AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Characterization and *in vitro* spread of mesquite (*Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M.C. Johnst.)

Ortiz-Robles, María D.[1]; Gómez-González, Adrián[2]; Flores-Benítez, Silvia[1]; Valera-Montero, Luis L.[1]; Romero-Amador, Lourdes D.[1]; Perales-Segovia, Catarino[1]; Silos-Espino, Héctor[1]

[1] TeCNM-Campus El Llano, Aguascalientes, Carretera Aguascalientes-San Luis Potosí km 18, El Llano, Aguascalientes, México, C. P. 20330.
[2] Colegio de Postgraduados, Campus San Luis Potosí, Iturbide 73, Salinas de Hidalgo, San Luis Potosí, México, C. P. 78600.
* Correspondence: silosespino@hotmail.com

ABSTRACT

Objective: To analyze fruit characteristics, genetic diversity, and *in vitro* spread of mesquite genotypes (*Prosopis laevigata* (Humb. & Bonpl.) ex Willd.) from the plains of Ojuelos-Aguascalientes, Mexico.

Design/Methodology/Approach: Fruit and leaf samples were collected from 20 mesquite genotypes to analyze and extract DNA to determine their diversity using RAPDs. Simultaneously, *in vitro* spread tests were performed.

Results: The Ojuelos de Jalisco genotype stood out for the weight of its fruits, while La Presa genotype stood out for its degrees Brix. Genotypes were grouped according to their best growth condition for their genetic analysis. In *in vitro* spread, AG_3 with IBA allowed stem/shoot elongation and root formation; meanwhile, $AgNO_3$ prevents leaf fall, allowing rooting and transfer to the soil.

Study Implications/Limitations: This study about mesquite (*P. laevigata*) was limited to the plains of Ojuelos-Aguascalientes.

Findings/Conclusions: Mesquite plants from the Ojuelos-Aguascalientes subregion, Mexico, were identified and georeferenced; likewise, a methodology for its *in vitro* spread was developed.

Keywords: Microspread, pods, *Prosopis laevigata*, DNA, georeferencing.

Citation: Ortiz-Robles, M. D., Gómez-González, A., Flores-Benítez, S., Valera-Montero, L. L., Romero-Amador, L. D., Perales-Segovia, C., & Silos-Espino, H. (2022). Characterization and *in vitro* spread of mesquite (*Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M.C.Johnst.). *Agro Productividad*. https://doi.org/10.32854/agrop.v15i8.2345

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: April 11, 2022.
Accepted: July 19, 2022.
Published on-line: August 29, 2022.

Agro Productividad, 15(8). August. 2022. pp: 137-145.

INTRODUCTION

Mesquite (*Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M.C. Johnst.) belongs to the Fabaceae family and is a forest resource found in the arid and semi-arid zones of Mexico. It is threatened by irrational exploitation resulting in soil and drainage basin deterioration, as well as in the low food availability for wildlife (Rodríguez-Sauceda *et al.*, 2014). Eleven

Image by Michael at Pixabay

species of mesquites can be found in Mexico and *P. laevigata* is distributed in the north-central region of the country (Palacios, 2006). These species have different morphological and genetic characteristics (Luna-Castañón, 2014). Determining their genetic diversity and developing technology for their efficient management may allow a sustainable management of mesquite populations (Ríos-Saucedo *et al.*, 2011). Seeds are commonly used for its reproduction. *P. glandulosa* Torr. seeds have a rhomboid-flattened shape, are yellow-brown, measure 4.21×6.97 mm, have a 0.34 g average weight, a 62% viability, and 51% *in vivo* germination (Villareal-Garza *et al.*, 2013). Using the plant tissue culture technique, explants (stems) of *P. laevigata*, *P. glandulosa*, and *P. limensis* adult plants become necrotic and/or have a poor response; therefore, *in vitro* germination is preferred (Buendía-González *et al.*, 2007; Trejo-Espino *et al.*, 2011). Recently, *P. pallida* seeds subjected to a water immersion treatment at 80 ° C for 10 min recorded a 36.7% germination within 30 days, 2.4 shoots per axillary bud, 3.6 axillary nodes for each cultivated apical bud, 53.3% rooting of micro cuttings, and 100% acclimatized seedlings (Rivera-Curi *et al.*, 2020). Clearly, each species has specific morphological, genetic, and spread response behavior. Therefore, this work analyzed the pod characteristics and the DNA profile using RAPDs and tests were performed to establish the *in vitro* spread of mesquite from the Ojuelos-Aguascalientes subregion.

