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AP

Suspension of irrigation during the maturation phase of (*Saccharum* spp.) Sugarcane cultivation

pág. 59

Año 14 • Volumen 14 • Número 11 • noviembre, 2021

Morphological characterization of *Moringa oleifera* seeds from different crops of Mexico 3

Effect of grasshopper (*Sphenarium purpurascens* Charpentier) paste addition on rheological behavior of Mole Poblano 11

Lippia graveolens (Lamiales: Verbenaceae) and *Oryganum vulgare* (Lamiales: Lamiaceae) obtained by means of the *in vitro* propagation technique 19

Effect of plant growth promoting rhizobacteria (PGPR) on the growth of chili habanero pepper (*Capsicum chinense* Jacq.) 29

Characterization of Persian lime production (*Citrus* × *latifolia*; Tanaka ex Q. Jiménez) 37

Determination of the point of zero charge of pineapple (*Ananas comosus* L.) peel and its application as copper adsorbent 45

y más artículos de interés...

Colegio de Postgraduados

Contenido

Año 14 • Volumen 14 • Número 11 • noviembre, 2021

3	Morphological characterization of *Moringa oleifera* seeds from different crops of Mexico
17	Effect of grasshopper (*Sphenarium purpurascens* Charpentier) paste addition on rheological behavior of Mole Poblano
25	*Lippia graveolens* (Lamiales: Verbenaceae) and *Oryganum vulgare* (Lamiales: Lamiaceae) obtained by means of the *in vitro* propagation technique
35	Effect of plant growth promoting rhizobacteria (PGPR) on the growth of chili habanero pepper (*Capsicum chinense* Jacq.)
43	Characterization of Persian lime production (*Citrus* × *latifolia*; Tanaka ex Q. Jiménez)
53	Determination of the point of zero charge of pineapple (*Ananas comosus* L.) peel and its application as copper adsorbent
59	Suspension of irrigation during the maturation phase of sugarcane (*Saccharum* spp.) cultivation
69	Morphometric characteristics and seed germination of *Dalbergia granadillo* Pittier
79	Ecophysiology and nutrition of cabezona pineapple (*Ananas comosus* L. Merril) in Chontalpa, Tabasco, Mexico
89	Reproductive aspects of the female jaguar (*Panthera onca* L.)
99	Use of agricultural technology, yields and economic profitability of small-scale producers (*Coffea arabica* L.)
107	*Trichoderma harzianum in vitro* mycoparasitism on *Peronospora belbahrii* in basil (*Ocimum basilicum*)
115	Greenhouse cultivation of Serrano pepper (*Capsicum annuum* var. Acuminatum): dry hydroponics
123	Long-term response planting method on wheat under conservation agriculture
133	Yield and forage quality in maize (*Zea mays* L.) inbred lines
141	Native palms as an economically important non-timber forest product among rural communities in the Yucatán peninsula, Mexico
149	Evaluation of different antioxidants during *in vitro* establishment of allspice (*Pimenta dioica* L. Merrill): a recalcitrant species
159	Diversity of VAM in soils used to cultivate sugarcane (*Saccharum* spp.) in Tabasco, Mexico
169	Optimal fertilization dose in castor bean (*Ricinus communis* L.) using partial budgets analysis
177	Productive and technological characteristics of goat farmers in Comondú, Baja California Sur

Bases de datos de contenido científico

Directorios

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Morphological characterization of *Moringa oleifera* seeds from different crops of Mexico

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ABSTRACT

Objective: to characterize the seeds of *Moringa oleifera* present in various crops in Mexico morphologically.

Design/Methodology/Approach: An analysis of variance, principal components, and conglomerates of qualitative morphological descriptors (shape, color, presence of wings and wing color) and quantitative (length, width, and weight) were carried out.

Results: Significant statistical differences ($P<0.05$) were found in the seeds' length, width, weight, and almonds. The principal component analysis indicated that components 1 (70.58%) and 2 (25.59%) contributed 96.17% of the variation, and the cluster analysis identified four groups. The evaluated populations showed qualitative variation (shape, color, presence of wings, and color of wings) and quantitative (length, width, and weight).

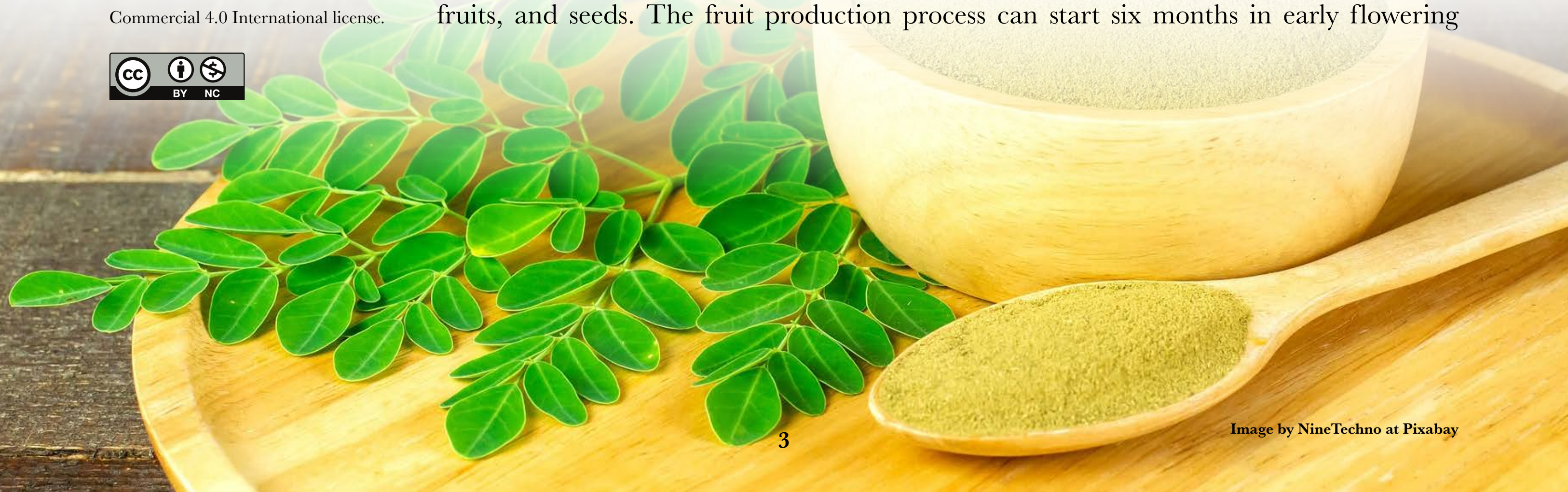
Study limitations/implications: the germination percentage could not be evaluated because no information was obtained on the age of the seed and the storage conditions.

Result/Finding/Conclusion: This information enriches the knowledge of *Moringa oleifera* in Mexico and serves as a basis for selecting materials of most significant interest.

Keywords: characterization, seed, *Moringa oleifera*.

INTRODUCTION

Moringa oleifera Lam. is a species that is currently cultivated in different types of ecosystems worldwide. It tolerates high temperatures, low humidity conditions, and soils with low nutrients (Paliwal *et al.*, 2011). It presents high production of biomass, flowers, fruits, and seeds. The fruit production process can start six months in early flowering



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varieties and more than one year in late varieties (Leone *et al.*, 2015). In addition, flowering is also influenced by different humidity levels in the soil (Muhl *et al.*, 2011). It has been reported that it can flower once or twice a year, and in optimal humidity conditions, it can produce fruits throughout the year (Zaku *et al.*, 2015). Fruit production can reach up to 411 plant^{-1} fruits with a yield of 51.3 t ha year^{-1} (Ndubuaku *et al.*, 2014). The fruits can be 30 to 120 cm long (Paliwal *et al.*, 2011), have a diameter of 1.49 to 2.55 cm, and have 12 to 23.5 seeds (Zhigila *et al.*, 2015). The tender fruits are green and pale green, ripe brown, dark brown, or golden (Ramos *et al.*, 2010; Popoola *et al.*, 2016). The seeds can be oval, round, or triangular, brown or dark brown, and with or without the presence of wings. The length of the seed can be from 1.0 to 1.5 cm, with 0.8 to 1.1 cm wide (Zhigila *et al.*, 2015). The weight of the seed fluctuates between 0.101 and 0.274 g (Shaltout, 2017). Each moringa tree could produce between 15,000 and 25,000 seeds plant^{-1} (Makkar & Becker, 1997). These seeds contain a high content of oil, protein, and chemical compounds that can be used for flocculation, elimination of heavy materials in the water, biodiesel production, and prevention of chronic diseases such as obesity, diabetes, hypertension, and cancer (Pirrò *et al.*, 2016).

Studies previously carried out by Ramos *et al.* (2010), Zhigila *et al.* (2015), and Popoola *et al.* (2016) showed that there is a high degree of morphological variation in moringa seed and environmental and edaphic factors modulate the diversity. In Mexico, there is little information about the degree of morphological variation in moringa seeds. This lack of information limits the development of projects focused on the integral use of the seed and the genetic improvement of the existing populations. Therefore, the objective of this research was to determine the morphological diversity of *M. oleifera* seeds from different crops in Mexico.

MATERIALS AND METHODS

During the months of May and June 2021, moringa seeds were collected from 22 crops located in the states of Chiapas, Guanajuato, Guerrero, Hidalgo, Michoacán, Nuevo León, Oaxaca, San Luis Potosí, Veracruz and Yucatán (Table 1, Figure 1).

Edaphology, climatology, vegetation, and land use files were downloaded from the CONABIO pages (http://www.conabio.gob.mx/informacion/gis/) and UNIATMOS (http://uniatmos.atmosfera.unam.mx/) to determine the climatic and edaphic variables.

The ArcGIS version 10.5 was used to obtain the variables that influence the morphology of moringa seeds. Table 2 shows the minimum, average, and maximum temperature values, annual precipitation, type of climate, altitude, humidity range, dominant soil, and soil moisture regime in each geographical area sampled.

Biological material

Five hundred grams of moringa seeds were collected to determine the morphological descriptors; 75 healthy seeds were randomly selected from each sampled crop. Quantitative descriptors: The length and width of the seeds were obtained with a vernier. The individual weight, almond weight, and weight of 100 seeds were quantified with an analytical balance of the Ohaus Adventurer® brand. Quantitative descriptors: the variables of seed color,

Table 1. Geographical locations of the *M. oleifera* crops evaluated.

Crop	State	Municipality	Community	Longitude	Latitude
CHI1	Chiapas	Tuzantán	Villa Hidalgo	−92.374722	15.108056
CHI2	Chiapas	Tuxtla Gutiérrez	Colonia La Salle	−93.0868889	16.7429444
GRO1	Guerrero	Acapulco de Juárez	Bejuco	−99.6977778	16.8216667
GRO2	Guerrero	Acapulco de Juárez	Parrotillas	−99.61558371	16.8787834
GRO3	Guerrero	Acapulco de Juárez	Concepción	−99.66028879	16.8799601
GRO4	Guerrero	Tecpán de Galeana	Mitla	−99.89343517	16.87894246
GTO1	Guanajuato	Soledad de Gasca	Celaya	−100.8146904	20.502528
HGO1	Hidalgo	San Felipe Orizatlán	Ahuatitla	−98.6660845	21.1630165
MICH1	Michoacán	Benito Juárez	El Rodeo	−100.4708226	19.3055772
MICH2	Michoacán	Múgica	Múgica	−102.180997	18.928047
NL1	Nuevo León	Escobedo	Francisco I. Madero	−100.2847444	25.7854473
OAX1	Oaxaca	Santa Cruz Xoxocotlán	San Juan Bautista	−96.7280556	16.9791667
OAX2	Oaxaca	Santa María Huatulco	La Herradura	−96.3658333	15.7772222
OAX3	Oaxaca	Mariscala de Juárez	Guadalupe la Huertilla	−98.1088889	17.8513889
OAX4	Oaxaca	Tuxtepec	San Juan Bautista	−96.1286697	18.087694
SLP1	San Luis Potosí	Tanlajas	Guayajox	−98.73666667	21.7144444
VER1	Veracruz	Soledad de Doblado	El Progreso	−96.4022719	19.0818742
VER2	Veracruz	Paso del Macho	Loma Pelada	−96.5398368	18.9258796
YUC1	Yucatán	Tzucacah	Tzucacah	−89.0391111	20.0720278
YUC2	Yucatán	Mérida	Frac. el Parque	−89.5872222	20.9711111
YUC3	Yucatán	Peto	Teshan	−88.62125	20.1486389
YUC4	Yucatán	Baca	Felipe Carrillo Puerto	−89.60700993	20.9954688

wing color, presence of wings, the fragility of the wings, and hardness of the seeds were determined.

Statistical analysis

An analysis of variance was performed to identify significant differences ($P<0.05$) among the states where seeds were collected, and then the Tukey test ($P<0.05$) was performed for the quantitative descriptors using the Infostat version 2020e software. The correlation coefficient, principal component analysis, and cluster analysis were performed through hierarchical clustering with a coefficient of variation in the unweighted pair group (Euclidean distance). The two-dimensional scatter diagram was performed using the percentage of variation in the first two principal components. The PAST 3.0 software was used.

RESULTS AND DISCUSSIONS

Climatic variables

An average temperature of 24.71 °C was identified, with a minimum of 10.46 °C and a maximum of 34.80 °C. Moringa cultures tolerate temperatures of 12 to 48 °C (Roloff *et*

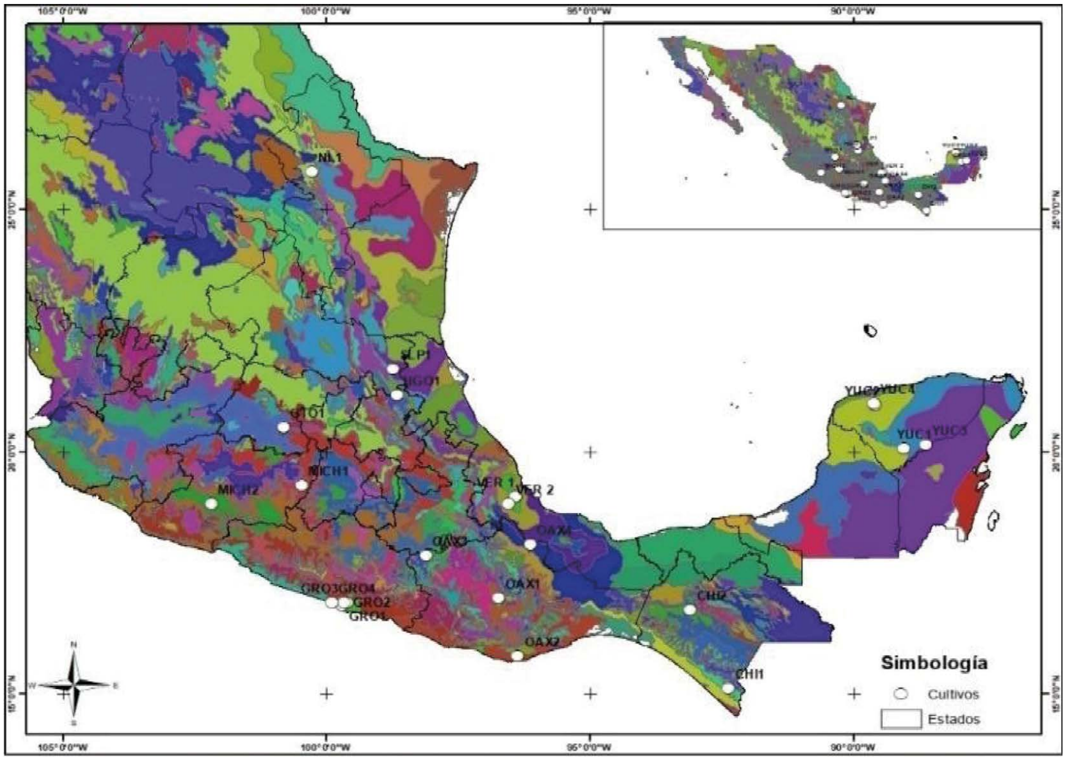

Figure 1. The geographical location of *M. oleifera* crops sampled. The types of climates present in the Mexican Republic are observed.

al., 2009). However, temperatures below 16 °C affect pollen viability (Radice & Giordani, 2018) and lower than 10 °C prevent flowering; therefore, they require a range of 25 to 35 °C to guarantee the production of biomass and fruits (Pandey *et al.*, 2011). Moringa responds positively under this temperature range because winter is not so cold and favors flowering and seed production (Adebayo *et al.*, 2011). The minimum and maximum precipitation regimes identified were 521 and 3005 mm year^{-1}. These values are within the range (250 to 2000 mm per year) for its establishment (Adikuru *et al.*, 2011). However, the water requirement is a function of each phenological stage of the plant, being 1000 mm the optimal precipitation for seed production (Olson and Alvarado, 2016). The climate with the most significant predominance in the sampled crops was the Awo, which is warm-humid with an average annual temperature greater than 22 °C and the humidity range most frequent was subhumid (w1). Muhl *et al.* (2011) mention that moringa predominates in sub-humid tropical climates because it is the most recommended for production purposes.

Regarding altitude, the minimum value identified was 11m, and the maximum was 1,755 m. Olson and Alvarado (2016) pointed out that crop performance decreases as altitude increases being 500 m the maximum altitude for the establishment of crops (Velázquez-Zavala *et al.*, 2016). Despite having excellent acclimatization capacity, factors such as precipitation, humidity range, wind speed, and altitude affect morphological variations in moringa (Martínez *et al.*, 2014). Förster *et al.* (2015) mentioned that environmental factors influence morphometric traits, phenology, and production. Ledea-Rodríguez *et al.* (2018)

Table 2. Climatic data of the *M. oleifera* crops sampled.

