Colegio de Postgraduados

Morphological characterization and genetic evaluation using RAPD molecular markers of *Agave maximiliana* Baker

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ABSTRACT

Objective: To study perform morphological characterization and use RAPD molecular markers in individuals of the species *Agave maximiliana* to detect its genetic variation.

Design/methodology/approach: This study was conducted with 40 samples from individuals collected in four locations in the municipalities of Mixtlán and Mascota, Jalisco. Morphological characterization of the selected individuals was performed by measuring seven foliar variables. Genetic characterization was performed using the RAPD technique, using primers OPA02 and OPA03 to obtain amplicon patterns. Cluster analysis, AMOVA, PERMANOVA, and Mantel analysis were performed based on the data obtained.

Results: The first three principal components explain 99.64% of the observed variability. Additionally, molecular variables show greater genetic variation within populations (96%) than between populations (4%). These values yield a p-value of 0.2717 and a PhiPT of 0.052, indicating a moderate level of genetic differentiation among the studied populations. The PERMANOVA showed a p-value of 0.0793, and the Mantel test resulted in a p-value of 0.3305.

Limitations on study/implications: The samples of *A. maximiliana* correspond only to an area called the "mountainous raicilla zone" in Jalisco, Mexico, and the results of this study are exclusive to that region.

Findings/conclusions: The analysis of morphological diversity showed no significance among the sampling populations and the individuals were not separated into groups defined by geographical origin. Furthermore, molecular analysis showed that there is more variability within local populations than among sampled populations.

Keywords: *Agave* sp., raicilla, morphological characterization, population variability, RAPD.

INTRODUCTION

Agaves comprise more than 200 species worldwide, and Mexico has approximately three-quarters of them; some are endemic, although their distribution throughout the country is uneven (Delgado-Lemus *et al.*, 2014; López-Pujol *et al.*, 2016; Eguiarte *et al.*,

2021). They are used as raw materials in the production of various industrialized products. Mexico is the source of agave species and also a center of domestication. It is believed that their historical domestication began 9,000 years ago, and they were used as food, beverages, clothing, and other uses (Arellanes *et al.*, 1997; Bellon *et al.*, 2009; Trejo *et al.*, 2018; Pérez-Zavala *et al.*, 2020). Currently, 53 species of agave have been registered that are used for the production of distilled beverages (Torres *et al.*, 2015), to mention such as *A. tequilana* Weber, *A. angustifolia* Haw., *A. karwinski* Zucc., *A. marmorata* Roezl, *A. potatorum* Zucc., *A. americana* L. var. *oaxacensis* Gentry, *A. cupreata* Trel. and Berger, *A. rhodacantha* Trel., *A. salmiana* Otto ssp. *crassispina* (Trel.) Gentry, *A. wocomahi* Gentry, *A. durangensis* Gentry and *A. maximiliana* Baker (García-Mendoza, 1998; Colunga-GarcíaMarín *et al.*, 2017).

Among the most profitable agave alcoholic products are distilled spirits such as tequila, mezcal, bacanora, and raicilla (Carrillo-Trueba, 2007; Lucio-López, 2022). In the case of raicilla, it is mainly consumed in Mexico and, to an incipient extent, it is exported to the USA. It is produced in a specific region of Mexico using mainly the species *A. maximiliana* and *A. angustifolia* and to a lesser extent the species *A. inaequidens*, *A. rhodacantha*, and *A. valenciana*. This region is defined by the municipalities of Mascota, Mixtlán, Cabo Corrientes, and Talpa de Allende in the state of Jalisco and the municipality of Bahía de Banderas in the state of Nayarit (IIEG, 2024a; IIEG, 2024b).

Distilled beverages are made from adult individuals before they complete flowering (Zizumbo-Villarreal *et al.*, 2013), which impairs the reproduction of this species, reduces its populations and reduces genetic variability (Aguirre & Eguiarte, 2013). This leads to a loss of diversity, limiting its adaptation to environmental changes and increasing its risk of extinction (Bourguiba *et al.*, 2012). In these cases, it is important to conduct genetic diversity studies, focusing on actions for the sustainable use of economically valuable species (Huang *et al.*, 2009; Zizumbo-Villarreal *et al.*, 2013). To achieve this, it is necessary to evaluate genetic variation using molecular techniques to distinguish plant types and their origins (Alfaro *et al.*, 2007; Rodríguez-Garay *et al.*, 2008; Torres-Morán *et al.*, 2010).