MATERIALS AND METHODS

Mesquite plants

The sampling was carried out in the subprovince of the Llanos de Ojuelos-Aguascalientes (Mexico's Mesa Central) identified as: typical plateau (43.77%), desert plain with rocky or cemented soil (22.86%), hillocks at the foot of mountains (16.30%), desert plain (14.20%), old alluvial hillocks (2.07%), and sierra with plateaus (0.80%) (INEGI, 2009). Mesquite plants adjacent to access roads were selected and georeferenced (Table 1).

Morphological analysis of pods

Fifty pods —which can be used for food— were collected per tree; then, length, diameter and weight were determined.

Genetic analysis of mesquite plants

The CTAB method (Doyle and Doyle, 1987) was used to extract DNA from fresh leaves. DNA quality was verified with agarose gel electrophoresis (1.5%), using 1X TBE buffer at 80 volts for 1.5 h, stained with ethidium bromide, and observed using an imaging system (BioDoc-ltTM Imaging System).The RAPD technique (Williams *et al.*, 1990) was used for the analyses, with 17, 10, 4, and 4 primers from the Operon A, B, F, and G series, respectively. The 12.5-μL reaction mixture consisted of 5 μL of sterile double-distilled water, 6.5 μL of reaction mixture (Gotaq® Green Master Mix), 0.5 μL of each primer (10 pmol), and 0.5 μL of genomic DNA (10 ng μL^{-1}). The terms of the reaction were the following: one cycle at 95 °C for 5 min; 40 cycles at 95 °C for 30 s, 36 °C for 30 s, 72 °C for 1 min; and one cycle at 72 °C for 4 min. For the amplification reactions, the Techne® Endurance TC-512 Gradient Thermal Cycler equipment was used. The

Table 1. Georeferenced location of mesquite plants in the Ojuelos-Aguascalientes subregion, Mexico.

Number	Community	Latitude (N)	Longitude (O)	Altitude (m)
1	Ojuelos de Jalisco	21° 52' 37.65"	101° 36' 19.45"	2237
2	Matancillas	21° 53' 16.02"	101° 38' 56.69"	2193
3	Chinampas	21° 47' 58.16"	101° 40' 36.79"	2098
4	La Presa	21° 46' 36.11"	101° 49' 49.38"	2094
5	La Troje	21° 45' 27.99"	101° 54' 27.22"	2069
6	Los Vergeles	21° 46' 08.56"	101° 56' 31.36"	2037
7	El Tepetatillo	21° 47' 21.18"	102° 00' 03.08"	2001
8	La Loma	21° 48' 47.25"	102° 04' 32.27"	1997
9	ITEL	21° 49' 13.75"	102° 06' 06.95"	2009
10	El Retoño	21° 50' 18.62"	102° 09' 15.15"	2013
11	Norias de Paso Hondo	21° 51' 18.64"	102° 12' 15.24"	1980
12	La Pona	21° 53' 18.93"	102° 16' 03.32"	1896
13	Pirules	21° 51' 45.65"	102° 19' 31.86"	1844
14	Isla San Marcos	21° 51' 46.66"	102° 19' 15.93"	1840
15	Ciénega Grande	22° 11' 24"	102° 01' 20"	2150
16	Los Campos	22° 01' 41"	101° 50' 34"	2110
17	Cerro de Montoro	22° 01' 29"	101° 49' 54"	2112
18	El Rusio (Los campos)	22° 01' 39"	101° 50' 44"	2110
19	Pabellón de Arteaga	22° 06' 17"	102° 18' 16"	1900
20	San Francisco de los Romo	22° 01' 18"	102° 13' 20"	1880

generated fragments (RAPD) were used to determine the profiles of the mesquite plants, a similarity matrix was calculated with the Bray-Curtis coefficient, and cluster analyzes were performed with the arithmetic mean method, using NTSYS pc 2.1 (Exeter Software, Setauket, New York, U S A) and Past 2.03.