Crop	Meant* (°C)	Mint (°C)	Maxt (°C)	Rainfall (mm)	Climate type	Humidity	Altitude (m)	Dominant soil	Soil moisture regime
CHI1	27.03	20.69	33.37	3005	Am	humid (m)	169	Acrisol	Udico 270 to 330 DDH*
CHI2	25.32	18.84	31.80	985	Awo	Subhumid (w0)	529	Leptosol	Xerico 90 to 180 DDH
GRO1	26.63	20.83	32.43	1207	Awo	Subhumid (w1)	30	Arenosol	Xerico 90 to 180 DDH
GRO2	26.85	20.78	32.92	1162	Awo	Subhumid (w1)	39	Arenosol	Xerico 90 to 180 DDH
GRO3	26.78	20.81	32.74	1179	Awo	Subhumid (w1)	56	Arenosol	Xerico 90 to 180 DDH
GRO4	26.22	20.88	31.56	1253	Aw1	Subhumid (w1)	217	Arenosol	Xerico 90 to 180 DDH
GTO1	18.74	10.46	27.02	638	BS1hw	Semiarid (BS1)	1755	Vertisol	Ustico 180 to 270 DDH
HGO1	23.45	17.80	29.10	1737	A(f)	Humid (m)	249	Regosol	Udico 270 to 330 DDH
MICH1	21.81	14.38	29.23	961	Aw1	Subhumid (w1)	1196	Feozem	Udico 270 to 330 DDH
MICH2	27.29	19.77	34.80	717	BS1(h')w	Árid (BS0)	265	Vertisol	Xerico 90 to 180 DDH
NL1	21.83	15.18	28.48	521	BS1hw	Semiarid (BS1)	478	Vertisol	Xerico 90 to 180 DDH
OAX1	20.26	11.99	28.53	688	BS1(h')w	Subhumid (w0)	1518	Regosol	Ustico 180 to 270 DDH
OAX2	25.86	19.38	32.34	1119	Awo	Subhumid (w0)	183	Arenosol	Aridico less than90 DDH
OAX3	23.92	15.59	32.25	699	BS1(h')w	Subhumid (w0)	1102	Leptosol	Xerico 90 to 180 DDH
OAX4	24.91	19.86	29.96	2242	Am	Humid (m)	32	Cambisol	Udico 270 to 330 DDH
SLP1	24.32	18.47	30.16	1316	Aw1	Subhumid (w1)	98	Vertisol	Ustico 180 to 270 DDH
VER 1	25.23	19.53	30.93	1143	Awo	Subhumid (w1)	68	Vertisol	Xerico 90 to 180 DDH
VER 2	24.72	19.07	30.37	1144	Awo	Subhumid (w1)	216	Vertisol	Ustico 180 to 270 DDH
YUC1	25.53	18.44	32.62	1085	Awo(x')	Subhumid (w0)	36	Luvisol	Xerico 90 to 180 DDH
YUC2	25.78	19.09	32.47	1053	Awo	Subhumid (w0)	11	Leptosol	Aridico less than90 DDH
YUC3	25.34	18.46	32.22	1178	Aw1(x')	Subhumid (w1)	29	Leptosol	Xerico 90 to 180 DDH
YUC4	25.79	19.15	32.44	1030	Awo	Subhumid (w0)	12	Leptosol	Aridico less than90 DDH

T*: temperature, DDH*: number of humid days.

highlighted the importance of knowing the environmental conditions when studying aspects of the moringa seed, mainly to guarantee seed production under temperature conditions and optimal irrigation.

Quantitative descriptors. Significant statistical differences ($P<0.05$) were obtained in the seed length of the 22 populations evaluated (Table 3). The OAX1 and CHI2 populations obtained 0.98 and 1.54 cm, respectively. The value of the CHI2 population is similar to the 1.5 cm reported by Zhigila *et al.* (2015), where he evaluated 30 accessions of moringa and higher than the 1.03 of Ramos *et al.* (2010) and 1.16 of López *et al.* (2018). Significant differences ($P<0.05$) were found in the width of the seed, and the range was 0.74 (VER2) to 1.11 cm (VER1) between the evaluated populations. These values are lower than the minimum (0.88 cm) and maximum (1.11 cm) reported by Zhigila *et al.* (2015) and similar to the 1.00 cm of Ramos *et al.* (2010) and 1.09 cm from López *et al.* (2018).

Significant differences ($P<0.05$) in length, wide and weight were observed among the evaluated materials. The 0.420 g observed in CHI2 is higher than the 0.300 g of Makkar and Becker (1997), 0.280 of López *et al.* (2018), 0.197 from Ramos *et al.* (2010), 0.274 g from Shaltout *et al.* (2017), and 0.305 from Popoola *et al.* (2016). Bezerra *et al.* (2004) mentioned that heavier seeds produced vigorous seedlings, and medium-weight seeds showed higher growth speed. This characteristic allows discarding materials of lower weight and thus guarantee each individual's gemination and survival processes (Popoola *et al.*, 2016). The quantitative variation identified in the seed weights it is the product of each crop's environmental effect and agronomic management, in addition to the individual potential of each genetic material modulated by environmental conditions (Ledea-Rodríguez *et al.*, 2018). The average weight of 100 seeds was 29.08 g with a minimum and a maximum of 21.29 and 38.60 g. The average value is similar to the 29.94 g reported by López *et al.* (2018) and less than 30.59 g of (Popoola *et al.*, 2016). The almond weight percentage related to the total weight was in the range of 62.63 and 77.02% for the YUC1 and GRO4 populations, respectively. Makkar and Becker (1997) mentioned that the almond occupies 75% of the total weight of the seed, and Valdés *et al.* (2018) identified that the almond occupies 71% of the total weight of the seed. This attribute indicates that the seed can be favored in the germination process and serve as an elite material for improvement.

Correlation analysis. The strongest positive correlation was observed between the mean weight of 100 seeds, seed weight and Almond weight (%), the length and weight of the seed, and seed weight and almond weight ($r=0.84$) (Table 4). A correlation of $r=0.41$ was found between the mean temperature and the weight of the seed. Moreover, a correlation of $r=0.43$ between the maximum temperature and the weight of the seed. The multiple introductions of moringa material in the country, the genetic diversity, and its high tolerance to different types of stress may be influencing this low relationship (Olson and Fahey, 2011; Tian *et al.*, 2015). Dao and Kabore (2015) mentioned the non-existence of a significant correlation between geographic location, altitude, and precipitation on moringa morphology. However, this aspect can be clarified by evaluating the same material in different environments.

Table 3. Morphometry of *M. oleifera* seeds from different crops in Mexico.

Crop	Length(cm)	Width (cm)	Weight(g)	Weight of 100 seeds(g)	Almond weight (g)	Almond Weight (%)
CHI1	1.32^{G} ± 0.02	1.02^{HIJ} ± 0.01	0.380^{J} ± 0.01	37.96	0.290	70.65
CHI2	1.54^{H} ± 0.02	1.07^{JK} ± 0.01	0.420^{K} ± 0.01	38.60	0.320	70.36
GRO1	1.02^{ABC} ± 0.02	0.87^{BC} ± 0.01	0.240^{A} ± 0.01	22.55	0.190	74.41
GRO2	0.99^{AB} ± 0.02	0.85^{B} ± 0.01	0.240^{A} ± 0.01	21.29	0.180	72.88
GRO3	1.00^{AB} ± 0.02	1.07^{JK} ± 0.01	0.310^{EFG} ± 0.01	29.56	0.250	72.16
GRO4	1.17^{F} ± 0.02	1.06^{J} ± 0.01	0.370^{J} ± 0.01	35.68	0.310	77.02
GTO1	1.04^{ABC} ± 0.02	0.91^{CD} ± 0.01	0.250^{AB} ± 0.01	24.75	0.200	70.24
HGO1	1.04^{ABC} ± 0.02	0.88^{BCD} ± 0.01	0.250^{AB} ± 0.01	24.49	0.230	74.79
MICH1	0.99^{AB} ± 0.02	0.89^{BCD} ± 0.01	0.250^{AB} ± 0.01	22.99	0.190	69.15
MICH2	1.16^{EF} ± 0.02	0.98^{FGH} ± 0.01	0.340^{HI} ± 0.01	31.53	0.260	72.74
NL1	1.10C^{DEF} ± 0.02	0.93^{DEF} ± 0.01	0.290^{CDE} ± 0.01	26.76	0.200	71.79
OAX1	0.98^{A} ± 0.02	0.89^{BCD} ± 0.01	0.260^{ABC} ± 0.01	26.19	0.230	74.93
OAX2	1.14^{DEF} ± 0.02	1.00^{GHI} ± 0.01	0.320^{FGHI} ± 0.01	29.70	0.240	71.28
OAX3	1.30^{G} ± 0.02	1.03I^{J} ± 0.01	0.320^{EFGH} ± 0.01	29.64	0.220	69.82
OAX4	1.03^{ABC} ± 0.02	0.91^{CD} ± 0.01	0.260^{AB} ± 0.01	27.24	0.260	76.21
SLP1	1.06^{BC} ± 0.02	0.92^{DE} ± 0.01	0.270^{BCD} ± 0.01	27.08	0.220	71.95
VER1	1.31^{G} ± 0.02	1.11^{K} ± 0.01	0.380^{J} ± 0.01	37.71	0.290	70.4
VER2	0.97^{A} ± 0.02	0.74^{A} ± 0.01	0.300^{DEF} ± 0.01	30.30	0.240	70.4
YUC1	1.07^{BCD} ± 0.02	1.07^{JK} ± 0.01	0.280^{BCD} ± 0.01	27.41	0.160	62.63
YUC2	1.00^{AB} ± 0.02	0.90^{CD} ± 0.01	0.260^{AB} ± 0.01	25.12	0.210	74.99
YUC3	1.09^{CDE} ± 0.02	0.97^{EFG} ± 0.01	0.340^{GHI} ± 0.01	30.67	0.240	69.89
YUC4	1.15^{EF} ± 0.02	1.00^{GHI} ± 0.01	0.350^{IJ} ± 0.01	32.49	0.260	70.61

CHI: state of Chiapas, GRO: state of Gerrero, GTO: state of Guanajuato, HGO: state of Hidalgo, MICH: state of Michoacan, NL: state of Nuevo Leon, OAX: state of Oaxaca: SLP: state of San Luis Potosi, VER: state of Veracruz, YUC: state of Yucatan; 1,2,3,4: collection sites within each mexican state; ±: estandar error.

Table 4. Correlation matrix of the quantitative descriptors of *M. oleifera* seeds.

	Seed length (cm)	Seed width (cm)	Seed weight (g)	Almond weight (%)	Mean temperature (°C)	Minimum temperature (°C)
Seed width(cm)	0.68*					
Seed weight(g)	0.85*	0.7*				
Almond weight (%)	0.67*	0.48*	0.84*			
Mean weightof 100 seeds(g)	0.81*	0.68*	0.97*	0.88*		
Altitude (m)					−0.85*	-0.92*

*: P-value <0.05.

Qualitative descriptors. Three forms of seed were identified in the evaluated populations: triangular, spherical, and oval. Four colors in the seeds were observed: light brown, brown, dark brown, and black, with wings and semi-winged (Table 5).

A higher frequency of spheric and oval shape seeds was identified. The triangular shape seeds were identified only in four populations. Zhigila *et al.* (2015) identified oval and isometric seeds with predominant wings, while Makkar and Becker (1997) mentioned that the seeds are spheric with a semi-permeable layer of brown to black color with three white wings spaced at 120°. In this investigation, a higher frequency of brown and dark brown seeds was found. However, other works have reported dark brown (Palada, 1996), brown (Makkar and Becker, 1997; Ramos *et al.*, 2010), brown to black (Parrotta, 2009), pale yellow to creamy, and white seeds (Anwar *et al.*, 2006: Anwar and Rashid, 2007). Makkar and Becker (1997) mentioned that white seeds have low viability, representing a negative aspect of germination. White wings were identified in most of the evaluated crops; the above is in line with the results of Zhigila *et al.* (2015) when identifying the presence of white wings in their evaluation. Ramos *et al.* (2010) mentioned that the wings were light brown.

Principal component analysis

The principal component analysis demonstrated the morphological variability of the seed in the 22 moringa populations (Table 6).

In the dispersion figure of components, four groups were observed. Group 1 integrated the GRO1, GRO2, GTO1, HGO1, MICH1, NL1, OAX1, OAX4, SLP1, and YUC2. Group 2 integrated the GRO3, MICH2, OAX2, OAX3, VER2, YUC3, and YUC4 crops. In group 3, only the YUC1 crop was included, and group 4 included GRO4, CHI1, CHI2, and VER1 cultures (Figure 2).

Cluster analysis

The cluster analysis identified four groups. Group 1 integrated subgroup A (GTO1 and MICH1) and subgroup B (HGO1, YUC2, OAX1, OAX4, NL1, SLP1, GRO1, and GRO2).

Table 5. Qualitative descriptors were used to evaluate the morphological diversity in *M. oleifera* seeds.

<table>
<tr><th>Crop</th><th>Seedshape</th><th>Color of testa</th><th>Seeds</th><th>Color of wings</th><th>Brittle wings</th><th>Seedcoat</th><th>Almond color</th><th>Pest presence</th></tr>
<tr><td>CHI1</td><td>Oval</td><td>Brown</td><td>Winged</td><td rowspan="18">Brownish</td><td>Yes</td><td rowspan="14">Hard</td><td rowspan="22">White</td><td rowspan="14">No</td></tr>
<tr><td>CHI2</td><td>spherical</td><td>Brown</td><td>Winged</td><td rowspan="4">No</td></tr>
<tr><td>GRO1</td><td>Oval</td><td>Brown</td><td>Semi-winged</td></tr>
<tr><td>GRO2</td><td>Spherical</td><td>Brown</td><td>Winged</td></tr>
<tr><td>GRO3</td><td>Triangular</td><td>Brown</td><td>Semi-winged</td></tr>
<tr><td>GRO4</td><td>Spherical</td><td>Brown</td><td>Semi-winged</td><td>Yes</td></tr>
<tr><td>GTO1</td><td>Oval</td><td>Light brown</td><td>Semi-winged</td><td>No</td></tr>
<tr><td>HGO1</td><td>Spherical</td><td>Brown</td><td>Winged</td><td rowspan="5">Yes</td></tr>
<tr><td>MICH 1</td><td>Spherical</td><td>Brown</td><td>Winged</td></tr>
<tr><td>MICH 2</td><td>Spherical</td><td>Dark Brown</td><td>Winged</td></tr>
<tr><td>NL1</td><td>Spherical</td><td>Brown</td><td>Winged</td></tr>
<tr><td>OAX1</td><td>Oval</td><td>Black</td><td>Semi-winged</td></tr>
<tr><td>OAX2</td><td>Triangular</td><td>Brown</td><td>Winged</td><td>No</td></tr>
<tr><td>OAX3</td><td>Triangular</td><td>Dark Brown</td><td>Semi-winged</td><td rowspan="2">Yes</td></tr>
<tr><td>OAX4</td><td>Oval</td><td>Dark Brown</td><td>Semi-winged</td><td rowspan="2">Membranous</td><td>Yes</td></tr>
<tr><td>SLP1</td><td>Spherical</td><td>Brown</td><td>Winged</td><td rowspan="2">No</td><td rowspan="3">No</td></tr>
<tr><td>VER 1</td><td>Oval</td><td>Brown</td><td>Winged</td><td rowspan="2">Hard</td></tr>
<tr><td>VER 2</td><td>Spherical</td><td>Brown</td><td>Semi-winged</td><td rowspan="3">Yes</td></tr>
<tr><td>YUC1</td><td>Triangular</td><td>Dark Brown</td><td>Winged</td><td>Brown</td><td>Membranous</td><td>Yes</td></tr>
<tr><td>YUC2</td><td>Oval</td><td>Brown</td><td>Winged</td><td rowspan="3">Brownish</td><td rowspan="3">Hard</td><td rowspan="3">No</td></tr>
<tr><td>YUC3</td><td>Spherical</td><td>Brown</td><td>Winged</td><td rowspan="2">No</td></tr>
<tr><td>YUC4</td><td>Spherical</td><td>Dark Brown</td><td>Winged</td></tr>
</table>

Group 2 integrated the GRO3, OAX2, OAX3, VER2, YUC3, MICH2, and YUC4 populations. Group 3 only to the YUC1 population and group 4 to the VER1, CHI1, and CHI2 populations (Figure 3). Tian *et al.* (2015) mentioned that morphological diversity is associated with genetic diversity.

The identification of morphological diversity allows the selection of characters of greater adaptive and productive importance. The information obtained through morphological descriptors helps breeders select materials with desirable traits for farmers and consumers (Gitonga *et al.*, 2008).

CONCLUSIONS

There was significant morphological variation in Moringa oleifera seeds grown in Mexico. The qualitative and quantitative descriptors used were highly informative to clarify

Table 6. Principal component analysis of morphological characters of *M. oleifera* seeds.

Characteristic	CP* 1	CP 2	CP 3	CP 4	CP 5	CP 6	CP 7
Seed length (cm)	0.023	−0.002	−0.002	0.016	0.021	0.024	0.068
Seed width (cm)	0.013	−0.005	−0.016	−0.026	0.026	0.053	0.005
Seed weight (g)	0.010	0.000	−0.011	0.002	0.008	−0.001	0.016
Almond weight (%)	0.007	0.007	−0.001	−0.001	0.005	0.004	0.000
Percentage of the almond weight inrelation to the total weight (%)	−0.118	0.982	−0.060	−0.077	0.020	0.094	0.041
Mean weight of 100 seeds (g)	0.992	0.118	0.011	−0.021	−0.002	0.007	−0.006
Seed shape	−0.009	0.109	0.771	0.595	−0.112	−0.107	−0.112
Color of testa	−0.009	−0.056	0.544	−0.489	0.356	0.215	0.533
Seeds	0.015	−0.053	−0.215	0.458	−0.246	0.608	0.552
Color wings	−0.001	−0.049	0.032	−0.091	−0.125	0.136	−0.138
Brittle wings	0.008	−0.017	−0.127	0.330	0.855	0.259	−0.242
Seed coat	0.009	0.028	−0.123	0.167	0.143	−0.587	0.468
Almond color	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pest presence	0.006	0.027	−0.163	0.211	0.176	−0.362	0.308
Eigenvalue	25.498	9.245	0.435	0.356	0.261	0.164	0.131
% variance	70.581	25.590	1.204	0.984	0.722	0.453	0.363

CP*: Principal Components.

