	-96.4022	19.0818
MoV2	Veracruz	Misantla	Santa Cruz Hidalgo	-96.8628	19.9555
MoO1	Oaxaca	Santa María Huatulco	La Herradura	-96.3658	15.7772
MoO2	Oaxaca	Mariscala de Juárez	Guadalupe la Huertilla	-98.1088	17.8513
MoO3	Oaxaca	Santa Cruz Xoxocotlán	San Juan Bautista la Raya	-96.7280	16.9791
MoG1	Guerrero	Tecpan de Galeana	Mitla	-99.8934	16.8789
MoC1	Chiapas	Tuzantán	Villa Hidalgo	-92.3747	15.1080
MoC2	Chiapas	Tuxtla Gutiérrez	Colonia La Salle	-93.0868	16.7429
MoY1	Yucatán	Mérida	Frac. El Parque	-89.5872	20.9711
MoY2	Yucatán	Baca	Felipe Carrillo Puerto	-89.6070	20.9954

Table 2. Climatic conditions of the Moringa oleifera Lam. population locations analyzed in this study.

Commercial plantation	Planting year	Altitude (m)	Minimum temperature (°C)	Average temperature (°C)	Maximum temperature (°C)	Annual rainfall (mm)
MoV1	2016	67	20	26	32	1039
MoV2	2009	202	18	23	29	1886
MoO1	2011	175	19	26	33	1016
MoO2	2013	1081	16	24	32	623
MoO3	2015	1507	13	21	29	634
MoG1	2009	37	17	27	36	941
MoC1	2015	134	21	27	33	3456
MoC2	2015	525	18	25	32	930
MoY1	2013	8	20	27	33	1002
MoY2	2016	20	16	26	39	1291

1.38 mm), width (10.27 ± 0.87 mm), and weight (368.36 ± 63.08 mg) were recorded. The seeds were disinfested with 5 % domestic-grade sodium hypochlorite for 5 min, rinsed with distilled water, and placed on moistened paper for germination at 25 °C. Twelve days later, germination was checked, and 10 seedlings per population were selected at random for molecular characterization.

DNA extraction and quantification

Genomic DNA was extracted from seedling leaves using ChargeSwitch™ gDNA Plant Kit (Invitrogen™) coupled to the extraction robot KingFisher Flex (Thermo Scientific, Waltham, MA, USA) according to the manufacturer instructions. A total of 150 mg of tissue per individual were used for the extraction. The integrity of the DNA was verified on 0.8 % agarose gel, and the concentration of the DNA was determined with a NanoDrop ND2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA),