Agave maximiliana is found in the states of Sonora, Chihuahua, Durango, and Sinaloa, at an altitude of 930 to 1850 meters above sea level in pine and oak forests (Eggli & Nyffeler, 2020). It is a species whose stems with detached leaves ("pineapple", or the heart of the agave plant) are used in the production of the distilled raicilla, in the mountainous area of the state of Jalisco (Colunga-GarcíaMarín *et al.*, 2007; Vázquez *et al.*, 2007; Cabrera *et al.*, 2020); individuals present wide morphological variability. Furthermore, in the mountainous area of the raicilla region of Jalisco, the species *A. maximiliana* exhibits high morphological variability. Therefore, the presence of ecotypes (Cachúa-Torres *et al.*, 2025) was observed, which could indicate high genetic variability in this species. Therefore, the objective of this study was to perform morphological characterization and use RAPD molecular markers to establish variation among populations of *Agave maximiliana* in this region. Although it is assumed that for farmers this species is the same throughout the raicilla region, its morphological variability would be expected to correspond to its genetic variability measured with molecular techniques in *Agave maximiliana*.

MATERIALS AND METHODS

Plant Material

In situ, morphological measurements were made, and leaf samples were collected from 40 individuals identified by raicilla producers as the *Agave maximiliana* species. This work was carried out in four locations within two municipalities in the state of Jalisco: one location in Mixtlán and three in Mascota (Table 1). Collections of each site were considered for this study as separate populations. Sampling in the agricultural fields was entirely randomized based on the material each producer allowed us to collect, since most of the selected plants are grown for commercial purposes, thus minimizing the impact on owners' incomes. Although there is no physical distance that guarantees that the *A. maximiliana* used by farmers are not related, given that some pollinators (bats and insects) can travel great distances, the Mixtlán site was considered sufficiently distant in a straight line from the three Mascota sampling sites (33.6-42.2 km). With these distances, it was expected that the sampled specimens would not have any related kinship between site 1 and sites 2-4.

Morphological Characterization

Individuals were described, measuring seven morphological variables in centimeters: leaf length, leaf width (maximum), leaf thickness, terminal spine length, lateral spine length, and lateral spine width; the number of lateral spines was measured in the middle of the leaf (considering 10 cm of leaf length). These data were used to calculate principal components and scatter plots. Furthermore, a dendrogram was also created using Paired group algorithm (UPGMA) and the analyses were performed using the statistical software PAST 4.12. The variables were standardized to perform the principal components analysis (PCA). PCA loadings plot for principal components 1 and 2 were included as a visualization that shows how original variables contribute to the principal components (PCs) by plotting them as vectors. Also the PCA loading matrix is included in this work.

Genetic characterization

DNA extraction

Approximately 10 cm of the terminal part of the leaf adjacent to the bud was taken from the 40 plants. These samples were placed in a cooler, transported to the laboratory

Table 1. Locations, number of samples per location and type of population.

Municipality	Geographic coordinates	Number of samples	Population type (key)
Mixtlán	20° 33' 14.1" N 104° 28' 37.8" W	12	Cultivated (MXC)
Mascota I	20° 31' 16.9" N 104° 51' 08.3" W	13	Cultivated (MTC)
Mascota S	20° 31' 03.6" N 104° 51' 13.8" W	10	Wild (MTS)
Mascota II	20° 33' 05.4" N 104° 53' 06.7" W	5	Cultivated (MSC)

of the TecNM ITEL Llano Aguascalientes, and stored at −20 °C. DNA extraction was carried out using the method of Coelho *et al.*, (2009) with modifications. PVP was added to the extraction buffer, where it was added to the sample when it was ground with liquid nitrogen. The samples were incubated at 65 °C for 30 minutes, the sample was fractionated twice with phenol-chloroform-isoamyl alcohol mixture (25:24:1), and centrifuged at 10,000 rpm for 10 minutes. The aqueous phase was recovered and combined with 700 μL of cold isopropanol and incubated at −20 °C for 30 minutes to precipitate the DNA. It was centrifuged at 10,000 rpm for 10 minutes and the resulting pellet was resuspended with 300 μL of TE buffer plus 150 μL of 5 M NaCl and 800 μL of absolute ethanol and incubated at −20 °C for 30 minutes. It was centrifuged again at 10,000 rpm for 10 minutes. The supernatant was discarded, the pellet was washed with 70% ethanol and centrifuged at 10,000 rpm for 5 minutes. The pellet was dried at room temperature and resuspended in 50 μL of TE buffer. This standardized DNA extraction method used by our work group has allowed us to obtain high-purity DNA in sufficient quantities for agaves and other plant species, as verified by PCR analysis. In this study the extracted DNAs were previously tested by positive amplification of a 610 bp fragment of the b-actin gene. This was used to validate that the PCR reaction was accurate and that there were no inhibitors in the sample that could affect the reaction.