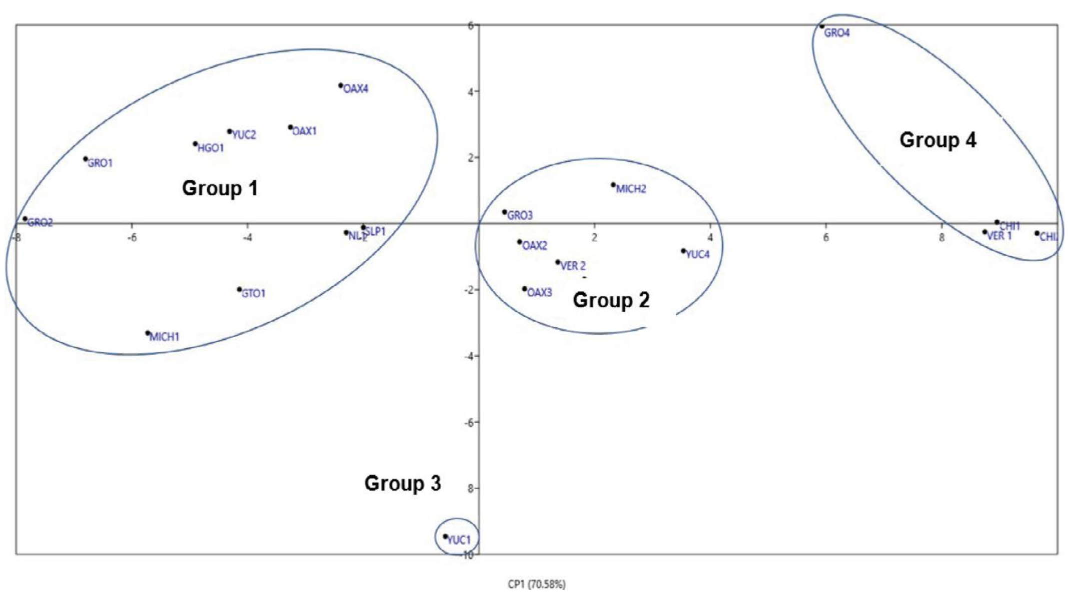

Figure 2. Dispersion of components 1 and 2 for the 22 cultures of *M. oleifera*.

the variation among the *Moringa oleifera* populations; in this sense, the seeds from the state of Chiapas (sites 1 and 2) and Veracruz (site 1) showed the highest weight suggesting that heavier seeds produced vigorous seedlings, and medium-weight seeds have shown higher

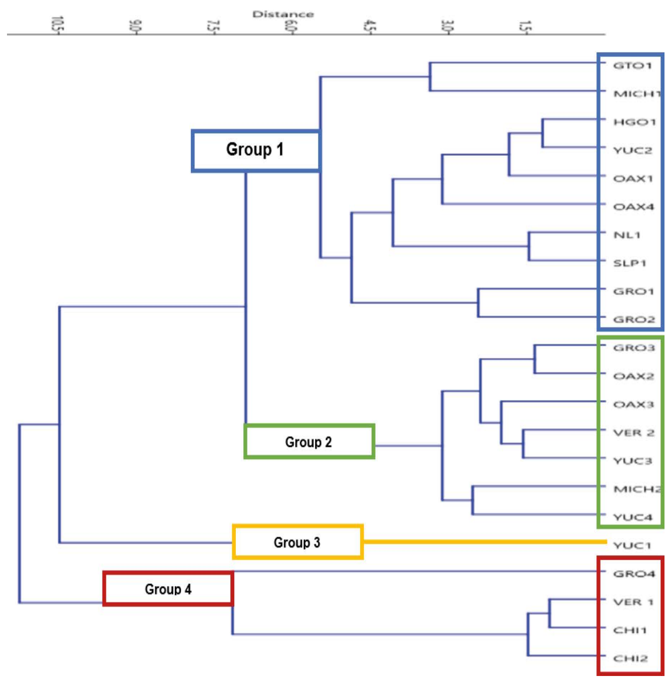

Figure 3. Cluster analysis based on the morphological descriptors of *M. oleifera* seeds

growth speed. This information contributes to the knowledge of this multipurpose species and serves as the basis for creating conservation and genetic improvement programs.

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REFERENCES

Adebayo, A.G., Akintoye, H.A., Olufolaji, A.O., Aina, O.O., Olatunji, M.T., & Shokalu, A.O. (2011). Assessment of organic amendments on vegetative development and nutrient uptake of *Moringa oleifera* Lam in the nursery. *Asian Journal of Plant Sciences 10*(1): 74-79.

Adikuru, N.C., Okafor, O.E., Ojiako, F.O., & Ibeawuchi, I.I. (2011). Prospects and challenges in the utilization of *Moringa oleifera* Lam. for agroecosystem sustainability in Nigeria. *International Journal of Agriculture and Rural Development 14*(2): 660-665.

Anwar, F., & Rashid, U. (2007). Physico-chemical characteristics of *Moringa oleifera* seeds and seed oil from a wild provenance of Pakistan. *Pakistan Journal of Botany 39*(5): 1443-1453.

Anwar, F., Zafar, S., & Rashid, U. (2006). Characterization of *Moringa oleifera* seed oil from drought and irrigated regions of Punjab, Pakistan. *Grasas Y Aceites 57*(2): 160-168.

Bezerra, A., M E., Momenté, V.G., & Medeiros, S.F. (2004). Germinacão de sementes e desenvolvimento de plántulas de moringa (*Moringa oleifera* Lam.) em funcão do peso de sementes e do tipo de substrato. *Horticultura Brasileira 22*(2): 295.

Dao, M.C.E., & Kabore, K.H. (2015). Morphological characteristic variation of eleven provenances of *Moringa oleifera* seedlings grown in the Northern Sudanese area of Burkina Faso. *African Journal of Plant Science 9*(10): 401-411.

Förster, N., Ulrichs, C., Schreiner, M., Arndt, N., Schmidt, R., & Mewis, I. (2015). Ecotype variability in growth and secondary metabolite profile in *Moringa oleifera*: Impact of sulfur and water availability. *Journal of agricultural and food chemistry 63*(11): 2852-2861. DOI: 10.1021/jf506174v

Gitonga, L., Kahangi, E., Muigai, A., Ngamau, K., Gichuki, S., Cheluget W. & Wepukhul, S. (2008). Assessment of phenotypic diversity of macadamia (*Macadamia* spp) germplasm in Kenya using leaf and fruit morphology. *African Journal of Plant Science 2*(9): 086-093.

Ledea-Rodríguez, J.L., Rosell-Alonso, G., Benítez-Jiménez, D.G., Arias-Pérez, R.C., Ray-Ramírez, J.V., & Reyes-Pérez, J.J. (2018). Producción de semillas de variedades de *Moringa oleifera* Lam. en el Valle del Cauto. *Agronomía Mesoamericana 29*(2): 415-423. http://dx.doi.org/10.15517/ma.v29i2.29545

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., & Bertoli, S. (2015). Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: an overview. *International Journal of Molecular Sciences 16*(6): 12791-12835. Doi: 10.3390/ijms160612791

López, S.E.M., Pazos, A., Rivero, G.A., Crespo, J.P.M., y Vargas, C.Z. (2018). Morfometría de fruto y semilla de *Moringa oleifera* Lam. "moringa". *Sciéndo Ciencia para el desarrollo 21*(2): 201-204.

Makkar, H.P.S. & Becker, K. (1997). Nutrients and antiquality factors in different morphological parts of the *Moringa oleifera* tree. *Journal Agriculture Science*. 128:311-322.

Martínez, P., González, S.F., Suárez, C.A.S. y Escobar, M.L.M. (2014). Adaptación y producción de biomasa de moringa (*Moringa oleifera* Lam.) en tres localidades del departamento de Santander. 6to Simposio Nacional Forestal 4 p.

Muhl, Q.E., Toit, E.S., & Petrus, J.R. (2011). Adaptability of *Moringa oleifera* Lam. (Horseradish) Tree Seedlings to Three Temperature Regimes. *American Journal of Plant Sciences* 2: 776-780. DOI:10.4236/ajps.2011.26092

Ndubuaku, U.M., Ndubuaku, T.C.N., & Ndubuaku, N.E. (2014). Yield characteristics of *Moringa oleifera* across different ecologies in Nigeria as an index of its adaptation to climate change. *Sustainable Agriculture Research 3*(1): 95-100.

Olson, M.E., y Fahey, J.W. (2011). *Moringa oleifera*: un árbol multiusos para las zonas tropicales secas. *Revista Mexicana de Biodiversidad* 82: 1071-82.

Olson, M.E., y Alvarado-Cárdenas, L.O. (2016). ¿Dónde cultivar el árbol milagro, ¿*Moringa oleifera*, en México? Un análisis de su distribución potencial. *Revista Mexicana de Biodiversidad 87*(3): 1089-1102.

Palada, M.C. (1996). Moringa (*Moringa oleifera* Lam.): A versatile tree crop with horticultural potential in the subtropical United States. *HortScience* 31: 794-797.

Paliwal, R., Sharma, V. & Pracheta, (2011). A review on Horse-radish tree (*Moringa oleifera*): a multipurpose tree with high economic and commercial importance. *Asian Journal Biotechnol 3*(4):317-328. DOI:10.3923/ajbkr.2011.317.328

Pandey, A., Pradheep, K., Gupta, R., Nayar, E.R., & Bhandari, D.C. (2011). 'Drumstick tree' (*Moringa oleifera* Lam.): a multipurpose potential species in India. *Genetic resources and crop evolution 58*(3): 453-460.

Parrotta, J.A. (2009). *Moringa oleifera* Lam., 1785. Enzyklopädie der Holzgewächse, Handbuch und Atlas der Dendrologie. DOI:10.1002/9783527678518.ehg2005015

Pirrò, S., Zanella, L., Kenzo, M., Montesano, C., Minutolo, A., Potestà, M., Sobze, M. S., Canini, A., Cirilli, M., Muleo, R., Colizzi V., & Galgani, A. (2016). MicroRNA from *Moringa oleifera*: Identification by High Throughput Sequencing and Their Potential Contribution to Plant Medicinal Value. *PloS one 11*(3): e0149495.

Popoola, J.O., Bello, O.A., & Obembe, O.O. (2016). Phenotypic intraspecific variability among some accessions of drumstick (*Moringa oleifera* Lam.). *Canadian Journal of Pure and Applied Sciences 10*(1): 3681-3693.

Radice, S. & Giordani. E. (2018). Flower development and pollen vitality of *Moringa oleifera* Lam. grown in a humid temperate climatic condition. *Advances in Horticultural Science 32*(4): 549-556. DOI: https://doi.org/10.13128/ahs-23504

Ramos, L.M., Silva, R.C., Vitti, F.M., y Conceição, R.S. (2010). Morfologia de frutos e sementes e morfofunção de plántulas de Moringa (*Moringa oleifera* Lam.). *Comunicata Scientiae 1*(2): 156-160.

Roloff, A., Weisgerber, H., Lang, U., and Stimm, B. (2009). Enzyklopädie der Holzgewächse, Handbuch und Atlas der Dendrologie. WILEY-VCH, Weinheim, Germany.

Shaltout, H. I., Mobarak, A. A., Ali, K., Baraka, D. & Aly, S. (2017). Morphological Variability Among *Moringa oleifera* (Lam.) Populations in Egypt. *Egyptian Journal of Botany 57*(1): 241-257. DOI: 10.21608/EJBO.2017.538.1020

Tian, Y., Yan, Z., Jing, Z., Cheng, Y.G., Liang, Y., Xuan, W., Chong-Ying, S., Jing, X., Tian, D., Lei, P., Yu, Z.H., AnNi, X., YeWei, H., JiaJin, Z., Xiao, M., Yang, D., ShuMei, H., & Jun. S. (2015). High quality reference genome of drumstick tree (*Moringa oleifera* Lam.), a potential perennial crop. *Science China Life Sciences 58*(7): 627-638. DOI: 10.1007/s11427-015-4872-x

Valdés, R.O.A., Pérez, A.V., y Muñoz, C.G. (2018). Efecto de peso y talla de semilla sobre plántulas de Moringa y Ricinus. *Revista Mexicana de Ciencias Agrícolas 9*(8): 1411-1422. DOI:10.29312/remexca.v9i7.734

Velázquez-Zavala, M., Peón-Escalante, I.E., Zepeda-Bautista, R. & Jiménez- Arellanes, A.M. (2016). Moringa (*Moringa oleifera* Lam.): potential uses in agriculture, industry and medicine. *Revista Chapingo. Serie Horticultura 22*(2): 95-116.

Zaku, S.G., Emmanuel, S., Tukur, A.A. & Kabir, A. (2015). *Moringa oleifera*: An underutilized tree in Nigeria with amazing versatility: A review. *African Journal of Food Science 9*(9): 456-461. https://doi.org/10.5897/AJFS2015.1346.

Zhigila, D.A., Mohammeda, S., Oladeleb, F.A., & Sawac. FBJ (2015). Numerical analyses of leaf and fruit external morphology in *Moringa oleifera* Lam. *Jurnal Teknologi (Sciences & Engineering) 77*(13): 123-131.

AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of grasshopper (*Sphenarium purpurascens* Charpentier) paste addition on rheological behavior of Mole Poblano

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ABSTRACT

Objective: Analyze the effect of grasshopper (*Sphenarium purpurascens* Charpentier) paste addition on the rheological behavior of Mole Poblano (MP) and its relation with the technological properties of the grasshopper paste.

Design/methodology/approach: The addition of grasshopper paste was done at different proportions as follows: T0=0% of grasshopper paste (GP) and 100% of mole Poblano (MP); T10=10% GP and 90% MP; T15=15% GP and 85% MP, T20=20% GP and 80% MP, T25=25% GP and 75 % MP, T30=30% GP and 70% MP. Water retention and emulsifying capacity of grasshopper paste were evaluated. Density, kinematic and apparent viscosity, and rheological behavior were analyzed at 25 °C; rheological parameters (consistency index (k) and flow behavior index (n)) were calculated by performing a regression analysis to adjust the graphs to a power-law model.

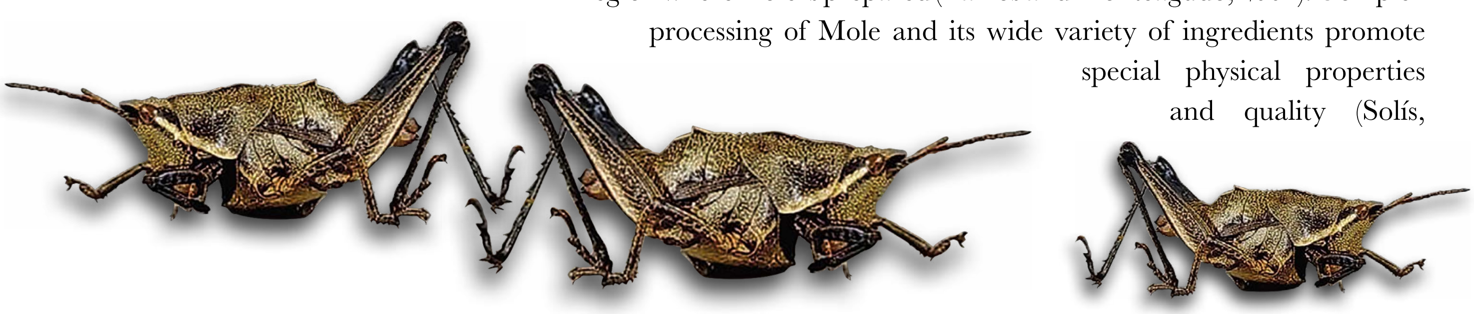
Findings/conclusion: Grasshopper paste had higher emulsifying capability than water retention capability. Apparent viscosity of all formulations decreased as shear rate increased, so all mixtures of GP and MP demonstrated No-Newtonian behavior and pseudoplastic performance. Index consistency increased as GP content increased, these results are related with protein content because GP had a good emulsifying capability.

Limitations on study/implications: More studies about the characterization of the proteins of GP and their interaction with other components are required.

Key words: mole poblano, grasshopper paste, viscosity, rheological parameters.

INTRODUCTION

Mole is a typical artisan food from México, which is a complex mixture of hot peppers ground with nuts, chocolate, oil, dry corn tortilla, and several spices in different proportions (Alvarez-Parrilla *et al.*, 2014; Barros, 2005), its formulation depends on the region where mole is prepared (Barros and Monteagudo, 2004). Complex processing of Mole and its wide variety of ingredients promote special physical properties and quality (Solís,



2008); paste, liquid, granule or comprised powder are the presentations of Mole in Mexican market (Hernández *et al.*, 2017). From physic-chemical point of view, this food is a complex colloid system, where emulsion and colloid suspension are mixed; fat, carbohydrates and proteins are related to the rheological behavior an although a low protein content in mole was described (2-3%) (Alvarez-Parrilla *et al.*, 2014), this component could has an important technological function as hydrocolloid or emulsifier, hence the addition of protein could be considered to the improvement of the technological and rheological properties.

Edible insects are commonly consumed in México, ants, crickets, worms, beetles, grasshopper among other insects are eaten. Also, grasshopper could provide environmental, economical and social benefit by its consumption, and its composition gives nutritional advantages because of protein, vitamins and minerals content, therefore it could be added to poor nutritional foods (García *et al.*, 2018). Regarding nutritional improvement, a previous study (Arcos-Estrada *et al.*, 2020) showed that the mixtures of grasshopper and mole poblano increased the protein content, this fact not only could modify its nutritional quality but also the physical characteristics as apparent viscosity and rheological performance which are related to the texture, sensory analysis, handling properties and acceptance or rejection of the food (Navas, 2006), therefore the aim of this study was evaluate the effect of the grasshopper paste addition on rheological behavior of Mole Poblano and its relation with the technological properties of the grasshopper paste.