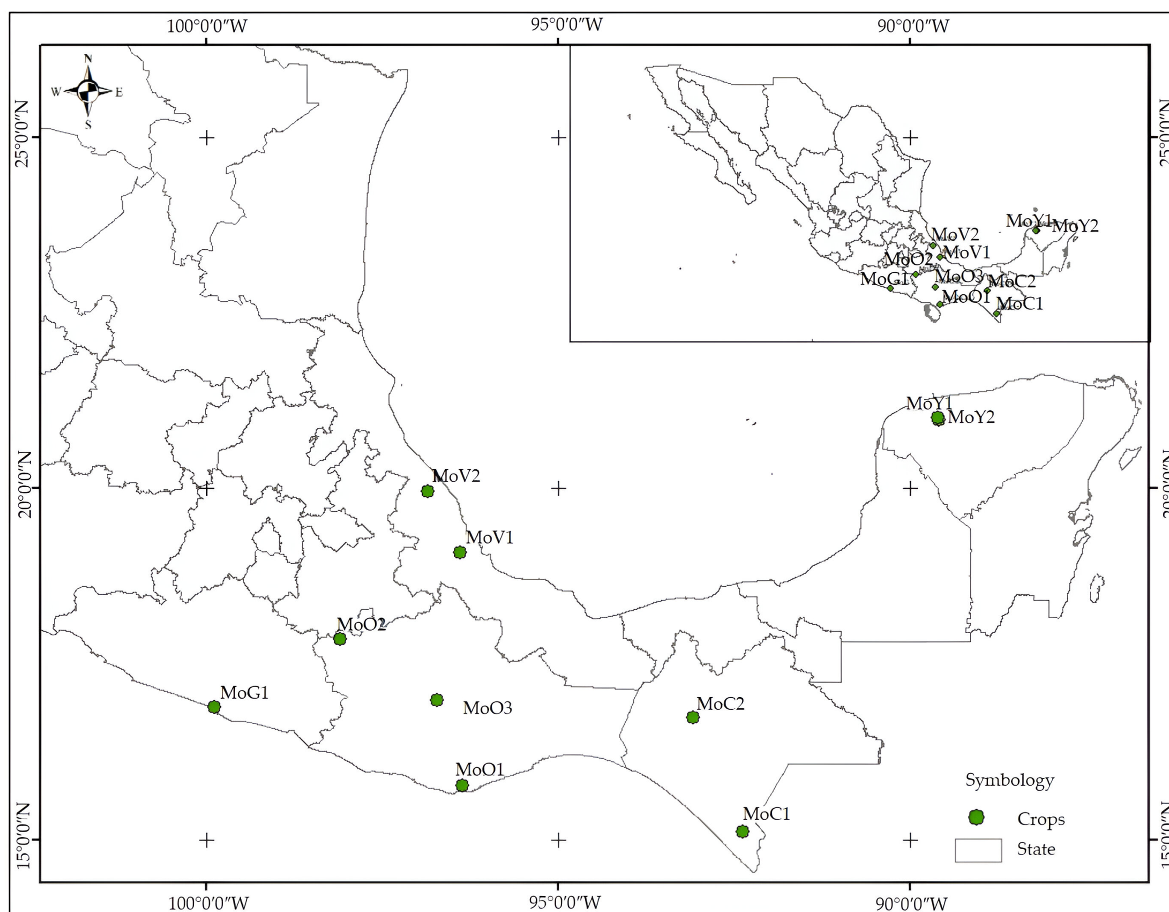

Figure 1. Sampling points of the *Moringa oleifera* Lam. populations from Southeastern Mexico characterized with simple sequence repeat (SSR) markers.

assuming that a unit of absorbance at 260 nm of DNA represents 50 ng mL^{-1} of double-stranded DNA.

Amplification of SSRs

Eight SSR microsatellite loci developed by Wu *et al.* (2010) were amplified by PCR; primers were synthesized at the Institute of Biotechnology of the National Autonomous University of Mexico (UNAM) (Table 3). PCRs were carried out in a 25 µL volume with 50 ng of genomic DNA, 1X Taq buffer, dNTPs (0.2 mM), $MgCl_2$ (0.8–2 mM) (Table 2), 50 pM of primers, and 1 U of enzyme (Go Taq® DNA Polymerase, Promega, WI, USA) using a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA).

Table 3. Simple sequence repeat (SSR) markers and reaction specifications used to characterize populations of *Moringa oleifera* Lam. cultivated in Southeastern Mexico.

Locus		Sequence (5´-3´)	Repeat unit	Alignment temperature	$MgCl_2$ concentration (mM)
MO8	F	GTAGATGGTGCAGCTACTCA	(CT)13	58	0.8
	R	TGGGGTTCTTGTTCTTTATT			
MO12	F	ACCGAAGATGATAAGGTGGG	(CT)11	58	0.8
	R	CAAAAGGAAGAACGCAAGAG			
MO13	F	TTTCGGGTTTTCTTTCACGG	(CT)15	54.9	1.0
	R	AGCTCACTTTCCATCTCCAT			
MO15	F	CCCCTCTATTTCCATTTTCC	(TC)10CCT(TC)6	54	0.8
	R	GCTCCATAAACCCTCTTGCT			
MO18	F	TTTTCCTCCCTTATTGTGCC	(GA)6A(AG)16	58	0.8
	R	CCGTTGCCCTTTGTGGTTCA			
MO48	F	AGAAGAACCCAACAGAGGAT	(TC)8C(CT)15A(AC)7	62	1.5
	R	CTTTTCACTAACCACCACCC			
MO58	F	TGGATTTCTTCTCCTGCTAT	(CT)6T(TC)9	56	2.0
	R	CACAGTTCTTATTGTATTGG			
MO61	F	TGTGGGTCCTGCCTTTTCTC	(TC)11	54.9	1.0
	R	CTTCTGTCTTTCTTCCTGCT			

The PCR program consisted of an initial denaturation at 94 °C for 5 min, followed by 30 cycles with denaturation at 95 °C for 1 min, alignment at 54–62 °C for 1 min (Table 3), extension at 72 °C for 1 min, and a final extension cycle at 72 °C for 5 min for MO13, MO15, MO43, MO53, and MO61 markers, while for MO8, MO12, and MO18 markers, the program included a denaturation cycle at 94 °C for 2 min, 5 cycles at 94 °C for 30 sec, 62–58 °C for 1 min (decreasing 1 °C per cycle), and 72 °C for 1 min, plus 30 cycles of 95 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min, and a final cycle at 72 °C for 8 min.