The PCR conditions were: 1.5 μl of DNA resuspended in TE 1X buffer, 2.5 μl of primers (100 μM), 6.25 μl of PlatinumTM PCR SuperMix High Fidelity (Invitrogen), and 14.75 μl of water were mixed in each PCR tube. For PCR, a Techne TC-512 gradient thermocycler was used, with a program consisting of an initial heating at 94 °C for 5 minutes and 40 cycles, as follows: denaturation (1 min, 94 °C), annealing (1 min, 51 °C) and extension (3 min, 72 °C). The final extension was 10 min at 72 °C.

RAPD Molecular Marker Analysis

Primers 1 to 20 of the OPA series (Operon Technologies, Alameda, CA, USA) were tested. Primers OPA02 and OPA03 were selected because they presented more bands and greater polymorphism in a previous analysis. The molecular weight of the bands was determined using GelAnalyzer 19.1 software (Lazar and Lazar, 2023) using the molecular weight marker (GeneRuler 1 kb DNA Ladder, SM0311, ThermoFisher) as a reference, which was included in the gels. The amplification products were visualized on 1.2% agarose gels in 1X TBE buffer at 60 Volts for two hours and stained with ethidium bromide for 15 minutes.

Statistical analysis

For morphological characters measurements, mean and standard deviation was calculated. ANOVA was also performed on these measurements. Means were compared whenever significance was observed ($p<0.05$) with Tukey's test using Minitab 17®.

For banding patterns from RAPDs, the presence (1) or absence (0) of each band was recorded visually, captured in a binomial data matrix in Excel, and analyzed using the Paired Group Algorithm (UPGMA) to construct the dendrogram with the statistical program PAST 4.12 (Hammer *et al.*, 2001) and the AMOVA analysis was performed with GenAlEx 6.503 (Peakall & Smouse, 2012). In addition, a Principal Coordinate Analysis (PCoA), PERMANOVA, and the Mantell test were performed comparing the four populations, also using PAST 4.12 software. The distances between sampling sites in kilometers were determined in a straight line using Google Maps software, using the geographic coordinates of each site as a reference point.

RESULTS AND DISCUSSION

Morphological Analysis

Most of the characteristics measured in the sampled specimens had means that were statistically similar, with the exception of leaf thickness and leaf length, which presented $p<0.05$ (Table 2). Tukey's test on leaf thickness showed two groups (Table 3). However, the Tukey test for leaf length showed no differences, generating a single group ($\alpha=0.05$).

Principal component (PC) analysis performed on the seven variables showed that the first three components: PC1, PC2 and PC3, presented eigenvalues greater than 1.0, which together explained 99.64% of the variance in the original data (Table 4). More detail of the most influential PC is shown on the loadings matrix (Table 5). The study revealed noticeable phenotypic diversity among morphological variants of A. maximiliana, collected in plots and wild areas in the mountainous area of the state of Jalisco, Mexico. Other studies confirm that there are individuals of *A. maximiliana* in the mountainous raicilla zone showing wide morphological variations in their "pineapple" sizes, leaves, and spines. These cases were considered "ecotypes" that produce different contents of reducing sugars, ranging from 32% to 83% (Cachúa-Torres *et al.*, 2025).

Table 2. Counts and measurements in cm (mean and standard deviation) per site and observed significance (*p*) for each measured variable.

Variable	MXC	MTC	MTS	MSC	p
Lateral spines/10 cm	9.33±6.08	7.00±3.58	7.20±4.21	13.40±9.18	0.1345
Lateral spine length	0.63±0.26	0.75±0.46	0.86±0.3	0.92±0.37	0.3796
Lateral spine width	1.12±0.59	1.03±0.47	0.87±0.21	0.68±0.19	0.2457
Leaf thickness	2.04±0.65	2.23±0.63	1.45±0.36	1.90±0.54	0.0216
Leaf length	74.58±20.74	83.84±22.33	84.00±15.60	56.80±9.53	0.0491
Leaf width	15.83±4.21	19.76±5.91	17.80±4.70	16.20±1.48	0.2105
Terminal spine length	3.08±1.04	3.84±1.40	2.80±0.53	2.80±0.27	0.0765

Table 3. Tukey's comparisons for leaf thickness.