MATERIALS AND METHODS

Materials

Grasshoppers were recollected in Hidalgo State, México and all the ingredients of mole Poblano formulation were commercial.

Methods

Grasshoppers paste (GP) preparation. Grasshoppers were recollected in Hidalgo State, México. They were washed and dried in an oven at 50 °C during 8 h, 500 g of insects were milled (Aragón-García *et al.*, 2018) and the grasshopper paste was stored in freezing until their incorporation to mole Poblano.

Water retention and emulsifying capacities of the GP. These properties were evaluated according to the methods proposed by Villegas de Gante (2015); in the first analysis 5 g of GP was mixed with 8 ml of 0.6M NaCl solution, then it was cooled in an ice bath during 30 minutes after, mix was centrifuged at 10000 rpm during 5 minutes, and finally the retained water was measured and reported. In the second analysis, 25 g of GP was mixed with 75 ml of 1M NaCl solution in a mixer during 5 minutes at lowest velocity, after that, vegetable oil was added to the mix until it does not incorporate in the GP, the incorporated oil volume was registered and reported.

Mole Poblano paste (MP) preparation. Mole Poblano paste was prepared according to the recipe from El Museo Frida Khalo, from the book Las fiestas de Frida y Diego, “Recuerdos y recetas” (Rivera-Marín, 2005) (Table 1). After, six mixtures were obtained with different proportions of GP and MP: T0=0% GP and 100% MP; T10=10% GP and 90% MP; T15=15% GP and 85% MP; T20=20% GP and 80% MP; T25=25% GP and

Table 1. Formulation of mole poblano.

Ingredient	g/100g of Mole Poblano
Hot peppers	34.79
Onion	16.50
Chicken soup	12.46
Lard	9.97
Tomato	6.13
Almonds	4.69
Chocolate	4.61
Bread roll and Tortillas	2.84
Sesame	2.36
Raisins	2.24
Condiments	1.12

75% MP; T30=30% GP and 70% MP. The mixtures were stored in freezing until their analysis.

Apparent viscosity. Viscosity evaluation was done using a DV2T Brookfield viscosity analyzer. 300 g of the mixture was put into a glass vessel and the viscosity was evaluated at 3, 30, 60, 120 and180 rpm at 25 °C (Méndez and Ramos, 2008) with the needle 7.

Density. Paste density was determined as the ratio of the weight of a standard container (8 g/cm^3) filled with GP or MP mixes at 25 °C.

Data Analysis

Three independent measurements per analysis were done. ANOVA analysis was done and the Tukey test using Minitab 17.

RESULTS AND DISCUSSION

Figure 1 shows the relation between viscosity and shear rate from different paste mixtures of GP and MP. Non-Newtonian behavior is observed in all mixtures because viscosity changed as the shear rate is modified and also showed a shear-thinning performance because their viscosity decreased as the shear rate increased. Besides, initial viscosity values (Table 1) increased as the GP protein increased until T15 treatment (15% GP) after that, the initial viscosity values of the mixtures T20 and T25 were lower than T15 and similar between them, whereas T30 treatment had a lower value but it was bigger than the control mixture value (T0). This behavior could be attributed to the addition of GP paste because a higher protein content is presented in the mixture, and these proteins can act as hydrocolloids and emulsifiers. These technological properties were measured in the GP and results showed that it retains 0.321 ml of H_2O and 2 ml of oil per gram, this demonstrates that GP is capable to retain oil and water, which has the property of forming viscous dispersions and/or gels and could function as thickening agents, and gelling agents in food.

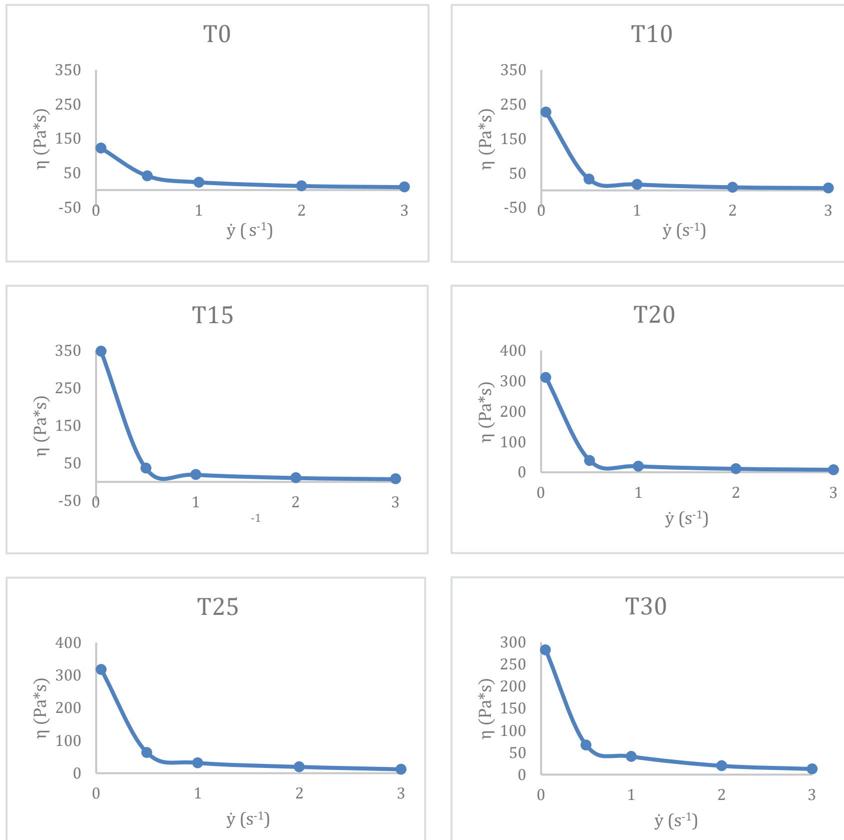


Figure 1. Relation between apparent viscosity and deformation velocity of different GP and MP mixtures at 25 °C.

Also, density values (Table 2) were higher than control except T25, these results are related to the non soluble solids of GP incorporated in the MP. Kinematic viscosity denotes the behavior of the paste as a fluid discarding the forces that generate the paste's motion and it represents better the viscosity of the static fluid, these values were higher than control and are related to the technological properties of GP (water retention and emulsifying capabilities).

On the other side, the data obtained were adjusted using the Otswald de Waele model (power model) (Eq. 1), which is used to describe to Non-Newtonian fluids, substituting the

Table 2. Density, apparent and kinematic viscosity of different GP and MP mixtures at 25 °C at 3 rpm.

Mixture	Density (kg/m^3)	Apparent Viscosity (Pa*s)	Kinematic viscosity (m^2/s)
T0	996.1	121.800	0.122
T10	1031.4	226.233	0.219
T15	1001.4	348.000	0.348
T20	1031.8	311.100	0.302
T25	986.5	317.300	0.322
T30	1035.8	282.700	0.273

viscosity equation (Eq. 2) in equation 1, we obtained a relation between apparent viscosity and shear rate (Eq. 3) in order to obtain the index consistency (k) values and flow behavior index (n). The last model was linearized using equation 4 (Eq. 4) (Talens Oliag, 2016).

$$\sigma = K\dot{y}^n \quad \text{Eq. 1}$$

$$\eta = \sigma / \dot{y} \quad \text{Eq. 2}$$

$$\eta = K\dot{y}^{n-1} \quad \text{Eq. 3}$$

$$\ln \eta = \ln K + (n-1)\ln \dot{y} \quad \text{Eq. 4}$$

Figure 2 shows a linearized curve of the all treatments and Table 3 shows the values of the consistency (k) and flow behavior index (n), where the all n values were lower than 1. The n value indicates if a fluid is near to the Newtonian behavior (n>1 is dilatant fluid, n=1 is Newtonian fluid and n<1 is pseudoplastic fluid) (Icarte, 2002). Different mixtures of GP an MP pastes had n values lower than 1 showing a Non-Newtonian and

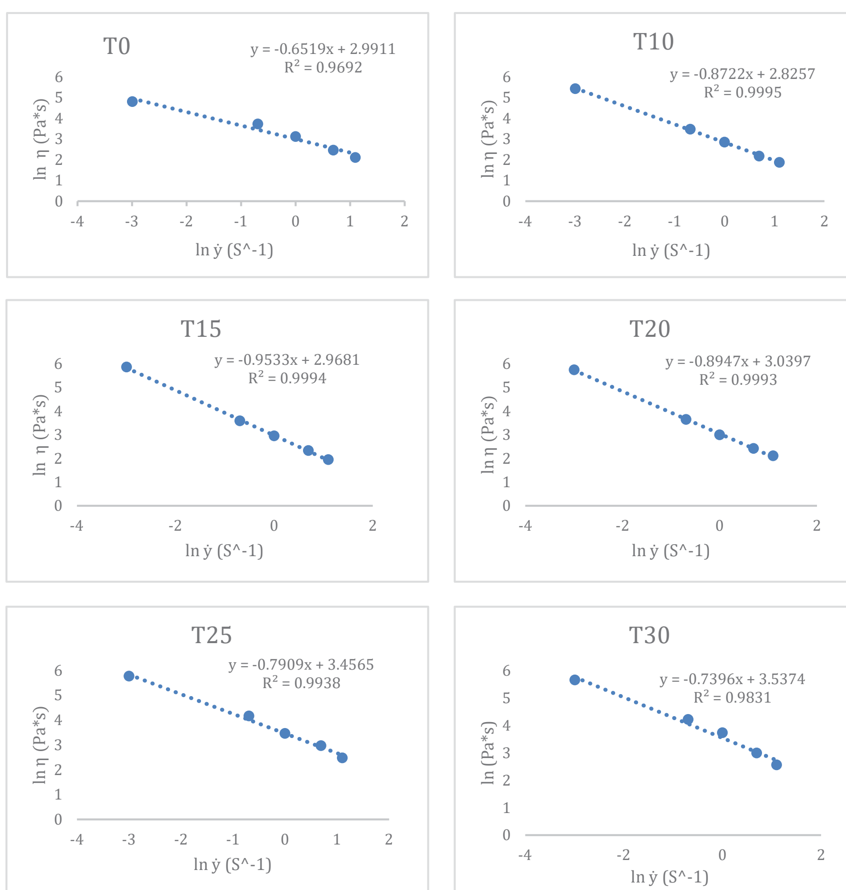


Figure 2. Linearized curves of the relation between apparent viscosity and shear rate of different GP and MP mixtures at 25 °C.

Table 3. Consistency and behavior indexes of different GP and MP mixtures at 25 °C.

Treatment	K	n
T0	19.907	0.348
T10	16.872	0.128
T15	19.455	0.047
T20	20.899	0.105
T25	31.706	0.209
T30	34.377	0.260

a pseudoplastic behavior, these results are according to other foods as honey (Gómez-Díaz *et al*., 2004), fruit puree (Miranda *et al*., 2011; Cardoso de Oliveira *et al*., 2012), and mayonnaise (Agueroa, 2016). Different factors influence the behavior index as content of ingredients, temperature, and particle size (Osorio, 2018). Mole is a complex mixture of different ingredient which have different technological functions that affect the viscosity and rheological behavior. The addition of GP paste increased the protein and polysaccharide contents and affect the n values of the mixtures. The values of the consistency coefficient (k) (Table 3) increased when the GP paste addition was increased, this indicates a lower ability of paste to flow when a higher content of GP paste is in the mixture. Similar values are observed in fruit pulps as mango (Ortega Quintana *et al*. (2015), and in fruit juices (Chin *et al*., 2009; Quek *et al*., 2013).

CONCLUSIONS

GP had higher emulsifying activity and water retention capacity, these technological properties were related to higher apparent and kinematic viscosity values of MP and GP mixes. Mole poblano added with grasshopper pastes had a Non-Newtonian and a pseudoplastic behavior.

REFERENCES

Agueroa, C. M. (2016). Viscosidad de una salsa de Tarwi (*Lupinus mutabilis*) libre de Gluten y lactosa utilizando gomas Guar y Xantan. *Revista de Investigaciones de la Universidad Le Cordon Bleu*, *3*(1), 29-40.

Aragón-García, A.; Rodríguez-Lima, D.R.; Pino-Moreno, J.M.; Aragón-Sánchez, M.; Carlos-Ángeles, S. y García-Pérez, A. (2018). Valor nutritivo de la harina de chapulín (*Sphenarium purpurascens* Charpentier) tostado o natural. *Entomología mexicana,* 5, 106−112. http://www.socmexent.org/entomologia/revista/2018/BHN/BHN%20106-112.pdf

Barros, C. y Monteagudo J.L.C. (2004). El mole. Aportaciones prehispánicas y Construcción y evolución del mole virreinal, en El mole en la ruta de los dioses. 6to Congreso sobre Patrimonio Gastronómico y Turismo Cultural (Puebla 2004). Memorias. México, Patrimonio Cultural y Turismo, Cuadernos, no. 12, pp. 11, 21, 34. [en línea]

Cardoso de Oliveira, Ricardo, & Rossi, Robson Marcelo, & Davantel de Barros, Sueli Teresa (2012). Estudo reológico da polpa de morango (*Fragaria vesca*) em diferentes temperaturas. *Acta Scientiarum. Technology, 34*(3), 283-288. [fecha de Consulta Junio de 2020]. ISSN: 1806-2563. Disponible en: https://www.redalyc.org/articulo.oa?id=3032/303226542005

Chin, NL, Chan, SM, Yusof, YA, Chuah, TG y Talib, RA (2009). Modelado del comportamiento reológico de concentrados de jugo de pomelo utilizando una curva maestra. *Revista de ingeniería alimentaria, 93*(2), 134-140.

Gómez-Díaz, D., Navaza, J. M., & Quintáns-Riveiro, L. C. (2004). Estudio viscosimétrico preliminar de mieles de bosque denominación específica “miel de galicia” viscosimetric study of multifloral honeys with specific guarantee “miel de galicia” estudo viscosímetrico de meles de bosque denominación específica “mel de galicia”. *CYTA-Journal of Food, 4*(4), 234-239.

Hernández, C. S., Hernández, M. Á. S., Montiel, L. G., & Pinacho, A. J. V. (2017). Capítulo 15. Propiedades fisicoquímicas del mole negro de Oaxaca. Índice, 188.

Icarte, R. M. (2002). Caracterización del comportamiento reológico de sopa de crema orientada a control de calidad. Valdivia, Chile.

Méndez, A. R., & Ramos, O. A. N. (2008). Comportamiento reológico de un fluido. Universidad del Mar. Oaxaca, México. Pág, 1-2.

Miranda, V.A.M. & Queiroz, Alexandre & Maria Feitosa de Figueirêdo, Rossana & Santos, Dyego. (2011). Viscosidade aparente de polpas de graviola com diferentes concentrações. *Revista Brasileira de Produtos Agroindustriais*. 13. 363-374. 10.15871/1517-8595/rbpa.v13n4p363-374.

Navas, J. S. (2006). Fundamentos de reología de alimentos.

Ortega Quintana, F. A., Salcedo Galván, E., Arrieta Rivero, R., & Torres Gallo, R. (2015). Efecto de la temperatura y concentración sobre las propiedades reológicas de la pulpa de mango variedad Tommy Atkins. *Revista Ion, 28*(2), 79-92.

Quek, M. C., Chin, N. L., & Yusof, Y. A. (2013). Modelling of rheological behaviour of soursop juice concentrates using shear rate–temperature–concentration superposition. *Journal of Food Engineering, 118*(4), 380-386.

Quirce Vázquez, C., Filippini, V., & Micó, E. (2013). La utilización de los insectos en la gastronomía, un taller nutritivo.*Cuadernos de biodiversidad*. 43.10.14198/cdbio.2013.43.02.

Rivera-Marín, G. (2005) Las fiestas de Frida y Diego. Recuerdos y recetas. Promexa, México, p.214.

Solís, Y. (2008). Evaluación Sensorial: Selección de Jueces (Doctoral dissertation). Instituto Politécnico Nacional. Unidad Profesional Interdisciplinaria de Biotecnología. México DF).

Talens Oliag, P. (2016). Caracterización del comportamiento reológico de un alimento fluido pseudoplástico.

AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Lippia graveolens (Lamiales: Verbenaceae) and *Oryganum vulgare* (Lamiales: Lamiaceae) obtained by means of the *in vitro* propagation technique

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ABSTRACT

Objective: The objective of this study was to establish the method of propagation of *Oryganum vulgare* and *Lippia graveolens* employing a plant tissue culture technique that decreased the phenolization percentages and increased the multiplication coefficients.

Design/methodology/approach: The *in vitro* germination percentage was evaluated in both MS and MS medium + activated carbon. Microcuttings (small shoots) of both species were established in base medium added with different antioxidant agents to decrease the phenolization of explants; the treatments were arranged in a completely randomized block design. For the propagation phase, a completely randomized factorial design was used, where the auxin/cytokinin phytoregulators, type of explants (axillary buds and leaves), and the species (*Lippia graveolens* and *Oryganum vulgare*) were considered as factors.

Results: Maximum germination (63.3% ±12.5) was obtained on day 15 in both culture media for *L. graveolens* and *O. vulgare*. The use of antioxidant agents mainly activated carbon, increased the in vitro establishment and activation of vegetative buds in both species by up to 90%. There were significant differences in the variables evaluated regarding the treatments, the explant, and the species in the multiplication phase. The combination 1.0/ 0.5 mg L^{-1} BA/AIB induces callus formation for both species. When used as leaf explants, callus formation was potentiated.

Study Limitations/Implications: The results presented are advances from a long-term experiment.