Electrophoresis

PCR products were analyzed in non-denaturing polyacrylamide gels (29:1) to 6 or 10 %. For this purpose, 5 μL of loading buffer (50 mM, Tris pH 8.0, 50 mM EDTA 0.5, 25 % sucrose, and bromophenol blue 25 mg mL^{-1}) were added to each reaction. From the resulting volume, 3 μL were run into the MGV-216-33 system (CBS Scientific®, USA) at 250 V for 2–4 h, using 1X TBE as running buffer (0.09 M Tris-borate, 2 mM EDTA pH 8.0) and 50 ng of 25 bp ladder (Invitrogen™, Thermo Scientific, Mexico) as reference of molecular weight. The staining and development of gels were carried out with silver nitrate, according to Sanguinetti *et al.* (1994). Each gel was documented in a MiniBis Pro16 mm transilluminator (Bio Imaging Systems®, Israel). The size of each band in base pairs was determined with GelAnalyzer software version 19.1 to build the molecular data matrix.

Data Analysis

The frequency of the most common allele, number of alleles, number of genotypes (i.e., combinations of alleles), observed heterozygosity (Ho), Weir-adjusted Nei´s gene diversity, polymorphic information content, as well as Wright F statistics, were obtained with PowerMarker V.3.25 software for both markers and populations. The number of different alleles, the number of alleles found in ≤ 25 and ≤ 50 % of populations, the number of private and effective alleles, the Shannon index, and the analysis of molecular variance (AMOVA) were obtained with the GenAlEx V.6.503 suite for Microsoft Excel. With this program, a plot from principal coordinate analysis (PcoA) was constructed with coefficients of differentiation (Fst). To visualize genetic relationships, a dendrogram was done using Nei's gene distances (Nei, 1972) and the unweighted pair group method with arithmetic mean (UPGMA) method (Sneath and Sokal, 1973) in the DARwin software version 6.0. The genetic structure was produced with the Admixture model of STRUCTURE software version 2.3.4. through a Bayesian analysis of individual allocation using 250 000 MCMC iterations (Monte Carlo Markov Chains) with 50 000 repetitions from K1 to K10, with 10 repetitions for each K (Pritchard *et al.,* 2000). Delta K value was obtained with Structure Harvester version 0.6.94 (Earl and vonHoldt, 2012).

RESULTS AND DISCUSSION

Diversity metrics

This work evaluated the diversity and genetic relationships of 10 populations of *M. oleifera* cultivated in Southeastern Mexico using SSR markers. The SSRs were chosen from a panel of 20 SSR markers developed for moringa by Wu *et al.* (2010) and reported as polymorphic in previous studies by Shahzad *et al.* (2013) and Popoola *et al.* (2017). Twenty-seven alleles were identified with a mean of 15.88 per locus. The MO15 locus amplified eight alleles, and markers MO12, MO48, and MO58 amplified 18 alleles each. The number of genotypes (i.e., combination of alleles per marker) ranged from 15 for the MO15 locus to 36 for the MO48 locus, with an average of 24.25 genotypes per locus. Allele frequencies ranged from 0.105 to 0.255 with a mean value of 0.196. Gene diversity, or expected heterozygosity, is defined as the probability of nonidentity of alleles taken at random (Nei, 1973) and ranged from 0.821 for MO15 to 0.919 for MO58, with a mean of 0.881. The PIC value ranged from 0.798 for MO15 to 0.914 for MO58, with a mean of 0.869 (Table 4).

The polymorphism of these markers was confirmed in this work with the value of the most common allele (0.196 on average). This variation was 0.19, 15.88, 0.88, and 0.86 for the allelic frequency, number of alleles per locus, gene diversity, and PIC value on average, respectively. In this regard, Ganessan *et al.* (2014) found values of 0.87, 1.77, 0.18, and 0.15 for these parameters in 12 moringa populations, and Popoola *et al.* (2017) observed values of 0.47, 7.75, 0.669, and 0.63 after examining 20 moringa populations. Similarly, Shahzad *et al.* (2013) found values of 0.49, 8.1, 0.64, and 0.59; Amao *et al.*

Table 4. Descriptive genetic information of eight simple sequence repeat (SSR)-screened loci in *Moringa oleifera* Lam. populations from Southeastern Mexico (n = 10).