Factor	N	Mean	Grouping
MTC	13	2.231	a
MXC	12	2.042	ab
MSC	5	1.900	ab
MTS	10	1.450	b

Means that do not share a letter are significantly different.

Table 4. Principal components (PC) of seven morphological variables of *A. maximiliana* from the root zone.

Principal component	Eigenvalue	% Variance
1	433.256	90.345
2	28.007	5.8402
3	16.5709	3.4555
4	1.13957	0.23763
5	0.359115	0.074884
6	0.161083	0.03359
7	0.0656927	0.013699

Table 5. Matrix loadings for principal components of morphological features of *A. maximiliana*.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Spines in 10 cm	−0.1	0.97	0.2	0.02	0.01	0.03	−0.01
Terminal spine length	0	−0.02	0.01	0.03	0.09	0.68	0.72
Lateral spine width	0.01	−0.03	0.01	0.11	0.25	0.68	−0.68
Leaf thickness	−0.01	−0.01	0.02	0.22	0.93	−0.26	0.11
Leaf length	0.99	0.12	−0.11	−0.01	0.01	0	0
Leaf width	0.13	−0.19	0.97	−0.02	−0.02	−0.02	0

Bartlett's test is to verify that different groups within a dataset exhibit similar levels of variability. The test for determinant matrix was very close to zero (1.49E-16), suggesting that the correlation matrices are not singular and that there are significant correlations between the variables (Table 6).

Furthermore, the p-value indicates a statistically significant difference from the null hypothesis that the correlation matrix is an identity matrix. Therefore, the null hypothesis is rejected, and it is concluded that the data are sufficiently correlated to proceed with multivariate analyses

The values obtained in our work are higher than those reported in wild populations of *Agave angustifolia* from Sonora, Mexico, which with the same number of PCs obtained 51.9% of the variance (Fragoso *et al.*, 2021). Morphological differences were observed, between and within plots and wild areas, in the characteristics of the measured agaves, the variables with the greatest weight were: leaf length in cm (PC1) and lateral spines in 10 cm (PC2) (Figure 1). Similarly, García-Núñez *et al.* (2020) also obtained 74% morphological

Table 6. Bartletts's sphericity test.

Determinant	1.49E-16
Chi^2	1364.8
df	27
p (spherical)	6.91E-271

Figure 1. Lateral spines of *A. maximiliana* leaves collected in the municipality of Mixtlán.

variability using only these two variables, which allowed them to more accurately distinguish the species and variants of "pulquero" agave in the states of Hidalgo and Mexico.

On the dispersion graph (scatter plot), the first two principal components were taken; in the results obtained, this graph did not show defined groups according to the collection site (Figure 2). These results differ from those reported by Esparza-Ibarra *et al.* (2015), this is because they worked with different species of "mezcal" agaves in the Potosino and Zacatecas plateau; in our work, only one of these species was considered.