Findings/conclusions: The germination of *L. graveolens* seeds can be achieved in MS medium after 15 days. Microcuttings of both *L. graveolens* and *O. vulgare* were successfully established in MS basal medium enriched with 1 g L^{-1} charcoal that showed low oxidation percentages and induced up to 90% the production of shoots

Image by ariesa66 at Pixabay

in the explants. The mixture of 1.0/0.5 mg L^{-1} BA/AIB induces callus formation for both species; when this medium is in contact with leaves as an explant, its formation is potentiated, achieving diameters up to 15 mm. In order to achieve the induction of shoots and roots, buds should be established in MS medium enriched with 0.5 mg L^{-1} IBA for both species; this mixture encreased the multiplication coefficients.

Keywords: Micropropagation, Mexican oregano, phenolization, multiplication coefficient, plant growth regulators.

INTRODUCTION

The most common oregans in Mexico are *Oryganum vulgare*, better known as common oregano or Greek oregano; it is a herbaceous species of the Lamiaceae family native from Europe (Arcila-Lozano *et al.*, 2004) and *Lippia graveolens*, better known as Mexican oregano (Clarenc *et al.*, 2020). The latter is a species that is distributed in the semi-arid climates of Mexico, mainly in the states of Hidalgo, Veracruz, Durango, Yucatán, Puebla, Coahuila, Oaxaca, San Luis Potosí, Querétaro, Guerrero (CONAFOR, 2011; Castellano-Hernández *et al.*, 2013; Cortés-Chitala *et al.*, 2021), in the vast majority of these areas this species is a wild resource in areas with a high degree of economic and social marginalization, making it necessary to establish adequate management of this resource (Castillo *et al.*, 2017; Díaz-De León *et al.*, 2020). Around 90% of *L. graveolens* production in Coahuila is obtained from wild areas (CONAFOR, 2009), causing natural regeneration and conservation of genetic variability of the species scarcity. An alternative for the conservation of wild oregans is the cultivation of plant tissues.

In vitro propagation studies of various oregans have been carried out, mainly via direct organogenesis (García-Pérez *et al.*, 2012) using MS medium (Murashige and Skoog, 1962) combined with the auxin cytosine complex. Sánchez-Pérez *et al.* (2021) reported the *in vitro* propagation of *L. graveolens* using axillary buds enriched with 2.5 mg L^{-1} of 6-benzyl aminopurine and evaluated different luminosity intensities to make the *in vitro* photosynthetic process more efficient. Well (2014) reported that the micropropagation of this species from vegetative explants (microcuttings) presents a high percentage of phenolization, which prevents cultivation. Castellano-Hernández *et al.* (2013) reported the generation of shoots of *L. graveolens* from axillary buds of plants germinated *in vitro* with cytokinins and the development of shoots with the addition of auxins. Therefore, the objective of the present work was to establish the propagation method of *Oryganum vulgare* and *Lippia graveolens* employing plant tissue culture technique or micropropagation that decreases the phenolization percentages and increases the multiplication coefficients.

MATERIALS AND METHODS

Selection of plant material

The seeds of *O. vulgare* were purchased from the company HortaFlor and mother plants from the Botanical Garden of Medicinal Plants of the Institute for Research and Training, Agriculture, Agriculture and Forestry of Mexico (ICAMEX) to validate their origin.

The seeds of *L. graveolens* were collected in the community of Higuerillas, municipality of Peñamiller, Querétaro. In addition, two specimens were selected as the mother plant.

For both species, the seeds were kept refrigerated at 10 °C until their establishment *in vitro*. The mother plants were kept under greenhouse conditions at the Lerma Autonomous Metropolitan University and the mother plant in a greenhouse of the Biotechnology Laboratory of the Protectora de Bosques del Estado de México (PROBOSQUE), where all the *in vitro* study was carried out.

In vitro establishment

The seeds and microcuttings were used as explants. The seeds were disinfected following the micropropagation process of *Thymus piperella* (Sáez *et al.*, 1994), with some modifications. The seeds were disinfected by soaking them in constant agitation for five minutes with two drops of commercial liquid soap; subsequently rinsed until the soap was removed with distilled water, followed by 30% v/v commercial sodium hypochlorite solution for 20 minutes and rinsed again with distilled water (three times). Afterward, they were placed in a laminar flow hood in a 70% ethanol solution for 30 seconds and rinsed with sterilized distilled water.

A completely randomized block design was established to evaluate the seed germination of both species, ten repetitions were used for each treatment, and each repetition contained five seeds. Two treatments of base culture medium were established: T1. 100% MS culture medium (Murashige and Skoog, 1962), with the whole mineral salts. T2. 100% MS culture medium plus 1 g L^{-1} of activated carbon. Both treatments were enriched with 30 g L^{-1} of sucrose, 7 g L^{-1} of bacteriological agar, and the pH was adjusted to 5.7 ± 0.1. The treatments were placed in Gerber-type flasks with 20 milliliters of medium each; they were sterilized in an autoclave at 120 °C for 15 minutes. Germination percentage was evaluated at 5, 10, and 15 days.

The disinfection of vegetative explants was carried out as Chanbe (2008) for common oregano microcuttings, changing the ascorbic acid by citric acid in the same concentration. As this type of species presents a high degree of oxidation due to the release of phenols at the cutting time, a completely randomized design was established with five treatments with ten repetitions per treatment to minimize phenolization. For the treatments, MS was used as the basal medium at 100% of its concentration enriched with 30 g L^{-1} of sucrose, 7 g L^{-1} of bacteriological agar supplemented with: O1 control; O2 with 2.5% lemon juice; O3 with 1 mg L^{-1} of citric acid; O4 with 1 g L^{-1} of activated carbon and O5 with 5% lemon juice. The oxidation percentage and the shoot regeneration percentage were evaluated at 30 days.

Multiplication and rooting stage

For the multiplication stage, a factorial design with five repetitions (five explants per repetition) was used, where the effect of the cytokinin-auxin complex for the stimulation of morphogenetic responses was evaluated. Factor A was the type of explant (axillary bud and leaf), and factor B was the components of the culture medium. For the latter, 6-benzyl amino purine (BAP) was used in concentrations 0, 0.5, and 1 mg L^{-1} combined with indol butyric acid at 0, 0.1, and 0.5 mg L^{-1} in MS base medium with 30 g L^{-1} of sucrose, 7 g L^{-1} of bacteriological agar. After 30 days of establishment, the following variables were evaluated: % oxidation, % of callus formation, callus diameter, % of shoot formation, shoot length, number and length of roots, and multiplication coefficient.

Statistic analysis

The variables for each phase were evaluated employing an analysis of variance. A Tukey test was performed for the variables that presented significant differences ($P<0.05$) (Steel and Torrie, 1980). The SAS software for Windows 10 was used.

RESULTS AND DISCUSSION

In vitro establishment

Regarding the *in vitro* establishment of seeds, *O. vulgare* did not germinate in any treatment. For *L. graveolens*, there were no significant statistical differences for the three evaluation dates, obtaining germination percentages of 30.0±7.8 and 26.76±8.3% in T1 and T2, respectively, at five days. Ten days after sowing, the trend was similar, with 56.67±10.0 and 50.0±11.4% germinated seeds. Maximum germination occurred 15 days after sowing with 63.3±12.5% for both treatments. These results were similar to those reported by Castellano-Hernández *et al.* (2013) for *L. graveolens*; they observed *in vitro* germination of seeds in MS medium to use them as initial micropropagation material, material that was subcultured in MS medium plus 0.5 mg L^{-1} of BA to activate 83.3±0.4% of buds. Regarding the microcuttings established as initial explants, there were significant statistical differences ($P<0.001$) between treatments, species, and treatment × species interaction in, both % of oxidation and % of explants that formed shoots.

The lowest percentage of oxidation was observed in the O4 treatment (20.5%±2.92), O1 showed 42.5%±8.68, the O2 and O3 treatments showed 46.0%±3.28 and 51.0%±3.3; respectively, and the highest oxidation was observed in O5 treatment (90.0%±3.55). The *L. graveolens* microcuttings showed a lower percentage of oxidation (42.80%±4.76) than *O. vulgare* (57.20%±3.78).

The treatment effect on the species is shown in Figure 1; it can be observed that treatment O4 (13.0±1.6%) and O1 (10.9±0.4%) had a significant effect on *L. graveolens*. On the other hand, *O. vulgare* showed less response to O4 treatment (28.00±1.7%) and O1, where the percentage of oxidation was 78.00%±2.9. Thus, indicating that microcuttings as the initial explant of Mexican oregano responded well to antioxidant agents. The addition of citric acid reduced the oxidation percentages, however, when it is used in high doses it is counterproductive.

The formation of shoots was inversely proportional to oxidation; when there was more oxidation, there was less presence of shoots. The presence of activated carbon (O4) considerably increased the presence of shoots (90.5%±3.52). *Lippia graveolens* regenerated more shoots than *O. vulgare* (66.24%±6.49 and 31.72%±7.92, respectively).

The results of Bueno (2014) showed the contrary because he added 2% ascorbic acid to the MS base culture medium and considerably reduced the phenolization of buds used as initial explants.

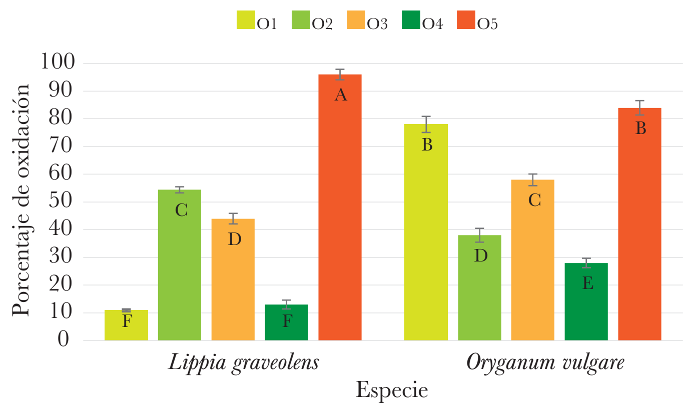

Figure 1. Effect of antioxidant agents in the culture medium for establishing microcuttings as an initial explant in *Lippia graveolens* and *Oryganum vulgare*. O1: control; O2: 2.5% lemon juice; O3: 1 mg L^{-1} of citric acid; O4: 1 g L^{-1} of activated carbon; O5: 5% lemon juice. Different letters present statistically significant differences P≤0.05.

Multiplication and rooting stage

For percentage of oxidation, percentage of callus formation, callus diameter, and percentage of shoot formation, there were significant statistical differences between the treatments, the species, and the explant ($P<0.0001$) except for the percentage of callus formation referring to the type of explant ($P=0.7022$) used (Table 1).

The treatments consisting of 0.5/0.5 and 1.0/0.5 mg L^{-1} BA/AIB presented the lowest oxidation percentages with 12.5 and 12.4% oxidation compared to the treatment without growth regulators that presented the highest percentage of oxidation, 32.9%. The 1.0/0.5 mg L^{-1} BA/AIB treatment was isolated entirely from the other treatments, causing callus formation in 69% of the explants, and the treatment without regulators presented only 7% of the explants. In the explants, the leaf presented a high degree of oxidation compared to the bud; both explants formed callus, but the diameter of the callus was more significant in the leaf than the bud, as shown in Table 2, the more significant number of sprouts were presented with oregano buds.

The *in vitro* behavior of *L. graveolens* was better than that of *O. vulgare*, from both the leaf and the bud had the best values for callus formation, the Mexican oregano leaf formed a

Table 1. Mean squares for the *in vitro* multiplication stage of *Lippia graveolens* and *Oryganum vulgare*.

	Oxidation	Callus formation	Callus diameter (mm)	Shoots formation
Treatment(T)	3068.3 *	2852.48 *	521.7 *	316.79 *
Species (SP)	194245 *	12520.06 *	4843.66 *	1355.3 *
Explant (E)	348668 *	30.81 NS	919.23 *	126271 *
T*SP	3003.2 *	1381.96 *	251.3 *	405.89 *
T*E	3212.4 *	187.70 *	49.62 *	121.11 *
SP*E	3212.4 *	295.23 *	830.09 *	0 NS
CV	39.00	25.64	35.50	34.70

* Significant at 0.05. NS. Not significant at 0.05.

Table 2. Percentages of oxidation, callus formation and diameter, and bud formation in *Lippia graveolens* and *Oryganum vulgare*.

		Oxidation (%)	Callus formation (%)	Callus diameter (mm)	Shoots formation (%)
Species	*Oryganum vulgare*	42.01 A	1.9 B	0.03 B	57.2 B
	Lippia graveolens	7.88 B	88.7 A	5.4 A	66.2 A
Explant	Leaves	53.1 A	56 A	5.1 A	1 B
	Bud	5.5 B	56 A	2.6 B	91.4 A
L. graveolens	Leaves	24.8 B	91.1 A	8.4 A	0 B
	Bud	6.3 C	87.6 A	4.1 B	94.6 A
O. vulgare	Leaves	95.6 A	5.6 B	0.1 C	2.2 B
	Bud	14.4 C	0 A	0 C	85.6 A

Different letters represent statistically significant differences P≤0.05.

large callus diameter (8.4 mm) (Figure 2), suggesting that the suspension cell culture of this species may be very viable to produce secondary metabolites from this explant. The leaf of *O. vulgare* regenerated shoots in a small proportion, thus producing direct organogenesis.

Regarding the generation of shoots by growing buds, statistical differences ($P<0.05$) were found between the treatments evaluated concerning the addition of growth regulators. For both species, the number of shoots and, therefore, the multiplication coefficient benefited when 1.0/0.0 mg L^{-1} BA/AIB was used; it increased almost twice for *L. graveolens* and triple for *O. vulgare* (Table 3). The length of the shoots of *L. graveolens* increased 22.8 mm with 0.0/0.5 mg L^{-1} BA/AIB concerning the control, in that same concentration, up to five roots per explant with an average length of 47 mm compared to the control that formed 1.3 roots per explant with approximately 3.5 mm.

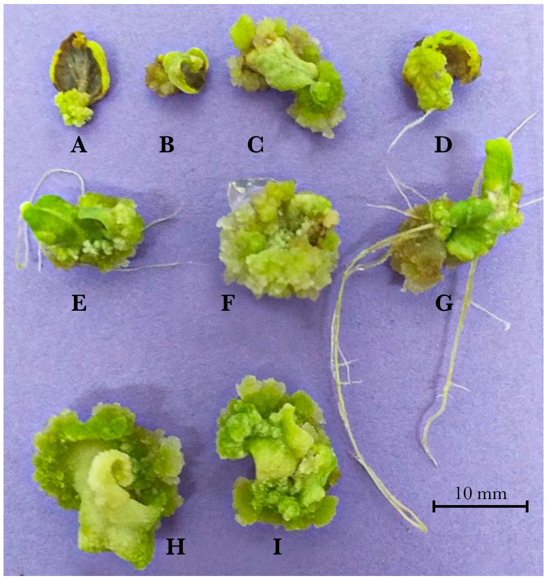

Figure 2. *Lippia graveolens* callus formation from leaf in MS basal medium added with 6-benzyl amino purine/indole butyric acid in mg L^{-1}. A: Control, B: 0.5/0.0, C: 1.0/0.0, D: 0.0/0.1. E: 0.5/0.1, F: 1.0/0.1, G: 0.0/0.5, H: 0.5/0.5, I: 1.0/0.5.

Table 3. Effect of cytosine/auxin in the multiplication and root formation stage in *Lippia graveolens* and *Oryganum vulgare* from buds.

Treatment BAP/AIB mg L^{-1}	Number of shoots	Shoot length (mm)	Root number/ explant	Root length (mm)	Propagation coefficient
Oryganum vulgare					
0.0/0.0	1.5 BC	16.6 AB	0.0 A	0.0 A	4.4 BC
0.5/0.0	1.9 ABC	12.8 B	0.0 A	0.0 A	5.1 ABC
1.0/0.0	**2.5 A**	18.2 AB	0.0 A	0.0 A	**7.3 A**
0.0/0.1	1.6 BC	17.4 AB	0.0 A	0.0 A	4.9 ABC
0.5/0.1	1.6 BC	12.5 B	0.0 A	0.0 A	3.9 BC
1.0/0.1	1.8 ABC	16.2 AB	0.0 A	0.0 A	4.8 ABC
0.0/0.5	1.1 C	22.9 A	0.1 A	1.0 A	3.4 C
0.5/0.5	1.4 BC	18.3 AB	0.0 A	0.0 A	3.8 C
1.0/0.5	2.0 ABC	16.3 AB	0.0 A	0.0 A	6.5 AB
Lippia graveolens					
0.0/0.0	1.8 DE	22.1 BC	1.3 C	3.5 C	4.0 D
0.5/0.0	3.0 CD	16.9 CD	0.2 D	0.1 C	8.7 BC
1.0/0.0	**4.4 A**	14.4 CD	0.0 D	0.0 C	**13.1 A**
0.0/0.1	1.7 DE	29.4 B	2.3 B	14.1 B	5.1 D
0.5/0.1	3.2 BC	19.2 C	0.0 D	0.0 C	10.1 BC
1.0/0.1	3.6 AB	9.0 D	0.0 D	0.0 C	4.3 D
0.0/0.5	1.3 E	44.9 A	5.1 A	47.0 A	5.9 DC
0.5/0.5	2.3 CD	23.0 BC	0.0 D	0.0 C	6.0 DC
1.0/0.5	2.0 DE	9.5 D	0.0 D	0.0 C	4.0 D

Different letters present statistically significant differences P≤0.05.

The results of this research agree with those reported by García-Pérez *et al.* (2012); via direct organogenesis, they obtained shoots of *O. vulgare* in MS medium enriched with 1 mg L^{-1} of BA. Castellano-Hernández *et al.* (2013) reported the induction of shoots of *L. graveolens* in MS medium enriched with 2.0 mg L^{-1} of BA, achieving 2.4±3.8 shoots per explant, due to a combination of 0.1/0.5 mg L^{-1} of IAA/BA of 6 mm and the induction of roots with 0.1 and 0.5 mg L^{-1} of IAA with 7.3±4 and 5.3±2.9 roots per explant. The increase in the shoot length (63±17mm) and roots (100.0±47 mm) and up to 15 roots per explant were reported by adding 1.5 mg L^{-1} of IBA.