SSR	Frequency of the most common allele	Number of genotypes	Number of alleles	He*	Ho*	PIC*
MO8	0.200	17	17	0.879	0.000	0.868
MO12	0.235	28	18	0.884	0.260	0.874
MO13	0.175	31	17	0.905	0.260	0.897
MO15	0.255	15	8	0.821	0.240	0.798
MO18	0.230	19	17	0.858	0.030	0.843
MO48	0.175	36	18	0.900	0.410	0.892
MO58	0.105	22	18	0.919	0.080	0.914
MO61	0.195	26	14	0.880	0.270	0.868
Mean	0.196	24.25	15.88	0.881	0.194	0.869

He*: expected heterozygosity; Ho*: observed heterozygosity; PIC*: polymorphic information content.

(2017) reported 0.57, 4.75, 0.52, and 0.48; and Rajalakshmi *et al.* (2017) found 0.36, 8.30, 0.72, and 0.68 when evaluating large collections of moringa germplasm.

On the other hand, the number of alleles varied from 8 to 18, a range higher than 6–13 (Shahzad *et al.*, 2013), 1–2 (Ganessan *et al.*, 2014), 4–11 (Rajalakshmi *et al.*, 2017), 2–9 (Amao *et al.*, 2017), and 4–15 (Popoola *et al.*, 2017), as documented by other studies of diversity in moringa. MO8, MO12, MO13, MO18, MO48, MO58, and MO61 markers contributed to the high values and fluctuation in the number of genotypes (15 to 36) in comparison with the 15 genotypes reported by Shahzad *et al.* (2013). Therefore, the number of alleles and genotypes showed the informative usefulness of the selected SSRs, as revealed by the PIC values (0.798 to 0.914) recorded after screening all eight SSRs in the 10 moringa populations.

In relation to the observed heterozygosity or proportion of heterozygous individuals (0.194), this value was higher than the 0.1 reported by Ganessan *et al.* (2014) but below figures reported in other studies, such as 0.57 (Shahzad *et al.*, 2013), 0.973 (Rajalakshmi *et al.*, 2017), and 0.972 (Popoola *et al.*, 2017). This result indicates that most of the SSR examined were in homozygous condition, that is, only one band per individual was recorded, which suggests that the mating between populations has been low and apparently these populations have remained genetically quasi-intact, probably due to low dispersion, the geographical distance that separates them, or a genuine intention of the farmer to keep their ecotypes identical.

Regarding populations, MoO1 and MoO2 recorded the highest value for the frequency of the most common allele, which was 0.4482 on average for all populations. MoO3 recorded the highest number of alleles and allele combination, with 6.37 alleles and 6.75 genotypes, although the most diverse population was MoV1 with a gene diversity of 0.757, observed heterozygosity of 0.313, and PIC value of 0.725 (Table 5).

Table 5. Descriptive genetic information of 10 populations of *Moringa oleifera* Lam. from Southeastern Mexico (n = 10).

Population	Frequency of the most common allele	Number of genotypes	Number of alleles	He*	Ho*	PIC*
MoV1	0.369	6.125	5.875	0.757	0.313	0.725
MoV2	0.456	4.875	4.250	0.634	0.163	0.589
MoO1	0.575	4.000	3.500	0.548	0.150	0.495
MoO2	0.538	3.375	3.375	0.575	0.000	0.508
MoO3	0.375	6.750	6.375	0.703	0.188	0.681
MoG1	0.456	4.875	4.750	0.668	0.275	0.618
MoC1	0.388	5.625	5.375	0.713	0.238	0.670
MoC2	0.519	4.750	4.375	0.609	0.163	0.554
MoY1	0.431	5.750	5.500	0.685	0.250	0.643
MoY2	0.375	5.750	5.125	0.714	0.200	0.676
Mean	0.448	5.187	4.850	0.660	0.194	0.615

He*: expected heterozygosity; Ho*: observed heterozygosity; PIC*: polymorphic information content.

It has been reported that a detailed molecular evaluation will largely depend on the type of marker. In the case of SSRs, these have not only been effective in measuring the magnitude of diversity but also in linking unobservable morphological differences to genetic variants (Wambugu *et al.,* 2018), establishing genetic-geographic links (Shahzad *et al.,* 2013), and associating high-performing genes for adaptive and performance traits (Govindaraj *et al.,* 2015).

In contrast to Shahzad *et al.* (2013), Ganessan *et al.* (2014), Rajalakshmi *et al.* (2017), Amao *et al.* (2017), and Popoola *et al.* (2017), who only reported the SSR diversity, in this research, the extent of diversity was also examined on the population level. Here, average values of 4.9, 3.6, and 0.6125 were obtained for different alleles, effective alleles, and unique alleles per population, respectively. This examination with the markers to the population level allowed to notice, for instance, that nine populations had individuals with SSR loci in heterozygous condition, standing out MoV1 population for its observed heterozygosity level (0.313). MoO2 stood out for having all SSR loci in a homozygous condition. Codominance is a desirable requisite for a molecular marker because it allows to identify heterozygous individuals and, therefore, to extend the details of the diversity to the nucleotide level.