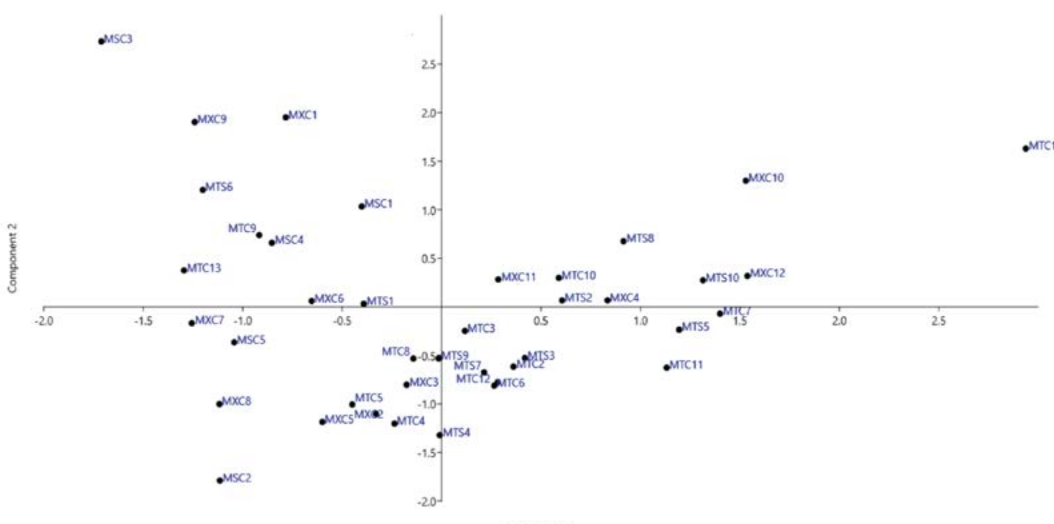

Figure 2. Scatter plot from the first two PCs in the analysis of seven variables measured in *A. maximiliana*, both wild and cultivated. Tags: MXC=Mixtlán cultivated, MTC=Mascota cultivated I, MTS=Mascota wild plants, MSC=Mascota cultivated II. The number at the end of the tag indicates the sample number. The units of the axes are indicated in eigenvalues.

In the cluster analysis (dendrogram) two defined groups were found that were composed of samples from more than two of the four sites (Figure 3). Sample MTC1 appeared separated because it was from the individual with the largest leaves, this variation can be explained according to the comments from more experienced producers, that agaves tend to cross with other agaves, and in this particular case, greater robustness was obtained in the plant.

Genetic Analysis

Of the 20 primers tested in our lab, the majority generated amplified products in all gels. The primers that showed the greatest band amplification were OPA02 (Figure 4), OPA03, OPA04, OPA09, and OPA10. We focused on analyzing the samples based on the amplifications obtained with primers OPA02 and OPA03. Primer OPA2 generated 17 bands ranging from 260 to 2350 bp, 10 of which were considered polymorphic (59%). Primer OPA3 generated 13 bands ranging from 370 to 2400 bp, 10 of which were considered polymorphic (77%). The polymorphic information content (PIC) were estimated as ranges: OPA2 PIC 0.095-0.499 and OPA3 PIC 0.049-0.480. These values ranged from moderately informative (0.25-0.5) to less informative (<0.25).

Alfaro *et al.* (2007) also used the same 20 OPA primers, and this study also agreed on the efficiency of using primer OPA02 instead of OPA20. They evaluated genetic diversity between and within populations in 10-year-old agaves, representative of variants of *Agave salmiana* and *A. mapisaga* from the northeast of the state of Mexico, using molecular markers and morphological characteristics, to obtain basic information for the development of conservation and genetic improvement strategies.

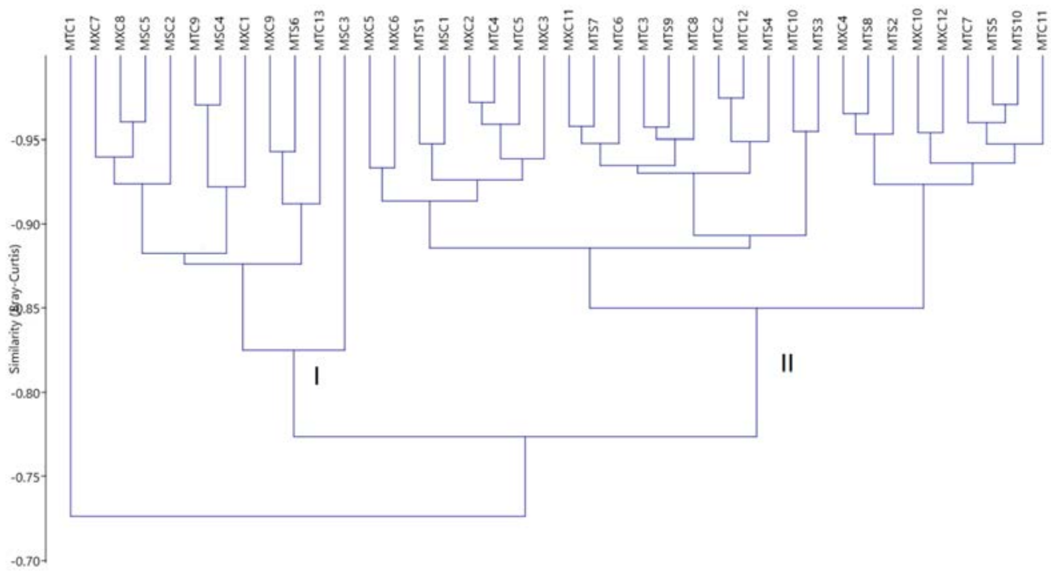

Figure 3. Dendrogram generated from the analysis of seven morphological variables measured in A. maximiliana. Dendrogram constructed using the Paired group algorithm (UPGMA) with the Bray-Curtis similarity index. Cophenetic Correlation Coefficient=0.742. Labels: MXC=Mixtlán, MTC=Mascota I, MTS=Mascota wild plants, MSC=Mascota II. The number at the end of the labels indicates the sample number.