In vitro spread procedure

Stem segments (1-2 cm) of 4-year old plants (established under nursery conditions) and seeds (mechanically scarified, 12-hour fungicide immersion), collected from plants analyzed in the morphological and genetic study were used, based on the following protocol: immersion in 3 g·Captan® L^{-1} for 30 min, 1.2 g·Benomyl® L^{-1} for 30 min, 0.05% colloidal silver for 10 min, 0.8% Cloralex® for 15 min, hydrogen peroxide (1:1, v:v) for 15 min, and finally 70% alcohol spray (at every change, the plants were rinsed with sterile distilled water). Stem explants were subjected to disinfectants, fungicides, and bactericides at different times and concentrations. The Murashige and Skoog (1962) basal medium was used with cefatoxime (350 mg·L^{-1}), growth regulators (BAP, 2,4-D, kinetin, and ANA) and antioxidants (silver nitrate). Then, 6.5 g of agar were added to the culture medium and it was sterilized at 121 °C for 15 min. Once the explants were established, they were transferred to the incubation room with temperatures from 20 to 28 ° C and a 16-h photoperiod of light. Rooted explants were established in previously sterilized substrate and placed under greenhouse conditions.

RESULTS AND DISCUSSION

Mesquite plants and their fruits

Twenty plants with clear morphological differences were chosen from nearby trees (height, diameter, leave and pod size, and growth pattern). In relation to the fruit (pods), the genotypes that stood out for their weight came from Ojuelos, Jalisco (570 g in 50 pods), while the tree of Los Vergeles from Lagos de Moreno, Jalisco stood out for its length and number of seeds per pod (18.88±2.50 cm and 24±1.62 seeds). The pods from Matancillas (Ojuelos, Jalisco) had a larger diameter (1.8±0.13 cm). The highest sugar concentration (° Brix) was found in the sample collected in La Presa (Lagos de Moreno Jalisco) (Table 2). Determining the mesquite pods characteristics allows us to infer their potential use as forages (Armijo-Nájera *et al*., 2019), timber, or as food for human consumption. For instance, García-López *et al*. (2019) reported a mean production of mesquite pod (*Prosopis laevigata*) of 3.7 ton·ha^{-1}, which was considered good, given the unfavorable precipitation and temperature conditions prevailing in the Altiplano Potosino.

Mesquite characterization by PCR-RAPDs

Out of the primers tested, only OPA16 (5'AGCCAGCGAA3) and OPG04 (5'AGCGTGTCTG3') were amplified (Figure 1A). Overall, fragments were amplified 250-

Table 2. Morphological characteristics of mesquite pods collected in Mexico's Mesa Central, Los Llanos de Ojuelos-Aguascalientes subprovince, Mexico.

Collection site	Weight of 50 pods (g)	Length (cm)	Diameter (cm)	Seeds per pod	°Brix
Ojuelos de Jalisco	570.0	17.6±1.80	1.7±0.15	20±1.33	15.5±1.3
Matancillas	433.8	14.2±1.86	1.8±0.13	18±1.93	27.0±1.3
Chinampas	294.2	16.2±1.85	1.3±0.23	16±2.94	18.0±1.1
La Presa	383.4	13.9±2.76	1.0±0.36	18±2.60	38.0±1.2
La Troje	120.2	9.4±1.75	1.3±0.45	9±2.77	20.0±0.8
Los Vergeles	272.2	18.8±2.50	1.6±0.19	24±1.62	25.0±1.8
La Loma	318.6	15.6±1.67	1.5±0.31	17±2.27	28.4±0.8
El Retoño	140.0	9.9±1.35	1.3±0.33	15±1.63	22.0±1.3
ITEL	290.6	15.7±1.66	1.0±0.30	15±1.94	17.5±0.7
La Pona	280.4	13.0±2.08	1.6±0.32	20±2.58	12.0±0.6
Norias de Paso Hondo	fruitless				
Isla San Marcos	269.0	18.2±1.95	1.5±0.32	20±1.58	11.5±0.1
Pirules	190.6	10.6±1.46	1.0±0.29	16±3.06	20.5±0.5
El Tepetatillo	294.4	18.0±1.33	1.4±0.47	19±1.60	21.5±0.6
Ciénega Grande	141.6	9.7±1.46	1.0±0.36	14±1.56	21.0±0.2
El Rusio (Los Campos)	290.0	15.3±2.04	1.3±0.35	21±1.79	25.0±0.9
Los Campos	235.0	12.0±1.18	1.2±0.40	14±1.70	25.5±0.5
Cerro de Montoro	250.4	14.8±0.94	1.0±0.36	20±1.89	20.5±0.9
Pabellón de Arteaga	290.6	15.03±2.05	1.4±0.36	18±3.40	19.0±0.4
San. Fco. de los Romo	395.0	17.9±1.25	1.5±0.25	22±1.33	7.0±0.3