Among populations, the number of alleles, or total allele count, ranged from 3.5 for MoO2 to 6.37 for MoO3, with an average of 4.91. The number of effective alleles, or number of alleles with equal frequency that would give the same level of diversity, ranged from 2.573 for MoO2 to 5.121 for MoO3, with an average of 3.662. This value is slightly less than the total number of alleles, which suggests that although there is diversity, some alleles are very frequent. The Shannon index ranged from 1.012 for MoO1 to 1.587 for MoV1, with an average of 1.32. This index is an easy-to-interpret

measure, where higher values indicate greater diversity; however, as it is affected by sample size and the relationship between categories, two data sets with the same value can have completely different structures. Even so, this index is a useful tool applicable to a wide range of fields. The number of private alleles, or alleles present only in a particular population, ranged from 0 for MoV2 to 2.5 for MoO3, with a mean of 0.61 (Table 6).

The analysis of molecular variance (AMOVA) indicated that the highest percentage of variation (% V) was found among individuals (% V = 58) and, to a lesser extent, among populations (% V = 20) (significant at p = 0.001, with 1000 permutations). Fisher's combined probability test by Raymond and Rousset showed that allele frequencies among pairs of populations were significantly different, with a 95 % confidence level ($p \leq 0.05$) (Table 7).

Table 6. Parental alleles among populations of *Moringa oleifera* Lam. from Southeastern Mexico.

Population	Number of alleles	Number of effective alleles	Shannon index	Number of private alleles	Number of alleles found (≤ 25 %)	Number of alleles found (≤ 50 %)
MoV1	5.875	4.391	1.587	0.250	0.500	3.500
MoV2	4.250	3.325	1.210	0.000	0.125	2.750
MoO1	3.625	2.669	1.012	0.125	0.625	2.625
MoO2	3.500	2.573	1.019	0.125	0.250	1.750
MoO3	6.375	5.121	1.572	2.500	1.125	2.625
MoG1	4.875	3.270	1.304	0.750	0.375	2.000
MoC1	5.375	4.243	1.451	0.625	0.250	2.500
MoC2	4.375	2.951	1.164	0.125	0.250	2.250
MoY1	5.625	4.033	1.442	1.125	0.625	2.750
MoY2	5.250	4.044	1.450	0.500	0.625	3.000
Mean	4.912	3.662	1.321	0.387	0.475	2.575

Table 7. Analysis of molecular variance (AMOVA) of 10 *Moringa oleifera* Lam. populations based on values obtained from simple sequence repeat (SSR) markers.

Variance	Degrees of freedom	Squares sum	Mean squares	Estimated variation	%
Among populations	9	174.910	19.434	0.721	20
Among individuals	90	450.650	5.007	2.106	58
Within individuals	100	79.500	0.795	0.795	22
Total	199	705.060		3.622	100

The values in coefficients of inbreeding (Fis and Fit) and genetic differentiation (Fst) showed high inbreeding in the populations (Table 8), with a remarkable inbreeding value of 1.0 for MoO2, which was in correspondence with its observed heterozygosity value (zero). For Fst, the lowest value was for MoV1 (0.09), and the highest value was for MoO1 (0.337), indicating that population genetic differentiation was from a moderate to a great level.

Table 8. Wrigth´s F statistics of 10 *Moringa oleifera* Lam. populations from Southeastern Mexico.

Population	Fis	Fit	Fst
MoV1	0.621	0.655	0.090
MoV2	0.766	0.821	0.232
MoO1	0.750	0.834	0.337
MoO2	1.000	1.000	0.294
MoO3	0.757	0.793	0.149
MoG1	0.621	0.696	0.198
MoC1	0.695	0.738	0.139
MoC2	0.757	0.821	0.262
MoY1	0.665	0.724	0.175
MoY2	0.744	0.779	0.136
Mean	0.738	0.786	0.201

FIS: coefficient of inbreeding within populations; FIT: coefficient of total inbreeding; FST: coefficient of differentiation.