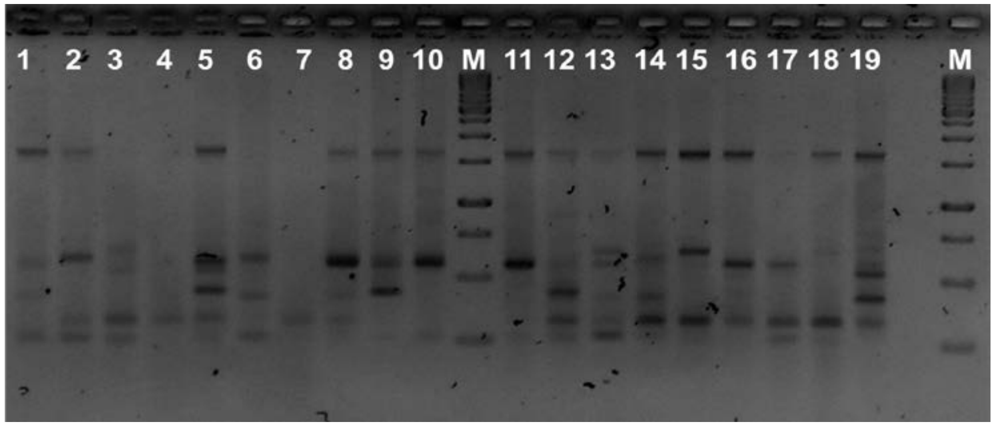

Figure 4. Amplicons were obtained from PCR using the OPA02 primer in individuals of *A. maximiliana* collected in the municipalities of Mixtlán (1 to 12) and Mascota (13 to 19). M indicates the molecular marker.

In the dendrogram, two main groups were formed (Figure 5). Genotypes from all of the four sampling sites were intermingled; despite being geographically distant, the genetic analysis indicates that they are genetically similar individuals. This is because part of the materials are shared between both locations, being the origin the wild areas and nurseries of institutions and among the same producers. For their part, Martínez-Velasco & Arzate-Fernández (2022) in their research using the RAPD primers (Y24 and Y4) and the ASSR (ASSR 20 and ASSR 29) obtained the necessary products to estimate the genetic integrity of *A. salmiana* and *A. marmorata* seedlings regenerated in vitro from leaf tissue of seedlings.

The PCoA scatter plot shows the first two principal coordinates as the x and y axes, showing a visual representation of how similar or dissimilar the samples are (Figure 6). As is evident in that figure, samples of the four sampling sites are dispersed and mixed without

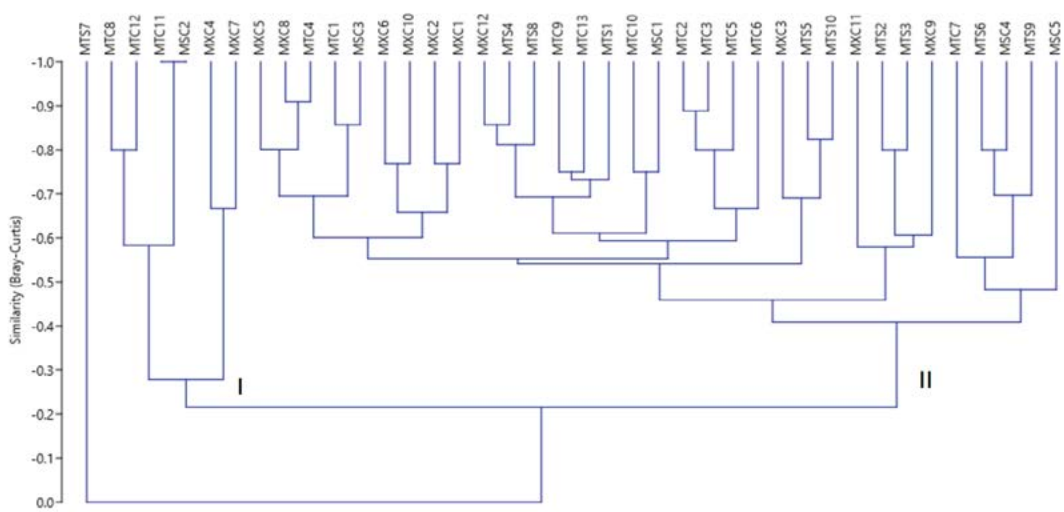

Figure 5. Dendrogram generated from the analysis of primers OPA02 and OPA03 in *A. maximiliana*. Dendrogram constructed using the Paired group algorithm (UPGMA) with the Bray-Curtis similarity index. Cophenetic Correlation Coefficient = 0.819. Labels: MXC=Mixtlán, MTC=Mascota I, MTS=Mascota wild, MSC=Mascota II. The number at the end of each label is the sample number.