2000 bp samples; La Loma and Norias de Paso Hondo specimens and Los Vergeles and El Retoño specimens showed an additional >2000 bp fragment with the OPA16 primer and the OPG04 primer, respectively. All the specimens coincided with two fragments: the first of 1000 and the second of approximately 1900 bp. A dendrogram was generated with the amplified fragments (Figure 1B) consisting of two large groups, distributed per agroecological region: one (A) that included three genotypes (Ojuelos de Jalisco, El Retoño, and El ITEL) and another composed of two subgroups (B) made up by seven genotypes (BI, Ciénega Grande, Chinampas, La Loma, Norias de Paso Hondo, Los Campos, and Cerro de Montoro) and eigh genotypes respectively (BII, Matancillas, El Rusio (Los Campos), Pirules, Los Vergeles, El Tepetatillo, San Marcos, La Pona, and La Presa). The Norias de Paso Hondo (fruitless) genotype showed an additional fragment that was not found in all

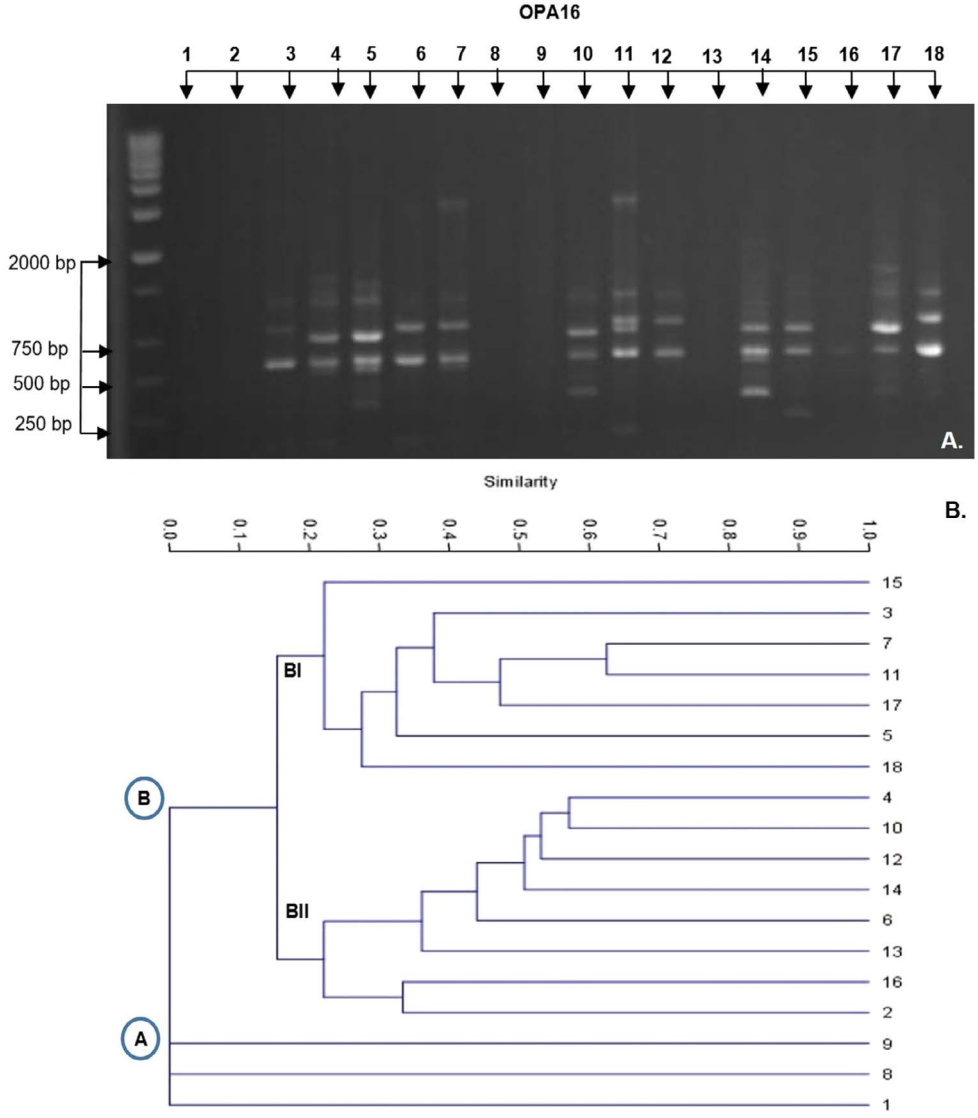

Figure 1. Amplification of DNA fragments by RAPDs in mesquite (*Prosopis laevigata*) from the plains of Ojuelos-Aguascalientes. A) Fragments with OPA16 and OPG04 primers. B) Corresponding dendrogram with groups A and B in blue circles.

other genotypes. Similarly, Luna-Castañón (2014) found a morphological difference in the same population (progenies vary in seedling height and the length-width of petiole, pinna, and pairs of leaflets), as well as a genetic difference between plants that are representative of the state of Aguascalientes. Morphometric and genetic diversity studies were carried out to identify salt tolerant *Prosopis* species (Roser *et al.*, 2014). The aim ot those studies was to design conservation and use strategies (Anand *et al.*, 2017) that help to identify threatened species (Moncada *et al.*, 2019; Contreras *et al.*, 2020) and to determine the species origin (Contreras-Negrete *et al.*, 2021). This study seeks to find mesquite genotypes with an outstanding characteristic, focusing on the fruit of wild plants.

Mesquite *in vitro* spread