Genetic Relationships

Three groups were revealed in the PCoA plot. Group A included populations of Yucatán and Veracruz (MoY1, MoY2, MoV1, and MoV2); Group B encompassed populations of Chiapas, Guerrero, and Oaxaca (MoC1, MoC2, MoO2, and MoG1); and Group C congregated cultivated populations of Oaxaca (MoO1 and MoO3) (Figure 2). Two dendrogram types were constructed based on the UPGMA method. The clustering was done at the population level and at the individual level. At the population level, the dendrogram showed four groups (Figure 3). Group A consisted of populations from Veracruz and Yucatán (MoV1, MoY1, and MoY2); Group B included populations from Chiapas, Guerrero, and Oaxaca (MoC1, MoC2, MoO2, and MoG1); Group C consisted of two populations, one from Veracruz and another from Oaxaca (MoV2 and MoO1); and Group D only included the MoO3 population. At the individual level, the same three groups were identified with the same populations as in the PCoA plot, with few individuals matched in different groups (Figure 4).

A more in-depth examination of the diversity displayed by the populations was made with the principal coordinate analysis and clustering by distances. Here, three clusters

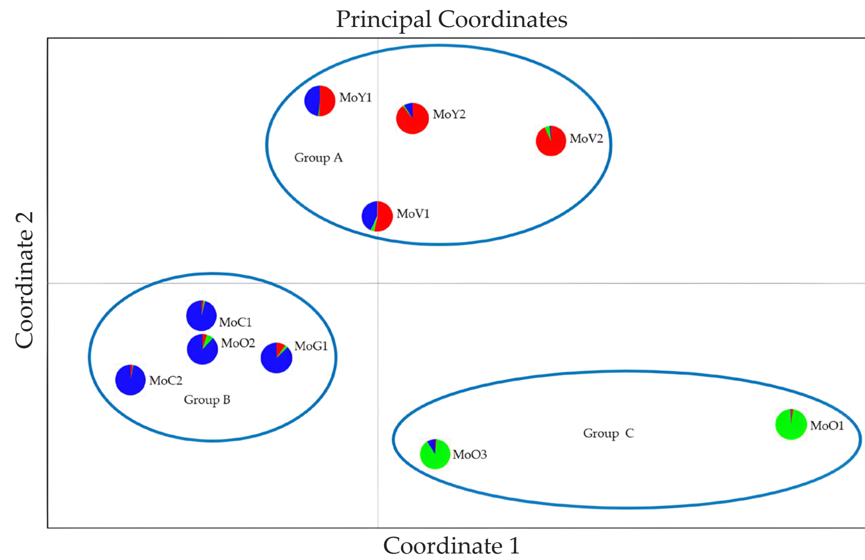

Figure 2. Principal coordinate analysis (PCoA) based on 10 *Moringa oleifera* Lam. populations. Colors show the share of genetic groups in populations (I: red, II: green, and III: blue) developed using the STRUCTURE software.

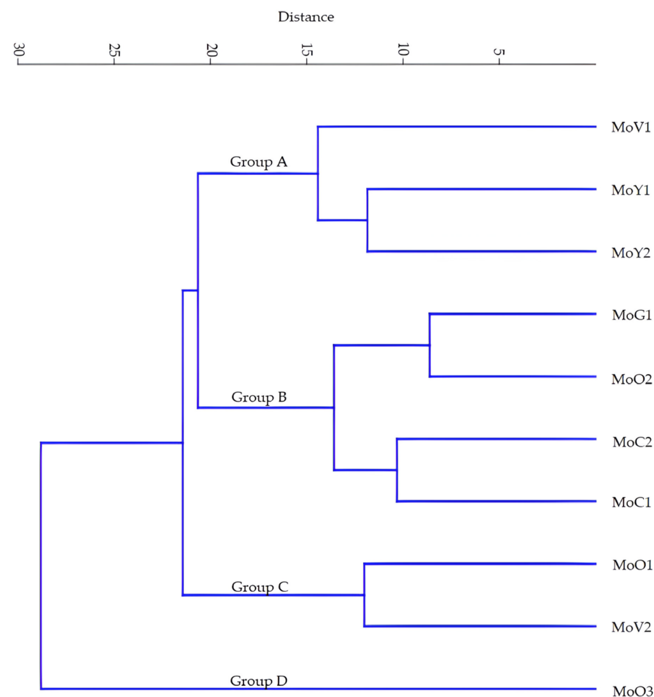

Figure 3. Dendrogram developed using the unweighted pair group method with arithmetic mean (UPGMA) method for 10 *Moringa oleifera* Lam. populations based on genetic Nei´s distance (1972) from eight SSR loci. Numbers in the scale indicate similitude values.