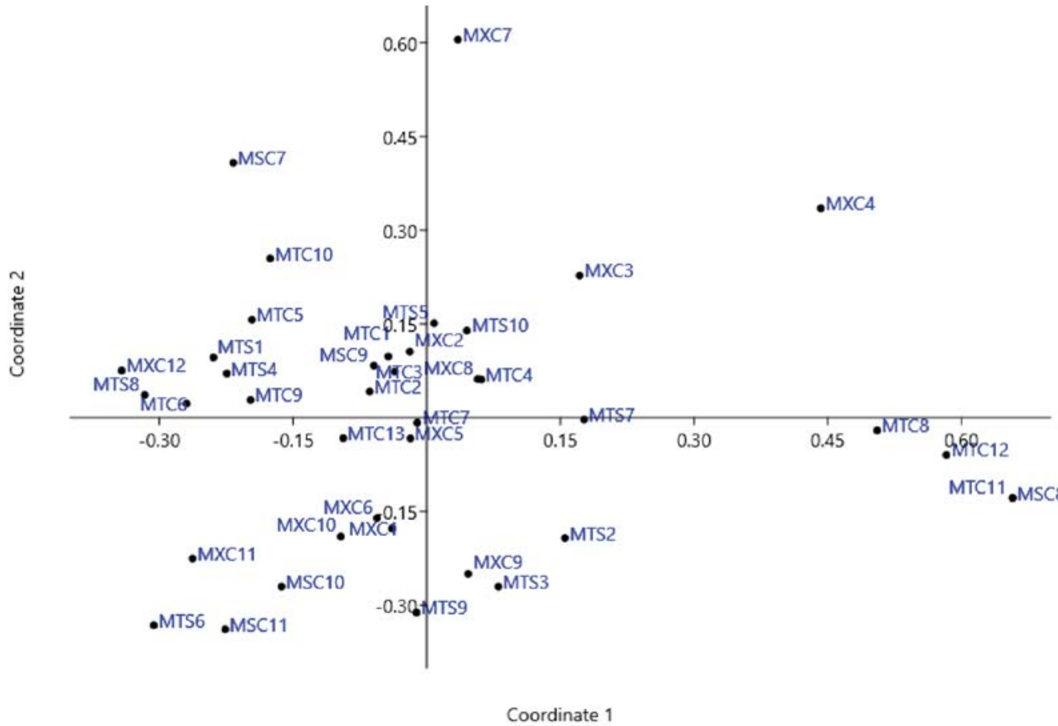

Figure 6. PCoA scatter plot from binary data obtained from RAPD amplicons of *A. maximiliana* using primers OPA02 and OPA03.

generating clear group conglomerates and showing that in general samples are genetically variable similar among genotypes even though these came from different sites. Values for the PCoA (Eigenvalues and Percent) per axis are shown on Table 7.

The analysis of molecular variance (AMOVA with 999 permutations) was performed considering the four populations according to the site of collection. The highest percentage of variation was detected within populations defined by the sampling site (96%), in contrast to the variation between populations (4%) (Table 8), p=0.2717 with a PhiPT=0.035 (PhiPT=AP/(WP+AP)=AP/TOT; AP=Est. var. among pops, WP=Est. var. within pops) (Figure 5). This PhiPT of 0.035 is a very low value, indicating little genetic differentiation among the populations being compared. Therefore, the genetic variation is mostly found within the populations rather than among them. Should be noticed that unbalanced sample sizes across populations may have induced bias by causing a systematic overestimation or underestimation of population parameters, making it difficult to draw accurate conclusions for all groups. Larger groups may bias the results, while smaller groups are more likely to have their characteristics measured less accurately and their specific outcomes underrepresented.

Considering that the traditional propagation system of the species is through seeds, this value reflects the variability that exists within each population and its degree of isolation. This result is very similar to the results obtained for *A. potatorum*, of 90% and 80% for *A. cupreata* among wild populations, reported by Aguirre & Eguiarte (2013). Félix-Valdez *et al*. (2016) also obtained lower values for *A. potatorum*, of 61%, due to the extraction of

Table 7. Table of values for the PCoA (Bray-Curtis similarity index).