The following asepsis protocol was used: Roma® detergent (5 g L^{-1}), 30% alcohol, Cloralex® (0.4%) and Cupravit® (3 g L^{-1}) for 5 min. As a result, an 85% asepsis was obtained from stem explants, which generated 5.6 (±1.5) shoots, as well as a 100% callus formation (Figure 2A) with MS (1962), with 2,4-D (3 g L^{-1}), ANA (2 g L^{-1}), and BAP (5 g L^{-1}). Unfortunately, explants became necrotic. With the *in vitro* seeds, 100% germination was achieved within 8 days (Figure 2B, 45-day plant).

The *in vitro* germinated explants also responded differently (Table 3), depending on the culture medium composition. In general, AIB generated oxidized callus, shoots, and roots. 2,4-D formed small necrotic calluses around the base. ANA induced an 80% callus formation, without shoots and roots. AG_3 with IBA promoted stem and shoot elongation, as well as root formation (Figure 2C). Regarding BAP, an 80% necrotic callus was formed and there was a low shoot formation and a lack of root formation (Figura 2D). Kinetin promoted callus (70%) and shoot formation (80%) but did not form roots. $AgNO_3$ prevented leaf fall (Figure 2E). Finally, the formed plants were established in soil and then transferred to greenhouse conditions (2F). The values obtained in this study are lower than those reported by Buendía González *et al.* (2007) for *P. laevigata* (MS with 2,4-D+BA); meanwhile Maldonado-Magaña *et al.* (2013) cultured cells in suspension with MS medium (1962) at half concentration with 2,4-D and kinetin. Minchala-Patiño *et al.* (2014) used the

Table 3. Behavior of mesquite explants in 100% MS to which growth regulators and antioxidant agents were added.

mg L^{-1}	Callus		Shoots		Root	
	(%)	Length (cm)	(%)	Length (cm)	(%)	Length (cm)
IBA (2)	100	3.29±1.90	43	1.45±2.43	33.25	0.26±0.20
2,4-D (3)	50	0.81±0.26	0.0	0.0	0.0	0.0
ANA (0.2)	100	0.7±0.2	0.0	0.0	0.0	0.0
AG_3 (Pri-vera)	40	0.74±0.33	27.5	1.65±1.2	0.0	0.0
BAP (X)	100	0.7±0.2	10	0.1±0.0	0.0	0.0
KIN (0.2)	100	0.6±0.2	40	0.4±0.2	0.0	0.0
KIN+AG_3 (2/4)	100	1.08±0.35	70	1.08±0.83	73.3	2.18±0.58
$AgNO_3$	100	0.58±0.3	45	0.4±0.3	86	1.5±0.9

Number of repetitions: 10.