The results of this work surpassed those reported for *L. graveolens* and *L. alba* (Bueno, 2014), where DM added with 2 mg L^{-1} of BA reported three shoots per explant at 28 days in *L. alba* and DM added with 0.5/0.1/3 mg L^{-1} of BA/AIB/phloridzin, they obtained 2.2 shoots per explant at 22 days. Sanchez-Velázquez *et al.* (2021) reported the micropropagation of *L. graveolens* from axillary buds in MS medium enriched with 2.5 mg L^{-1} of BA, obtaining shoots with great success.

CONCLUSION

The germination of *L. graveolens* seeds can be achieved in MS medium after 15 days. Microcuttings of both *L. graveolens* and *O. vulgare* were successfully established in MS basal medium enriched with 1 g L^{-1} charcoal that showed low oxidation percentages and induced up to 90% the production of shoots in the explants. The mixture of 1.0/0.5 mg L^{-1} BA/AIB induces callus formation for both species; when this medium is in contact with leaves as an explant, its formation is potentiated, achieving diameters up to 15 mm. In order to achieve the induction of shoots and roots, buds should be established in MS medium enriched with 0.5 mg L^{-1} IBA for both species; this mixture encreased the multiplication coefficients.

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REFERENCES

Acevedo, Diofanor, Navarro, Mario, & Monroy, Luis. (2013). Composición Química del Aceite Escencial de Hojas de Orégano (*Origanum vulgare*). *Información tecnológica, 24*(4), 43-48. https://dx.doi.org/10.4067/S0718-07642013000400005.

Arcila-Lozano, Cynthia Cristina, Loarca-Piña, Guadalupe, Lecona-Uribe, Salvador, & González de Mejía, Elvira. (2004). El orégano: propiedades, composición y actividad biológica de sus componentes. *Archivos Latinoamericanos de Nutrición, 54*(1), 100-111. Recuperado en 23 de febrero de 2021, de http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0004-06222004000100015&lng=es&tlng=es.

Barrales Cureño, H. J., & Ramírez Sepúlveda, M. F. (2013). Una revisión sobre la producción de taxoides anticancerígenos en cultivos *in vitro* de callos y células de *Taxus* spp. *Revista Colombiana de Biotecnología, 15*(2), 167-177. https://doi.org/10.15446/rev.colomb.biote.v15n2.41743.

Bueno, D.A.Y. (2014). Estimulación abiótica de la acumulación de compuestos bioactivos en dos especies de Lippia. Tesis doctoral en Ciencias y Tecnología en la Opción Terminal de Biotecnología Productiva. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado De Jalisco, A.C. http://ciatej.repositorioinstitucional.mx/jspui/handle/1023/487.

Castellanos-Hernández, O. A., Acevedo-Hernández, G. J., Torres-Morán, M. I., Zurita, F., Gutiérrez-Lomelí, M., Del Toro-Sánchez, C. L., & Rodríguez-Sahagún, A. (2013). *In vitro* clonal propagation and regeneration of the commercially important plant Mexican oregano (*Lippia graveolens*). In Vitro Cellular and Developmental Biology - Plant. https://doi.org/10.1007/s11627-013-9538-4.

Castillo, I.O., Almazán, A.J.S., Arellano, J.D.J.E., Vázquez, C. (2017). Recolección y Comercialización del Orégano (*Lippia* spp) en el Semi-Desierto Mexicano, Un Caso De Estudio: Reserva Ecológica Municipal Sierra y Cañón De Jimulco, México. *Revista Mexicana de Agronegocios*. 41:684-695.

Chambe, C. E. (2008). Introducción al sistema de cultivo in vitro y micro propagación de cinco ecotipos de orégano (*Origanum vulgare* L.) en condiciones de laboratorio. Tesis de grado.Facultad de Ciencias Agrícolas de la Universidad Nacional Jorge Basadre Grohmann de Tacna. http://repositorio.unjbg.edu.pe/handle/UNJBG/608

Clarenc Aarland, Rayn, Castellanos-Hernández, Osvaldo Adrián, Rodríguez-Sahagún, Araceli, & Acevedo-Hernández, Gustavo Javier. (2020). Efecto del estrés salino sobre la morfología y fitoquímica de orégano mexicano (*Lippia graveolens* Kunth) cultivado *in vitro*. *Biotecnia, 22*(3), 131-137. Epub 10 de febrero de 2021.https://doi.org/10.18633/biotecnia.v22i3.1223

Comisión Nacional Forestal CONAFOR. (2009). Fichas de información comercial de productos forestales. Coordinación General de Educación y Desarrollo Tecnológico, Gerencia de Desarrollo y Transferencia de Tecnología. Fondo Sectorial para la Investigación, el Desarrollo y la Innovación Tecnológica Forestal, SEMARNAT, México, D. F.

Comisión Nacional Forestal CONAFOR. (2011). Paquete tecnológico para la producción de orégano (*Lippia* spp.). Coordinación General de Educación y Desarrollo Tecnológico. Gerencia de Desarrollo y Transferencia de Tecnología. Zapopan, Jalisco, México. https://www.conafor.gob.mx/biblioteca/OREGANO.pdf

Cortés-Chitala, M. del C., Flores-Martínez, H., Orozco-Ávila, I., León-Campos, C., Suárez-Jacobo, Á., Estarrón-Espinosa, M., & López-Muraira, I. (2021). Identification and Quantification of Phenolic Compounds from Mexican Oregano (*Lippia graveolens* HBK) Hydroethanolic Extracts and Evaluation of Its Antioxidant Capacity. *Molecules, 26*(3), 702. doi:10.3390/molecules26030702.

Díaz-De León, C.I., González-Álvarez, M., Guzmán-Lucio, M.A., Núñez-Guzmán, G.R., & Moreno-Limón, S. (2020). El orégano de los géneros *Lippia* (Verbenaceae) y *Poliomintha* (Lamiaceae) en el Estado de Nuevo León, México. Polibotánica, (50), 1-18. Epub 25 de noviembre de 2020. https://doi.org/10.18387/polibotanica.50.1.

García-Pérez, Enrique, Fernando Francisco, Castro-Álvarez, Gutiérrez-Uribe, Janet Alejandra, & García-Lara, Silverio. (2012). Revisión de la producción, composición fitoquímica y propiedades nutracéuticas del orégano mexicano. *Revista mexicana de ciencias agrícolas, 3*(2), 339-353. Recuperado en 22 de febrero de 2021, de http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S2007-09342012000200010&lng=es&tlng=es.

Murashige, T. y Skoog, F. (1992). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum, Copenhagan* 15:473-497.

Pérez-Alonso, N., y Jiménez, E. (2011). Producción de metabolitos secundarios de plantas mediante el cultivo in vitro. *Biotecnología Vegetal*, 11(4).

Sáez, F., Sánchez, P. & Piqueras, A. (1994). Micropropagation of *Thymus piperella*. *Plant Cell Tiss Organ Cult*. 39: 269–272. https://doi.org/10.1007/BF00035981

Sánchez-Velázquez E. Rodríguez-Sahagún Araceli, Acevedo-Hernández Gustavo J., Aarland Rayn C. y Castellanos-Hernández Osvaldo A. (2021). Effect of light sources on the micropropagation of mexican origanum (*Lippia graveolens*). *e-CUCBA. 15*(8): 44-52.

AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of plant growth promoting rhizobacteria (PGPR) on the growth of chili habanero pepper (*Capsicum chinense* Jacq.)

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ABSTRACT

Objective: To evaluate the effect of plant growth promoting rhizobacteria (PGPR) on the growth and production of habanero pepper (*Capsicum chinense* Jacq.).

Design/Methodology/Approach: Twelve strains of PGPR were evaluated in habanero pepper seeds of the orange variety. The species of PGPR were *Rhizobium leguminosarum*: (CP Méx 46), *Pseudomonas* spp: (*P. fluorescens*, C2, A7, A9, A9m, Avm); *Azospirillum*, (Sp7, Sp 59, UAP 40, UAP154), plus a control treatment, giving a total of 13 treatments. The study variables were seedling emergence (SE), plant height (PH), white fruit incidence (WFI), virotic plants (VP), days to flowering (DF) and fresh fruit yield (FFY). The experimental design was random blocks with four repetitions.

Results: An effect on the growth of habanero pepper from PGPRs was found in all the variables studied. Seedling emergence and their height was favored by strains Sp9 (84.16%) and A7 (73.44). The number of white flies decreased with the inoculation of CP Méx 46, while the incidence of virosis decreased in plants inoculated with SP9 (32.00%). The highest yield of fresh fruit was found in plants with the strain AVM with 16636 kg ha^{-1}.

Findings/Conclusions: The effect of inoculation with PGPR is in function of the strain used and the study variable, growth stage and development stage of the habanero pepper plant.

Keywords: Inoculation, *Rhizobium*, *Pseudomonas* spp, *Azospirillum*.

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INTRODUCTION

Habanero pepper (*Capsicum chinense* Jacq.) is one of the most important crops of the Yucatan Peninsula in Mexico. The average surface cultivated annually is 700 ha; however, it has increased in various states in Mexico to cover the national and international demand. An ecologically acceptable alternative to increase the crop's yield is the inoculation with growth promoting microorganisms, called biostimulants or biofertilizers (Compant *et al.*, 2010).

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One of these microorganisms is plant growth promoting rhizobacteria (PGPR). Rhizobacteria exert beneficial effects on the plants through direct and indirect mechanisms, or a combination of both (Parray *et al.*, 2016). Direct mechanisms happen when the bacteria synthesize metabolites that ease or increase the availability of nutritional elements, required for their metabolism and nutritional process (Gómez-Luna *et al.*, 2012). Among the direct mechanisms, the following stand out: nitrogen fixation (N); synthesis of plant hormones, vitamins and enzymes, solubilization of phosphorus (P) (Esquivel-Cote *et al.*, 2013), while the indirect mechanisms are characterized by the PGPR causing the decrease or elimination of plant pathogen microorganisms, whether through the production of antimicrobial substances or antibiotics, lithic enzymes or a combination of these; from competition of nutrients or space in the ecological niche (Esquivel-Cote *et al.*, 2013). According to Constantino *et al.* (2008), the vegetative growth of plants and the fruit yield of *Capsicum chinense* were higher when inoculated with *A. brasilense*, *Azotobacter chroococum* and *Rhizophagus* spp. than in plants without inoculation. For their part, Reyes-Ramírez *et al.* (2014), in their evaluations with three strains of rhizobacteria in habanero pepper plants treated with *Pseudomonas* found greater height of the plant, stalk diameter and total dry biomass than the control plants 120 (ddt).

They also found that the yield was higher (899.84 g per plant) and the fruits had greater length, diameter and weight, concluding that the inoculation when transplanting with *Pseudomonas* spp. to habanero pepper plants increased their growth and the size of the fruit. In collections and evaluations of rhizobacteria strains evaluated in poblano chili pepper plants (*Capsicum annuum*), an increase of 20% was found in the weight of the aerial part of the plant, concluding that the rhizobacteria strains can be used to increase the quality of poblano chili seedlings, which could guarantee a better establishment and health of these in the field (González-Mancilla *et al.*, 2017). Complementary studies in tomato and pepper point to the use of rhizobacteria of the genus *Bacillus* spp. which increased the percentage of germination of pepper seeds in 7% and the biomass from 16 to 37% (Luna-Martínez *et al.*, 2013). In this context, the present study evaluated the effect of different experimental PGPR strains on the growth of habanero pepper (*Capsicum chinense* Jacq.).

MATERIALS AND METHODS

The experiment was conducted in Chiná, Campeche, Mexico (19° 45' 18" N and 90° 26' 23" W). The orange variety of habanero chili pepper was used. Twelve experimental strains of plant growth promoting rhizobacteria (PGPR) were evaluated: CP Méx 46 (*Rhizobium leguminosarum*), *P. fluorescens*, C2 (*P. fluorescens*), *P. putida*, A7 (*Pseudomonas* sp.), A9 (*Pseudomonas* sp.), A9m (*Pseudomonas* sp.), Avm (*Pseudomonas* sp.), Sp7 (*Azospirillum brasilensis*), Sp 59 (*Azospirillum brasilensis*), UAP 40 (*Azospirillum brasilensis*), and UAP154 (*Azospirillum brasilensis*).

The chili seeds were inoculated with bacteria suspensions of each strain evaluated with a bacterial suspension volume of 2.5 mL applied to each lot of seeds (10^9 cells per milliliter of suspension). Once the habanero pepper seeds were inoculated, they were sown manually in polystyrene trays with 200 cavities, depositing one seed per cavity at a depth of one centimeter.

The substrate used was Canadian peat moss™. Ten days after sowing (DAS) the emergence of the seedlings began, and the trays were placed in seed banks of a rustic greenhouse. The irrigation was applied according to the needs of the plant, and irrigating once daily was enough. Derosa® 1+Previcur® at a dose of 1.0 mL L^{-1} were applied, respectively. Raizal® 0.5 g L^{-1} of water was applied in later irrigation to promote the rooting of the seedling. Fertilization was carried out by the leaf using the formula 20-30-10 (N-P-K), giving on average two applications per week at a rate of 2 g L^{-1} of water.

Transplant was conducted at 60 DAS. The distances between plants were 50 cm and 1.5 m between furrows. In the field, habanero pepper plants were subjected to the agronomic management in fertigation recommended by Soria *et al.* (2002). The experimental unit consisted of three furrows 10 m long and 1.5 m wide, considering as a useful plot the central furrow constituted by 21 plants. The study variables were the seedling emergence (SE), which was done through visual counting of the sowing substrate expressed in percentage at 10 DAS; plant height (PH) prior to transplant, days to flowering, plants with virosis (PV) and total fresh fruit yield (FFY). A completely random block experimental design with four repetitions was used. The treatments were the 12 strains of rhizobacteria inoculated on habanero chili seeds and a control treatment without inoculation. The data obtained were subjected to a variance analysis and Tukey's means comparison test ($P \leq 0.05$), through the statistical analysis software SAS (Statistical Analysis System Institute).

RESULTS AND DISCUSSION

Plant emergence

The effect of the rhizobacteria on the emergence of habanero chili seedlings was variable. The two best treatments were the non-inoculation of seeds, with 84.36 plants emerged at 10 DAS, and the effect of strain Sp 59 (*Azospirillum* spp.) with 84.16 plants (Table 1). This effect is barely within the percentage of emerged plants necessary for the commercial production of habanero pepper plants in trays with 80%; therefore, the impact of PGPRs was not clear for the conditions of study. Lagunas *et al.* (2001) reported that the inoculation with *Bacillus firmus* increased their germination in 6.0%, effect of similar magnitude to that found in this assay. Similar results have shown that inoculation with *Bacillus* spp. in poblano chili (*Capsicum annuum*) promoted increments in germination of 7% (Jalili *et al.*, 2009). This improvement in germination can be explained by the reduction of the levels of ethylene in the seed and the increase of indole-3-acetic acid, stimulating the cell division and an increase in germination.

Plant height

The plant height of habanero chili at the time of transplant presented more evident effects regarding the rhizobacterial inoculation, which is why the inoculated plants presented a higher response to inoculation and therefore a greater plant height than those not inoculated (Table 1). The results showed that the strains with greatest effect were a A7 (*Azospirillum*) and Avm (*Pseudomonas*) with plants of 23.44 and 20.8 cm height, superior to those found at treatment without inoculation (14.02 cm).

Table 1. Seedling germination and plant height at plant transplant of *Capsicum chinense* Jacq. inoculated with PGPR.

Treatment (strain)	Species	Germination	Heigth (cm)
Cp Mex 46	(*Rizobium leguminozarum* by *phaseoli*)	79.34 d	16.95 def
P. fluorescens	*P. fluorescens*	72.34 b	14.38 ab
C2	(*P. fluorescens*)	78.30 d	18.33 f
P. putida	(*Pseudomonas putida*)	76.14 c	15.69 bcd
A7	(*Pseudomonas* sp. cepa 7)	74.06 b	23.44 i
A9	(*Pseudomonas* sp. cepa 9)	74.14 b	14.28 ab
A9m	(*Pseudomonas* sp. cepa A9m)	81.34 e	17.12 ef
Avm	(*Pseudomonas* sp. cepa Avm)	74.06 b	20.8 h
Sp 7	*Azospirrillum brasilensis* cepa Sp7	76.47 c	17.33 ef
Sp 59	*Azospirrillum brasilensis* cepa Sp59	84.16 f	15.05 abc
UAP 40	*Azospirrillum brasilensis* cepa UAP40	79.06 d	16.02 cde
UAP 154	*Azospirrillum brasilensis* cepa Sp154	81.32 e	15.43 abc
Not inoculated		84.36 f	14.02 a
DMS		1.16	1.39

Treatments with the same letter are statistically equal (Tukey $\alpha=0.05$).

This increase in the growth can be translated into more vigorous plants, better nourished than those that use less time to obtain the adequate height of transplant (12 cm). On average, the plants inoculated with strains of rhizobacteria presented a height at harvest of 22.07 compared to the 14.02 cm obtained in plants without inoculating, which implies less time in the production of plants without devaluing their quality. These results can be compared to those obtained by Castillo *et al.*, (2017) in their evaluations with different strains of experimental rhizobacteria in the production of habanero chili plants, who found that inoculation with rhizobacteria in habanero chili with rhizobacteria strains BSP1.1, R44 and P61 promoted a significant increase in the height of plants in tray (39, 35 and 25%, respectively), compared to the control plants. It is important to consider that colonization of rhizobacteria requires time and happens only when there is compatibility between the microorganisms and the intrinsic factors of the plant, such as root exudates (Khalid *et al.*, 2004, Trivedi *et al.*, 2012). In addition, it is necessary to consider the existence of other physiological mechanisms promoted by PGPRs such as better water absorption and efficient use of mineral elements (Egamberdiyeva, 2007), production of indole-3-acetic acid (IAA), which promotes root or vegetative development (García *et al.*, 2010), with the consequent reduction of ethylene in addition to a better use of the nutrients in the growth substrate (Husen *et al.*, 2011).