Axis	Eigenvalue	Percent
1	23.05	18.32
2	18.66	14.82
3	13.62	10.82
4	12.2	9.69
5	10.62	8.44
6	9.09	7.22
7	7.17	5.7
8	6.81	5.41
9	5.21	4.14
10	4.25	3.38
11	4.23	3.36
12	2.76	2.19
13	2.29	1.82
14	1.69	1.34
15	1.23	0.98
16	0.94	0.75
17	0.78	0.62
18	0.6	0.47
19	0.43	0.34
20	0.25	0.20.2

Table 8. AMOVA for the four populations of *A. maximiliana*.

Source	df	SS	MS	Est. Var.	%
Among Pops	3	12.781	4.260	0.116	4%
Within Pops	36	113.094	3.141	3.141	96%
Total	39	125.875		3.257	100%

individuals for mezcal production. Finally, it is worth mentioning that Cabrera-Toledo *et al*. (2020) in their study of populations of *A. maximiliana* collected in municipalities of Jalisco where raicilla is produced, in the states of Zacatecas, Nayarit, Sinaloa, and Durango, in their results they obtained little diversity between and within the morphological variables and in the AMOVA analysis 61% within populations and 38% between populations.

The PERMANOVA analysis across groups showed an overall p-value of 0.0793 (Table 9). When comparing sampling sites, only a significant p-value (p=0.0403) was found between the MXC and MTS data, which included cultivated and wild genotypes, respectively (Table 10). The Mantel Test showed an R=0.5122 value that indicates a moderate positive correlation between the two matrices being compared (genotype group *vs*. geographical distance among groups). Finally, a p=0.3305 for the Mantel test indicates that there is no statistically significant correlation between the two distance matrices previously mentioned (Table 11).

Table 9. PERMANOVA for the four populations of *A. maximiliana*.

Permutation N	9999
Total sum of squares	125.9
Within-group sum of squares	113.1
F	1.356
p (same)	0.0793

Table 10. PERMANOVA p-values matrix for the four sampling sites of *A. maximiliana*.

	MXC	MTC	MTS	MSC
MXC		0.0548	0.0403	0.4958
MTC	0.0548		0.2636	0.2081
MTS	0.0403	0.2636		0.5065
MSC	0.4958	0.2081	0.5065	

Table 11. Mantel Test for the four populations of *A. maximiliana*.

Permutation	N=9999
Correlation	R=0.5122
p (uncorr; onetailed):	p=0.3305

CONCLUSIONS

Briefly, it was found that both the morphological analysis and the RAPD analysis show that there is more variability among individuals within the same group than between groups.

More in detail, comparison of morphological traits showed that almost all of these variables were statistically identical, with the exception of lateral spine width; however, only two locations differed. Furthermore, it was found that all genotypes were intermixed when placed on a two-dimensional principal component plane, with PC1 and PC2 contributing the most to the characteristics of leaf length and lateral spines present in 10 cm.

According to the morphological study, a 99.64% variability index observed in *A. maximiliana* in the raicilla region was explained solely by three principal components obtained from foliage measurements such as leaf length, lateral spines/10 cm and leaf width, that are related to the plant's overall appearance. Bartlett's analysis showed that the matrices used were not singular and that multivariate analysis of the data could proceed without problems.

The dendrogram on morphological characteristics only formed two groups, which included mixtures of genotypes from all sampling sites, indicating that relative similarity was found between most of the genotypes.

The molecular results showed that the RAPD primers OPA02 and OPA03 used in this work were effective in differentiating the studied individuals, as observed in *A. maximiliana* from the state of Jalisco. When analyzing RAPDs in clusters as a dendrogram, two groups were found, one of which included genotypes from all four sites. Furthermore, the PCoA scatter plot showed the samples to be dispersed, indicating dissimilarity without generating clear clusters. AMOVA analysis showed little genetic differentiation between the compared populations (p=0.2717, PhiPT=0.035). The AMOVA analysis of the RAPD data revealed that the greatest diversity was found among individuals within the populations of the selected localities, but only slight diversity was found among populations. PERMANOVA showed a non-significant p-value (p=0.0793), and the Mantel test showed an R=0.5122 indicating a moderate positive relationship between the distance matrices and genetics.

Finally, it can be concluded that the variability from both the cultivated and wild genotypes collected in the raicilla region are relatively homogeneous among sites.

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