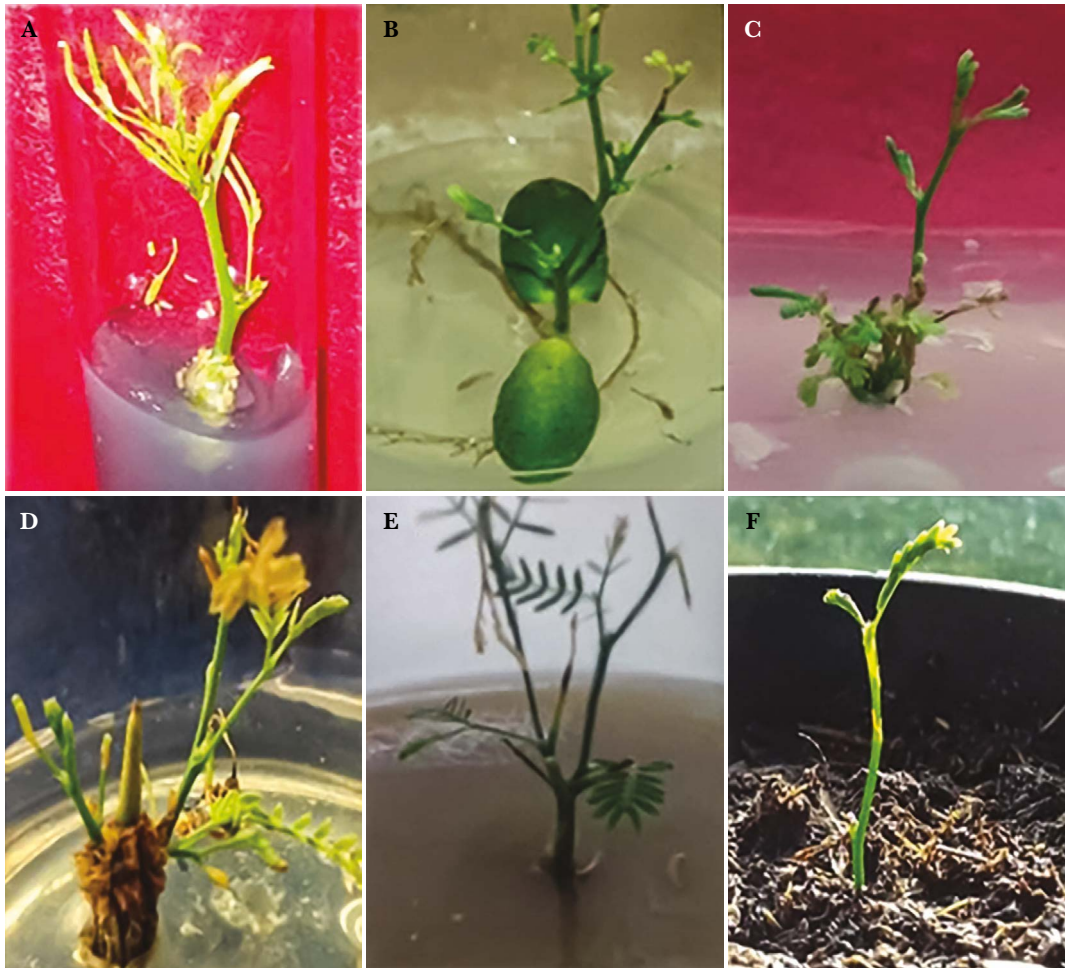

Figura 2. Evidence of the *in vitro* spread of mesquite (*Prosopis laevigata*) from the subprovince of Los Llanos de Ojuelos-Aguascalientes. A. Asepsis of stem explants generated shoots, as well as callus formation. B. *In vitro* seed germination. C. AG_3 with IBA elongated stems and shoots and formed roots. D. BAP formed necrotic callus, low shoot formation, and no root formation. E. $AgNO_3$ prevented leaf fall.

kinetin+adenine sulfate combination at a rate of 1 and 25 $g \cdot L^{-1}$. Recently, Rivera-Curi *et al.* (2020) obtained good results (number of shoots and roots) with the *P. pallida* species in a cytokinin-free medium, putting cotton on the lid to remove ethylene; for its part, 0.5 mg IBA L^{-1} was enough to achieve rooting.