Incidence of white fly

The plants inoculated with the strains CP Mex 46, *P. fluorescens* and C2 (*Pseudomonas*) presented lower incidence of white fly (Table 2), showing on average 17, 16.5 and 17 insects per trap, respectively. The variation of the results found can be attributed to the effects of each strain of rhizobacteria in the study and the rhizosphere interaction in the cultivation site, as well as the physical and chemical conditions of the soil. With relation to this variable, authors such as Chiquito (2002) in jalapeño pepper and tomato crops, found that plants inoculated with rhizobacteria presented low incidence of white fly, attributing that to the presence of siderophores, hydrogen cyanide, and salicylate in plants. The low incidence of white fly can also be attributed to the suppression of substances that attract the white fly in inoculated plants (Zendher *et al.*, (1997). According to Martínez and Carrillo (1990) the genera *Pseudomonas*, *Rhizobium* and *Azotobacter* are the ones that activate most the synthesis of siderophores.

Number of virotic plants

The number of virotic plants from the effect of inoculation with rhizobacteria varied significantly (Table 2). On average, the percentage of virotic plants was lower in plants with PGPR (58.88%) compared to the plants not inoculated (62.25%). The results showed that the strains *Sp9 and Avm* presented lower percentage of virotic plants, 32% and 34%, respectively, significantly lower incidence than the control treatment (62.25%).

These percentages could be favored by the intense precipitations of the prior agricultural cycle, which abated drastically the populations of white fly in the field, giving as a result a reduction of the transmission of virosis. It should be considered that the resistance induced is associated with the capacity of PGPRs to promote the growth of plants and protect them against the attack from pathogens. The effect of repelling the white fly or keeping the plant from becoming a host can be attributed to the induced resistance provoked by a large variety of microorganisms such as *Pseudomonas fluorescens* and *Pseudomonas putida*,

Table 2. Incidence of white fly (*Bemisia tabasi*), virotic plants, and day to flowering *Capsicum chinense* Jacq. plants inoculated with PGPR.

Treatment (strain)	Species	Whitefly incidence (PL)	Virotic plants (%)	Flowering (days)
Cp Mex 46	(*Rizobium leguminozarum* by *phaseoli*)	17.00 a	54.67 d	62.27 d
P. fluorescens	*P. fluorescens*	16.75 a	68.25 h	58.24 a
C2	(*P. fluorescens*)	17.25 a	60.67 df	63.24 e
P. putida	(*Pseudomonas putida*)	19.75 ab	64.05 g	62.72 de
A7	(*Pseudomonas* sp. cepa 7)	30.50 b	60.67 df	58.70 ab
A9	(*Pseudomonas* sp. cepa 9)	24.00 b	61.37 f	58.72 ab
A9m	(*Pseudomonas* sp. cepa A9m)	38.50 c	69.00 h	59.27 b
Avm	(*Pseudomonas* sp. cepa Avm)	29.00 b	34.62 b	58.11 a
Sp 7	*Azospirrillum brasilensis* cepa Sp7	19.50 ab	58.57 f	58.76 ab
Sp 59	*Azospirrillum brasilensis* cepa Sp59	21.00 ab	32.00 a	59.24b
UAP 40	*Azospirrillum brasilensis* cepa UAP40	34.75 b	40.35 c	58.29 a
UAP 154	*Azospirrillum brasilensis* cepa Sp154	38.50 c	62.25 fg	58.24 a
Not inoculated		38.50 c	89.30 i	61.05 c
DMS		3.51	2.35	0.85

Treatments with the same letter are statistically equal (Tukey $\alpha=0.05$).

species used in this study, which could produce various metabolites as a defense system, among which the following stand out: AS, lipopolysaccharides (LPS), siderophores, cyclic lipopeptides, 2,4-diacetylphloroglucinol, homoserine lactones, and volatile compounds such as acetoin and 2,3-butanediol (Molina-Romero *et al.*, 2015). Also, it is important to consider that the plants have genes that codify to generate chemical defense mechanisms against the attack of pathogenic organisms, whether by decreasing or impeding such an attack. This biological phenomenon has been called systemic resistance (Gómez and Reis, 2011).

Days to flowering

The effect of inoculation with PGPR on 50% of the flowering of habanero chili plants was variable, and the following strains were found to be outstanding: Avm (*Pseudomonas* sp.) with 58.11 days, followed by UAP 154Y *P. fluorescens* with 58.24 days for both, less time than that found with the control treatment of 61.05 days.

The difference of 2.94 days between AVM and the control seems small, although in three days the prices of the fruit of the habanero chili can vary drastically. The results found could be influenced by the management of the crop, fertilization, water availability, temperature, presence of diseases, or some pest insect, conditions that could induce the reproductive stage earlier. With relation to this, Molina-Romero *et al.* (2015) established that PGPRs have the capacity to produce growth promoting substances (auxins; gibberellins; and cytokinins), and with this to promote the floral induction, floral differentiation, and floral development, processes that entail metabolic changes in the plant, such as gibberellin production (Yuri *et al.*, 2002).

Fresh fruit yield

The habanero chili pepper yield was favored in different magnitude from rhizobacterial inoculation. The best treatment found was inoculation with the strain Avm (*Pseudomonas* spp.) with average yield of 16636.30 kg ha^{-1}, compared to the treatment without inoculating with a yield of 12554.80 kg ha^{-1} with a difference of 4081.50 kg ha^{-1} (Figure 1).

Recent studies with habanero chili in greenhouse conditions inoculated with different strains of rhizobacteria showed that *Pseudomonas* spp. induced a significant difference in fruit yield compared to the control treatment of 899.84 g per planta. Rini and Sulochiana (2006) documented the significantly higher growth and yield of the *C. annuum* plants inoculated with *Pseudomonas fluorescens* compared to the control without inoculating. For their part, Constantino *et al.* (2008) observed a higher growth and yield in the *Capsicum chinense* plants treated with *Azospirillum brasilense* and a consortium of *Rhizophagus* spp. compared to plants not inoculated.

Authors such as Reyes-Ramírez *et al.* (2014) found an increase in habanero chili in the fruit yield and number of fruits ($p \leq 0.05$) from inoculation with *Pseudomonas fluorescens*. This effect on the fresh fruit yield of the chili has possibly been observed in different plant species such as tomato from inoculation with *Bacillus* spp., *Aeromonas* spp. and *Pseudomonas lini*, plus an effect of the rhizobacteria-substrate interaction.

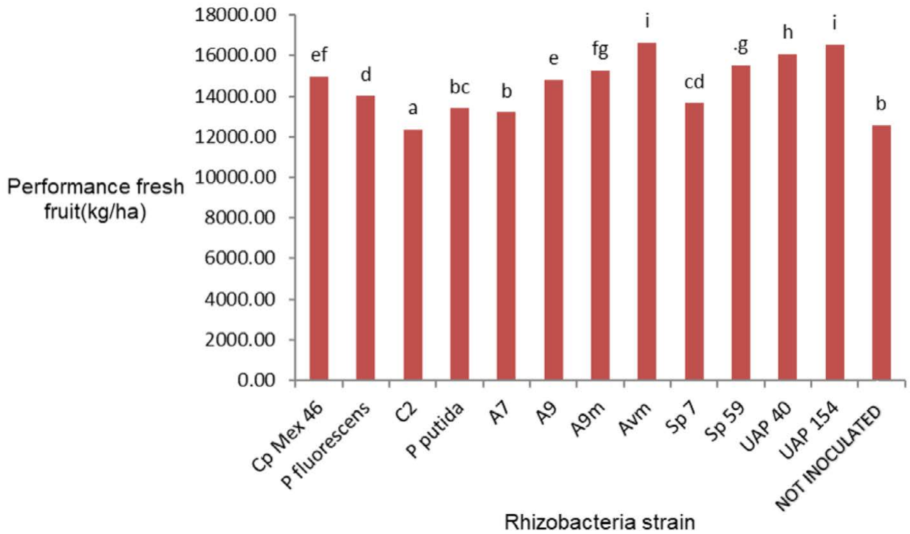

Figure 1. Yield by treatment of fresh fruit of habanero pepper. Treatments with the same letter are statistically the same (Tukey α=0,05). DMS=392.04

CONCLUSIONS

The results in this study indicated that there is a promotion of the growth of the habanero chili plant from the effect of the inoculation with rhizobacteria with variation due to the strain and the variable or the state of development of the plant. It is concluded that the *Pseudomonas* genus is potentially usable in the production of habanero chili seedling and fruit.

REFERENCES

Castillo-Aguilar C de la C, J J Zúñiga-Aguilar, A A Guzmán-Antonio, R Garuña.2017. La inoculación von PGPR mejora el crecimiento, l absorción de nutrientes y parámetros fisiológicos de las plantas de capsicum chinense. ϕyton 9457 (86): 199-204.

Compant, S. C. Clement, and A. Sessitsch. 2010. Plant growthpromoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospect for utilization. *Soil Biol. Biochem.* 42: 669-678.

Constantino M., R. Gómez-Álvarez, J. D. Álvarez-Solís, V. Geissen, E. Huerta, and E. Barba. 2008. Effect of inoculation with rhizobacteria and arbuscular micorrhizal fungi on growth and yield of *Capsicum chinense* Jacquin. *J. Agric. Rural Develop. Trop. Subtrop*. 109: 169-180.

Chiquito C RG. 2002. Impacto de PGPR en la uniformidad, vigor y condición fitosanitaria de chile (*Capsicum annum* L) y jitomate (*Licopersicon esculentjum* Mill) determinado al momento del trasplante. Tesis de Maestría en Ciencias, Colegio de Postgraduados. Montecillo, Texcoco, Edo. De México.

Egamberdiyeva, D. (2007). The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. *Applied Soil Ecology* 36: 184-189.

Esquivel-Cote, R., Gavilanes-Ruiz, M., Cruz-Ortega, R. y Huante, P. (2013). Importancia agrobiotecnológica de la enzima ACC desaminasa en rizobacterias, una revisión. *Revista Fitotecnia Mexicana, 36*(3), 251-258.

García F, H Muñoz, C Carreño, G Mendoza (2010) Caracterización de cepas nativas de *Azospirillum* spp. y su efecto en el desarrollo de *Oryza sativa* L. "arroz" en Lambayeque. Sci. Agropec. 1:107- 116

Gómez-Luna, B. E., Hernández-Morales, A., HerreraMéndez, C. H., Arroyo- Figueroa, G., VargasRodríguez, L. y Olalde-Portugal, V. (2012). Aislamiento de bacterias promotoras del crecimiento de la rizósfera de plantas de guayaba (*Psidium guajava*). *Ra Ximhai, 8*(3), 97-102.

Gómez, D. E. y Reis, E. M. (2011). Inductores abióticos de resistencia contra fitopatógenos. *Química Viva*, 10(1), 6-17.

González- Mancilla A, J J Almaraz- Suárez, R Ferrera-Cerrato1, M P Rodríguez- Guzmán, O R Taboada-Gaytán, A Trinidad-Santos, A Alarcón, R I Arteaga-Garibay. 2017. Caracterización y selección de rizobacterias promotoras de crecimiento en plántulas de chile poblano (*Capsicum annuum* L.). *Rev. Int. Contam. Ambie. 33*(3) 463-474.

Husen E, A T Wahyudi, A S Gianto (2011). Soybean response to 1-aminociclopropano-1-carboxylate deaminase-producing *Pseudomonas* under field soil conditions. *Amer. J. Agric. Biol. Sci*. 6:273-278.

Jalili F, K Khavazi, E Pazira, A Nejati, H A Rahmani, H R Sadaghiani, M Miransari (2009) Isolation and characterization of ACC deaminase-producing fluorescent pseudomonads, to alleviate salinity stress on canola (*Brassica napus* L.) growth. *J. Plant Physiol*. 166:667-674.

Khalid, A., S. Tahir, M. Arshad & Z.A. Zahir (2004). Relative efficiency of rhizobacteria for auxin biosynthesis in rhizosphere *vs*. non-rhizosphere soil. *Australian Journal of Soil Research* 42: 921-926.

Lagunas L J, M E Zavaleta, K S Osada, O S Aranda, R I Luna, H H Vaquera (2001). *Bacillus firmus* como agente de control biológico de *Phytophthora capsici* Leo. en jitomate (*Lycopersicon esculentum* Mill.). Rev. Mex. Fitopat. 19:57-65.

Luna-Martínez L, R A Martínez-Pacheco, M hernández Iturriaga, S M Arvizu-Medrano, J R Pacheco-Aguilar. 2013. caracterización de rizobacterias aisladas de tomate y su efecto en el crecimiento de tomate y pimiento. *Rev. Fitotec. Mex. Vol. 36* (1): 63-69.

Martínez C B, G Carrillo-Castañeda. 1990. Estudio sobre el efecto de *Pseudomonas fluorescems* en plantas de *Phaseolus vulgaris* L. *Agrociencia* 3:127-139

Molina-Romero, D., Bustillos-Cristales, M. R., Rodríguezandrade, O. y Elizabeth, Y. (2015). Mecanismos de fitoestimulación por rizobacterias, aislamientos en América y potencial biotecnológico. *Revista de La DES Ciencias Biológico Agropecuarias, 17*(2), 24-34.

Parray, J. A., Jan, S., Kamili, A. N., Qadri, R. A., Egamberdieva, D. & Ahmad, P. (2016). Current Perspectives on Plant Growth-Promoting Rhizobacteria. *Journal of Plant Growth Regulation, 35*(3), 877-902. doi:10.1007/s00344-016-9583-4.

Reyes-Ramirez A, M López arcos, E Ruiz-Sánchez, L latournerie-Moreno, A Pérez-Gutierrez, M G Lozano-Contresras, M Zavala-León. 2014. Efectifidad de inoculantes microbianosen el crecimiento y productividad de chile habanero (*Capsicun chinense*). *Agrociencia* 48:285-294.

SAS Institute Inc. 2004. SAS/STAT User's guide, Version 9.1. SAS Institute. Cary, NC.

Soria F. M., A. Trejo, J. Tun, R. Saldívar (2002). Paquete tecnológico para la producción de chile habanero (*Capsicum chinense* Jacq.), Secretaría de Educación Pública/ SEIT/Instituto Tecnológico Agropecuario de Conkal, Yucatán. 21p.

Trivedi, P., A. Pandey, and L. M. Palni. 2012. Bacterial inoculants for field applicactions under mountain ecosystems: present initiatives and future prospects. In: Maheshwari, D. K. (ed). Bacteria in Agrobiolog: Plant Probiotics. Springer-Verlag. pp: 15-44.

Yuri, J., Lobos, G. y Lepe, V. 2002, sept. Pomáceas. Boletín técnico. Universidad de Talca. 2 (5): 1-4.

Zehnder G, J KJloepper, C yan, G wel. 1997. Induction of systemic resistance in cucumber against cuucumber beetles by plant growth promoting rhizobacteria. *J Econom.Entomology* 89:510-514.

AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Characterization of Persian lime production (*Citrus × latifolia*; Tanaka ex Q. Jiménez)

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ABSTRACT
Objective: To characterize the production system of Persian lime (*Citrus × latifolia*; Tanaka ex Q.Jiménez) in Martínez de la Torre, Veracruz, Mexico.
Design/Methodology/Approach: A semi-structured survey was applied with 50 Persian lime producers, through a directed sample. For this study a logistic regression was conducted: logit model, through measurement of the binary or dummy dependent variable, where the dependent variable takes values of "0 and 1".
Results: Lime producers are mostly men with an average age of 54 years, the type of property is ejido and they have been producing lime from 15 to 30 years, the knowledge used for the production are rainy periods and soil fertility. Most use intolerant rootstock. Regarding the econometric result, variables with higher significance were obtained that are related to the dependent variable.
Study Limitations/Implications: It offers elements for the production of Persian lime.
Conclusions: Finally, there is low participation of women in agricultural tasks. When it comes to the application of fertilizers, pruning and an adequate use of fertilization influence the production yield.

Keywords: Production, Agriculture, Logit Model.

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INTRODUCTION

Agricultural activities tend to be destined to the production of foods and to obtaining vegetables, fruits, leafy vegetables and cereals. Agriculture implies the transformation of the environment to satisfy the needs of man (Vivero & Álvarez, 2020). "Most poor people in the world live in rural zones. Agriculture is a source of livelihood for 86 percent of the people who live in rural areas" (Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO), 2020). According to estimations by FAO (2020), agriculture provides employment to around 1,300 million smallholders and landless workers throughout the world. Nine out of ten farming operations in the world are managed by families and close to 80% of foods in the world are produced by family farms that are operated and nearly all depend on family workforce.

Image by fernando zhiminaicela at Pixabay

Small-scale farmers and family farmers have economic links with the rural sector, so they promote employment to a large degree, especially in developing countries where agriculture still concentrates the majority of the workforce (FAO, 2020). Concerning the data obtained by the Agriculture and Livestock National Survey (INEGI, 2020), for 2019 it reports that 17% of the positions of day laborers are occupied by women and 83% by men; from 100 producers, 17 are women responsible for the management and decision making in agriculture and livestock production units.

Regarding agriculture in Veracruz, this state has a solid agrifood production. Agriculture in Veracruz contributes 50.6% of the agrifood production of the state, followed by livestock production which contributes 47%, and lastly the fishing sector that contribtues 2.4%. It is the second state with greatest contribution to the national total (Agroproductores, 2020). The district Martínez de la Torre is known as the citrus capital; it is the district with most citrus activity. Table 1 shows that by 2019, Persian lime production occupies the first place with a surface of 15,584 harvested hectares, followed by the cultivation of orange with 9,571 hectares of harvested surface, and grapefruit occupies the third place with 2,280 hectares.