Figure 4. Dendrogram based on simple sequence repeat (SSR) data for 100 individuals from 10 populations of *Moringa oleifera* Lam. from Southeastern Mexico.

were identified, all including the same populations (Figures 2 and 3). In addition, three genetic original groups were also identified in the genetic structure analysis. These groups, defined by the K value, were more than those reported by Rajalakshmi *et al.* (2017) and below K = 5 reported by Ganessan *et al.* (2014). The number of genetic original groups could be attributed to the size of the geographical area under study, the number of populations evaluated, its limited dispersal, and the lack of crossing with wild populations due to its recent introduction (Louwaars, 2018). This way, moringa populations from Veracruz and Yucatán share alleles from two genetic backgrounds, and populations from Chiapas, Guerrero, and one from Oaxaca share alleles of one of these genetic backgrounds. Alleles of a third genetic original background are shared by the other two populations from Oaxaca. Therefore, these three genetic groups are the foundation of the genetic diversity of all the ten populations examined in this study.

Genetic Structure

Bayesian analysis of assignment of individuals showed that the most probable number of original genetic groups is K = 3, since the highest value of the rate of change in the likelihood ratio in comparison to K was obtained here. Accordingly, each individual from each population was allocated into one of the three genetic groups. Delta K values were calculated from Evanno *et al.* (2005) (Figure 5).

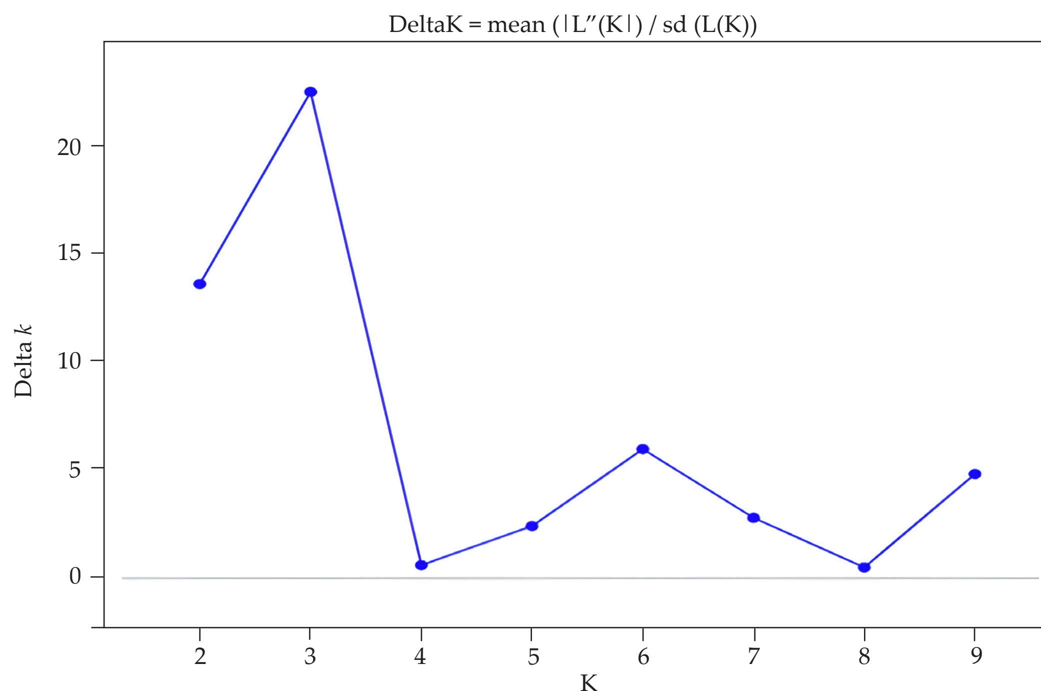

Figure 5. Delta K values for developing STRUCTURE analysis of *Moringa oleifera* Lam. populations from Southeastern Mexico.

The genetic structure analysis identified three original genetic populations (red, blue, and green color) (Figure 6). Two populations from Veracruz and Yucatán (MoV1 and MoY1) had a very similar background with two original populations (red and blue); another two populations of the same geographical states (MoV2 and MoY2) had a composition most similar to one original population (red). Populations of Oaxaca, Chiapas, and Guerrero (MoO2, MoC1, MoC2, and MoG1) were all also similar and showed a constitution of one original genetic population (blue). Finally, two populations of Oaxaca (MoO1 and MoO3) revealed to bear the genetic background of mainly one original population (green).