CONCLUSIONS

In this research, mesquite (*P. laevigata*) genotypes were characterized based on fruit size and DNA profile grouped by RAPDs, obtaining a methodology for *in vitro* spread. The Ojuelos of Jalisco, Los Vergeles, Matancillas, and La Presa genotypes were remarkable. The genetic profile grouped the individuals according to the proximity and/or orographic physical barrier of the studied subprovince. Within *in vitro* spread, AG_3 with IBA resulted in the elongation of stems, shoots, and roots, while $AgNO_3$ prevented leaf fall, achieving rooting and transfer to soil. Therefore, we infer that, even though mesquite populations

belong to "the same species", the cross-pollination process enables a great interaction between individuals in their natural environment.

REFERENCES

Anand, S. S., Thakur, S., Gargi, M., Choudhary, S., Bhardwaj, P. (2017). Development and characterization of genomic microsatellite markers in *Prosopis cineraria*. *Current Plant Biology*, (9-10), 37-42. Doi: 10.1016/j.cpb.2017.03.001

Armijo-Nájera, M.G., Moreno-Reséndez, A., Blanco-Contreras, E., Borroel-García, V. J., Reyes-Carrillo, J. L. (2019). Vaina de mezquite (*Prosopis* spp.) alimento para el ganado caprino en el semidesierto. *Revista Mexicana de Ciencias Agrícolas, 10*(1), 113-122. Doi: 10.29312/remexca.v10i1.1728

Buendía-González, L., Orozco-Villafuerte, J., Cruz-Sosa, F., Chávez-Ávila, V. M., Vernon-Carter, E. J. (2007). Clonal propagation of mesquite tree (*Prosopis laevigata* Humb. & Bonpl. ex Willd. M.C. Johnston). I. Via cotyledonary nodes. *In Vitro Cellular Devlopment Biology,43*(3),260–266.Doi:10.1007/s11627-007-9027-8

Contreras-Negrete, G., Letelier-Gálvez, L.E.A., Piña-Torres, J., González-Rodríguez, A. (2021). Genetic structure, phylogeography and potential distribution modeling suggest a population expansion in the mesquite *Prosopis laevigata* since the last interglacial. *Plant Systematics and Evolution, 307*(2). Doi: 10.1007/s00606-021-01744-5.

Contreras, R., van den Brink, L., Burgos, B., González, M., Gacitúa, S. (2020). Genetic characterization of an endangered Chilean endemic species, *Prosopis burkartii* Muñoz, reveals its hybrids parentage. *Plants, 9*(6),1-29. Doi:10.3390/plants9060744.

Doyle, J. J., Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin, 19*(1), 11-15.

García-López, J. C., Vicente-Martínez, J. G., Rendón-Huerta, J. A., Ruiz-Tavares, D., López-Aguirre, S., Lee-Rangel, H. A., Pinos-Rodríguez, J. M., Álvarez-Fuentes, G., de-Nova, J. A., Durán-García, H. M., Salinas-Rodríguez, M. (2019). Producción y contenido nutrimental de vainas de tres variantes de mezquite (*Prosopis laevigata*) en el Altiplano Potosino, México. *Agrociencia, 53*(6), 821-831.

INEGI. (2009). Prontuario de información geográfica municipal de los Estados Unidos Mexicanos. Instituto Nacional de Estadística y Geografía. Disponible en: https://docplayer.es/96682324-Prontuario-de-informacion-geografica-municipal-de-los-estados-unidos-mexicanos-ojuelos-de-jalisco-jalisco-clave-geoestadistica-14064.html

Luna-Castañón, R. (2014). Variabilidad morfológica y genética del mezquite *Prosopis laevigata* (Humb. & Bonpl. ex Willd.) en el Estado de Aguascalientes. Tesis de Maestría, Universidad Autónoma de Aguascalientes, Aguascalientes, Aguascalientes, Mexico. 90 p.