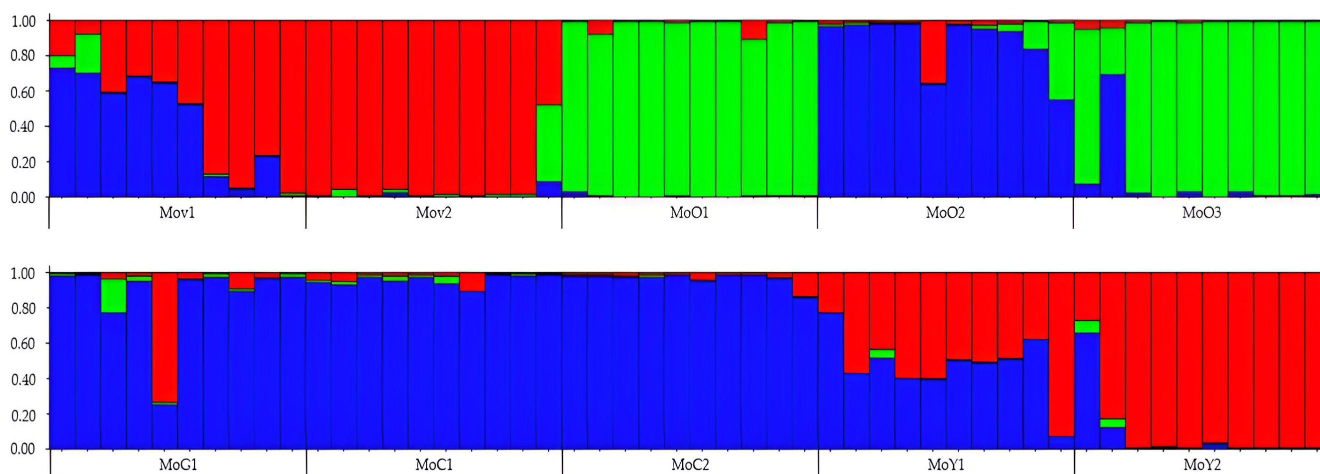

Figure 6. Clustering model for 100 genotypes of 10 *Moringa oleifera* Lam. populations using simple sequence repeat (SSR) data (K = 3). The numbers on the ordinate axis indicate the coefficient of ancestry of populations.

Structure analysis allows to identify the degree of genetic background. Populations with scores above 0.8 are considered pure, and values below 0.8 are considered mixed. MoV2 (0.930) and MoY2 (0.898) populations from group I (red) are pure, MoO1 (0.967) and MoO3 (0.899) populations from group II (green) are pure, while MoC1 (0.959) and MoC2 (0.967) belong to group III (blue) (Table 9).

According to Tian *et al.* (2015), genetic variability represents an immediate or future potential for conservation and sustainable development of moringa. For conservation, the genetic profile with the eight SSR markers can be useful to monitor these populations and perhaps to design management and conservation programs. For sustainable use, variability recorded in all 10 populations could be used to generate novel genetic combinations through breeding programs. Particularly, the genetic diversity was 0.662 on average, comparable to 0.62 of maize, another allogamous species (Bedoya *et al.*, 2017).

Table 9. Estimated proportion of ancestry of the 10 studied populations of *Moringa oleifera* Lam. from Southeastern Mexico.

Population	Genetic Group		
	I	II	II
MoV1	0.532	0.036	0.432
MoV2	0.930	0.054	0.016
MoO1	0.023	0.967	0.010
MoO2	0.047	0.072	0.881
MoO3	0.012	0.899	0.089
MoG1	0.093	0.032	0.875
MoC1	0.024	0.017	0.959
MoC2	0.026	0.007	0.967
MoY1	0.509	0.010	0.482
MoY2	0.898	0.015	0.086

The diversity of moringa can result from its flowering type (Raja *et al.,* 2013), early sexual maturity, self and cross-pollination (Muluvi *et al.,* 2004), agroecological conditions, genetic material migration, reincorporation of material, and natural and artificial selection (Tak and Maurya, 2015). Even though moringa is not a plant native to Mexico, all 10 populations under study showed a high allelic richness coming from three genetic original groups. These populations and others not included may be the starting point for the development of new cultivars of this species, to give rise to a catalogue of new moringa genotypes with morphological, physiological, and chemical characters adapted to the environmental conditions of Southeastern Mexico.

CONCLUSIONS

A high gene diversity was found within the studied *Moringa oleifera* Lam. populations, with a wide variety of alleles at the eight loci analyzed. The proportion of individuals carrying two different alleles at a particular locus was low, which could indicate that populations have remained quasi-intact despite their reproductive nature. Furthermore, we found evidence of clear genetic clustering, suggesting the existence of three subpopulations. These results highlight the genetic diversity of moringa that has been introduced in Mexico.

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