Maldonado-Magaña, A., Orozco-Villafuerte, J., Buendía-González, L., Estrada-Zúniga, M. E., Bernabé-Antonio, A., Cruz-Sosa, F. (2013). Establecimiento de cultivos de células en suspensión de *Prosopis laevigata* (Humb. & Bonpl. ex willd)

M.C. Johnst para determinar el efecto del zinc en la absorción y acumulación de plomo. *Revista Mexicana de Ingeniería Química,12*(3), 489-498.

Minchala-Patiño, J., Poma-Angamarca, R., Muñóz-Chamba, L., Yaguana- Arévalo, M., González-Zaruma, D., Eras-Guamán, V. H., Rojas-Idrogo, C., Delgado-Paredes, G. E. (2014). Propagación *in vitro* de *Prosopis limensis* Benth. in Hook. (Fabaceae-Mimosoideae). *Quebracho, 22*(2), 88-99.

Moncada, X., Plaza, D., Stoll, A., Payacan, C., Seelenfreund, D., Martínez, E., Bertin, A., & Squeo, F. A. (2019). Genetic diversity and structure of the vulnerable species *Prosopis chilensis* (Molina) Stuntz in the Coquimbo Region, Chile. *Gayana Botanica, 76*(1), 91-104. Doi: 10.4067/S0717-66432019000100091

Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologic Plantarum, 15*(3). 437-497. Doi: 10.1111/J.1399-3054.1962.TB08052.X

Palacios, R. A. (2006). Los mezquites mexicanos: Biodiversidad y distribución geográfica. *Boletin de la Sociedad Argentina de Botánica, 41*(1-2), 99-121.

Ríos-Saucedo, J. C., Trucíos-Caciano, R., Valenzuela-Núñez, L. M., Sosa-Pérez, G., Rosales-Serna, R. (2011). Importancia de las poblaciones de mezquite en el Norte-Centro de México. INIFAP-SAGARPA: Distrito Federal, México. 230 p.

Rivera-Curi, J. C., Cabrera-Pintado, R. M., Bulnes-Soriano, F. (2020). Micropropagación de *Prosopis pallida* (Humb &Bonpl. Ex Willd.) Kunth a partir de yemas apicales. *Revista Colombiana de Biotecnología, 22*(1), 18-26. Doi: 10.15446/rev.colomb.biote.v22n1.70949

Rodríguez-Sauceda, E. N., Rojo-Martínez, G. E., Ramírez-Valverde, B., Martínez-Ruiz, R., Cong-Hermida, M. C., Medina-Torres, S. M., Piña-Ruiz, H. H. (2014). Análisis técnico del árbol del mezquite (*Prosopis laevigata* humb. & bonpl. ex willd.) en México. *Ra Ximhai, 10*(3), 173-193.

Roser, L. G., Ferreyra, L. I., Ewens, M., Vilardi, J. C., Saidman, B. O. (2014). Genetic and morphometric characterization of clones of *Prosopis alba*, Algarobia, selected for salt tolerance. *Tree Genetics & Genomes, 10*(2), 383–397. Doi: 10.1007/s11295-013-0693-x

Trejo-Espino, J. L., Rodríguez-Monroy, M., Vernon-Carter, E. J., Cruz-Sosa, F. (2011). Establishment and characterization of *Prosopis laevigata* (Humb. & Bonpl. ex Willd) M.C. Johnst. cell suspension culture: A biotechnology approach for mesquite gum production. *Acta Physiologiae Plantarum, 33*(5), 1687-1695. Doi: 10.1007/s11738-010-0705-5

Villarreal-Garza, J. A., Rocha-Estrada, A., Cárdenas-Ávila, M. L., Moreno-Limón, S., González-Álvarez, M., Vargas-López, V. (2013). Caracterización morfométrica, viabilidad y germinación de semillas de mezquite y huizache en el noreste de México. *Phyton. 82*(2), 169-174.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research, 18*(22), 6531-6535. Doi: 10.1093/nar/18.22.6531