Physiological characteristics of Persian lime (*Citrus latifolia*)

Persian lime is a tree that is planted with the use of the technique called pattern graft; that is, an active branch or shoot which is grafted in a pattern for its production. The pattern is a citrus variety resistant to root diseases and with the capacity to improve nutrient absorption (Agroproductores, 2020). The pattern selection will be done with the conditions of the producing agricultural zone and with the advice of an expert in the area. Some patterns used to graft Persian lime are: alemow (*Citrus macrophylla*), bitter orange (*Citrus aurantium*), volkamer lime (*Citrus volkameriana*), among others (Agroproductores, 2020).

MATERIALS AND METHODS

Description of the study site

The state of Veracruz is found in central-eastern Mexico, on the Gulf of Mexico coast. It has 212 municipalities and according to the most recent Population and Housing Census from INEGI (2020), the state is leader in sugarcane, orange, pineapple and maize grain production. Products such as lime and coffee are recognized globally due to their quality

Table 1. Main citrus fruits in Martínez de la Torre.

Cultivation	Sown	Harvested	Production volume (ton)	Yield (ton)	Price	Production value (thousands of pesos)
Lemon	15,584.00	15,534.00	229,221.60	14.76	6,560.29	1,503,761,018.82
Orange	9,571.00	9,530.00	121,939.50	12.80	2,369.04	288,879,580.80
Grapefruit	2,280.00	2,275.00	102,375.00	45.00	3,329.74	340,882,132.50
Tangerine	128.00	125.00	2,485.00	19.88	3,000.00	7,455,000.00

Source: Prepared by authors with information from the database (SIAP, 2020).

and exported to more than 20 countries in the world. The most important municipalities of the state in agricultural production are Tres Valles, Playa Vicente, Álamo Temapache, Coatepec and Isla, and in terms of Persian lime production they are Atzalán, Cotaxtla, Cuitláhuac, Carrillo Puerto, Martínez de la Torre, Misantla, San Rafael, Tlapacoyan and Papatlan (INEGI, 2020).

The municipality of Martínez de la Torre is located in the northern zone of the state, on coordinates 20° 04' latitude North and 97° 04' longitude West, at an altitude of 151 meters above sea level. It borders north with Tecolutla, Papantla and San Rafael, east with Nautla and Misantla, south with Atzalan, Misantla and Tlapacoyan, and west with Papantla and the state of Puebla; it has a surface of 815.13 Km^2 and occupies 1.07% of the territory in Veracruz; it is located at an approximate distance of 101 Km from Xalapa, capital of the state of Veracruz (H. Ayuntamiento de Martínez de la Torre, 2019). Regarding the study zone, it is represented by conventional producers of Persian lime, from the municipality of Martínez de la Torre in the state of Veracruz.

Research techniques

Fifty (50) semi-structured surveys were conducted through sampling directed toward different producers of Persian lime. In the first section of the survey, the sociodemographic characteristics of the producers were identified, and in the second section the economic characteristics that were used for the econometric model.

For the data analysis obtained, the IBM Statistical Package for Social Science (SPSS) Version 25 software was used, where statistical tests and the logit regression model were carried out. Relative frequency and absolute frequency were used for descriptive statistics.

Econometric model

For this study, a modelling process was conducted through the logistic regression econometric model: logit model, by measuring the dependent variable productivity, which is binary or dummy; that is, it is only characterized with two values, "0" for producers that have low productivity which are under the average of the yield obtained in the study zone and "1" for producers that obtain yields higher than the mean. The independent variables were: surface (ha), fertilizer 20-10-20, complex fertilizer and pruning.

The Logit model was considered a binary response model

$$P\left(Y=1\middle|X_1,X_2,,,Xk\right)=G\left(\beta_0+\beta_1X_1+\beta_2X_2+,,,+\beta_kX_k\right) \quad \text{Equation (1)}$$

To avoid the problems of the linear probability model, they are specified as $y=G(X\beta)$, where G is a function that takes values strictly between 0 and 1 (0<G(Z)<1), for all the real numbers of z, for the Logit model whose expression will be:

$$Si\ G(z)=\frac{e^z}{1+e^z} \quad \text{Equation (2)}$$

$$Y = G(z) = G(\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_k X_k) = \frac{e^{\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_k X_k}}{1 + e^{\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_k X_k}}$$

Equation (3)

The logistic regression coefficients can be used to estimate the ratio of the advantages of probability for success to the probability of failure of each independent variable of the model. Operatively, parameters αand αare estimated by maximum likelihood through the model (Logit binomial).

Maximum likelihood method

The method that was used to estimate the Logit model is the Maximum Likelihood Method. This method estimates the parameters of the model maximizing the likelihood function with regard to the parameters of the model, finding the values of the parameters that maximize the probability of finding the responses obtained in the survey, according to (Pérez, 2007), the logarithm of the conditional likelihood function is given by:

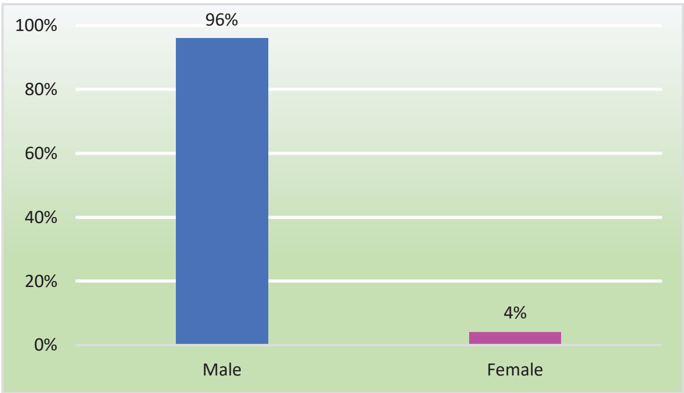
$$L(\beta) = \prod_{yi=1} P_j \prod_{yi=0} = (1 - Pj) = \prod_{i=1}^{n} G(X'\beta)^{Y^i} (1 - G(X'\beta))^{1=Y_i}$$

Equation (4)

RESULTS AND DISCUSSION

Descriptive analysis of the production indicators

A descriptive analysis of the information compiled through the questionnaire applied to Persian lime producers in Martínez de la Torre was made. Graph 1 represents the variable sex, and it shows that the women are only 4 percent, which is why Persian lime production is led by men with 96 percent; it should be highlighted that women have very low participation in the Persian lime production. When it comes to the variable of age, it is observed that 37 percent of the producers are men in ages between 50 and 69 years and



Graph 1. Sex of Persian lime producers.
Source: Prepared by authors based on field research 2020.

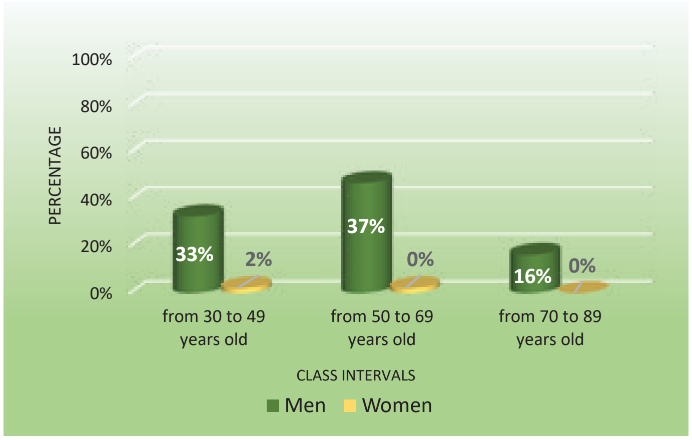
only 2 percent are women in ages between 30 and 49 years. Finally, age is not limiting to Persian lime production since in the results obtained they indicate that the maximum age is 84 years and the minimum 32 years; the mean age of producers of Persian lime is 54 years (Graph 2).

Concerning the type of property, Graph 3 shows that 86 percent of Persian lime producers have *ejido* property; this 86 percent is made up by 42 producers, and on the other hand, only 14 percent own private property.

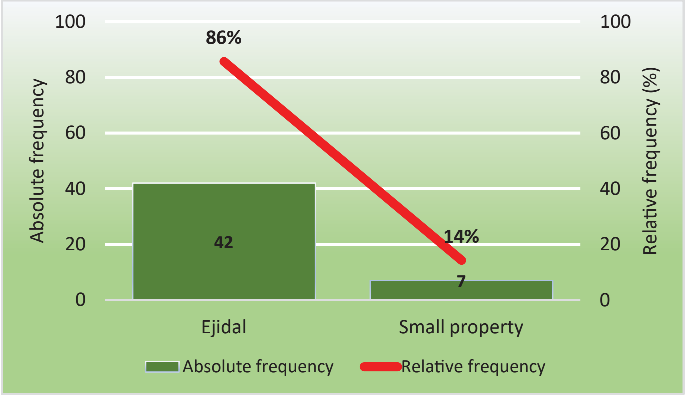
Graph 4 refers to the time that producers from Martínez de la Torre have been producing Persian lime, and it was observed that the class of highest predominance is between the class interval of 15 to 20 years, with this class being the one of highest percentage with 31 percent and an absolute frequency of 15 producers.

Graph 5 represents the type of knowledge that Persian lime producers apply in Martínez de la Torre, where they were qualified as: Do apply and Do not apply.

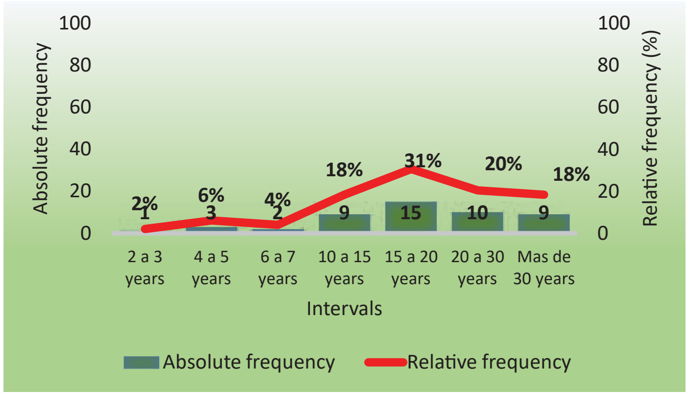
The analysis where they Do apply was the following: The producers are influenced in their totality, with 100 percent, with the class interval of rainfall since it is fundamental for



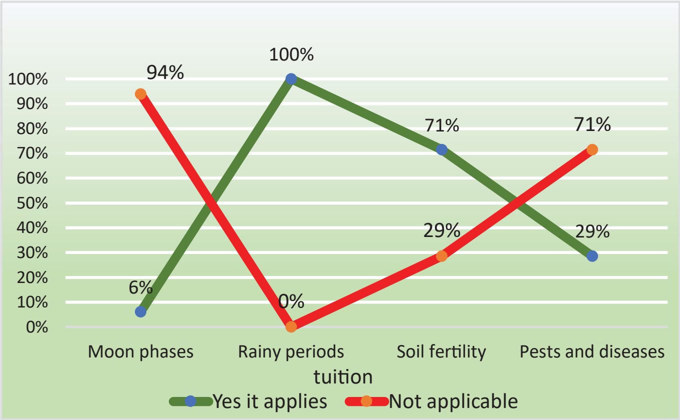
Graph 2. Age of Persian lemon producers.
Source: Prepared by authors based on field research 2020.



Graph 3. Type of residence property.
Source: Prepared by authors based on field research 2020.



Graph 4. Years as lime producer.
Source: Prepared by authors based on field research 2020.

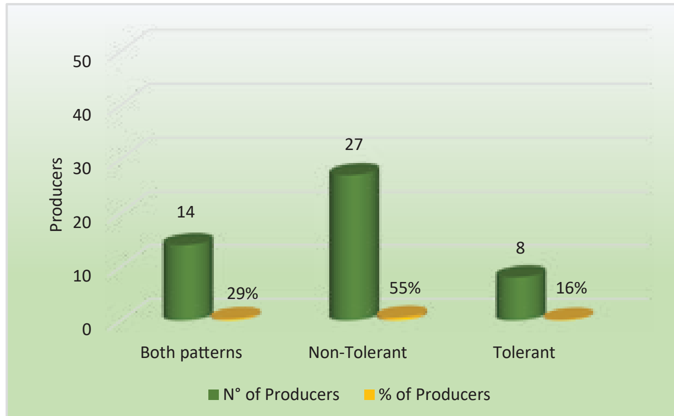


Graph 5. Type of applied knowledge.
Source: Prepared by authors based on field research 2020.

the crop's development; soil fertility is another important indicator where the producers are taken as reference for lime production and is represented by 71 percent of the total producers; for the class of pests and diseases, it represents 29 percent of the total producers; the lunar phases are applied by only 6 percent of the producers.

The analysis where they Do not apply is the following: For the class of pests and diseases, 71 percent of the total producers do not apply this knowledge, the class soil fertility is represented by 29 percent of the total producers, which pointed out that they do not apply this knowledge, and in terms of the class lunar phases, 6 percent of the total of producers do not apply this knowledge.

In relation to the pattern that they use for Persian lime shade, they are divided into two categories: tolerant and intolerant. The analysis showed the following: in Graph 6, 29 percent is represented by 14 producers who work with both patterns, it should be highlighted that the tolerant patterns most used for Persian lime production are: Swingle, Volkameriano and Macrofila, and the intolerant pattern is Cucho; 55 percent is represented by 27 producers who work with the intolerant pattern; 16 percent is represented by 8 producers, who are producing with tolerant patterns.



Graph 6. Type of pattern used.
Source: Prepared by authors based on field research 2020.

Results from the Logit regression model

From the econometric model of the Logit logistic distribution, the production is considered as dependent variable and as explicative independent variables: surface sown in (ha), fertilizer 20-10-20, complex fertilizer and they are declared in the following way:

$$PRODUCTIVITY = \alpha_0 + \beta_1 SURFACE - SOWN + \beta_2 FERT - 201020$$
$$+\beta_3 FERT - COMPLEX$$

The Logit regression for the first step shows that 64 percent of the cases were adjusted correctly as shown in Table 2.

Another information exit obtained by the Logit regression in Table 3 was the Hosmer and Lemeshow test. Normally it is recommended that the value be higher than .05 to point out that it is a reliable model; for this case the study showed that it is higher than .05, verifying that it is a reliable and well-adjusted model with a value of .783.

On the other hand, Table 4 shows in the first column the values B (β) that represent the coefficients, highlighting that these are not read as ordinary least squares coefficients, but

Table 2. Dependent variable leaderboard[ab].

Observed			Predicted		
			Productivity		Correct percentage
			Low produces	High production	
Step 0	Productivity	Low production	0	18	0.0
		High production	0	32	100.0
	Overall percentage				64.0

a. A constant is included in the model.
b. The cut value is .500

Source: Prepared by authors with Software SPSS v.25, field research 2020.

Table 3. Hosmer and Lemeshow test.

Step	Chi square	gl	Sig.
1	3.969	7	.783

Source: Prepared by authors with Software SPSS v.25, field research 2020.

rather they only serve to see if there is a positive or negative relation with the dependent variable. In the fifth column the Sig. values (*P value*) are found, which manifest if the independent variables are significant or not, where there should be values lower than .05, while those variables that have a value higher than .05 ought to be eliminated from the model because they are not significant. The variables that were statistically significant are SURFACE_SOWN, FERT_201020, FERT_COMPLEX.

Regarding the values of Odds Ratio for the SPSS software, it is Exp (B), which shows that: The values that are equal to 1 mean that they Do not have any relation or association with the dependent variable; the values that are higher than 1 mean that there is a relation or association with the dependent variable. When it comes to the result of the Exp (B) values, there is that the independent variable FERT_20-10-20 has a positive ratio, with the ratio value higher than 1, which means that an increase of a unit in the application of fertilizer 20-10-20 would increase the production; the independent SURFACE_SOWN variable also shows a positive relation higher than 1 with a ratio value of 1.36, meaning that an increase in one unit of the plantation surface can be predicted to increase 1.36 times the production. In terms of the independent variable FERT_COMPLEX, it shows a negative relation with a ratio value of .126 which is higher than 1 and therefore its result should be interpreted through its inverse which would be $+1/.126=7.93$, meaning that an increase of one unit of this fertilizer would cause a decrease of 7.93 times in production. On the other hand, the variable when pruning is done on the crop did not represent significance with the dependent variable.

CONCLUSIONS

Martínez de la Torre is the capital of citruses, it has great advantages such as its geographic location that allows commercialization in an efficient way, in addition to having the adequate climate and soil for the development of lime and other citruses, where its greatest characteristic is the quality of the fruit. Thus, they are arriving to more markets

Table 4. Variables in the equation.

		B	E.T.	Wald	gl	Sig.	Exp(B)
Step 1[a]	Surface_sown	.311	.134	5.370	1	.020*	1.365
	Fert_201020	3.646	1.253	8.472	1	.004*	38.321
	Fert_complex	−2.075	.951	4.761	1	.029*	.126
	Performs_ pruning	.274	.926	.087	1	.767	1.315
	Constant	−.903	.878	1.057	1	.304	.405

a. Variable (s) entered in step 1: SURFACE_SOWN, FERT_201020, FERT_COMPLEX, PERFORMS_ PRUNING.
* the correlation is significant at level 0.05
Source: Prepared by authors with Software SPSS v.25, field research 2020.

each day, and eighty percent of the production goes to the international market, mainly the United States, Europe and Japan.

Considering the results obtained from Persian lime producers in the municipality Martínez de la Torre, in synthesis most of the producers are men, with an average age of 54 years. In terms of the type of property, it is ejido, the land where they have Persian lime plantations is their own, and in most cases they have been producing lime for 15 to 30 years. Regarding the knowledge applied for Persian lime production, the producers are guided by the rainy periods and soil fertility. In relation to the type of pattern that they use, the one most used is the Cucho pattern, followed by tolerant patterns such as Swingle, Volkameriano and Macrofila.

In the analysis of production, the Logit econometric model determined that the factors that affect positively the production of Persian lime are: surface sown and fertilizer 20-10-20. Regarding the fertilizer complex, this variable was significant, but with a negative relation with the dependent variable since when increasing this fertilizer in one unit, the production decreases; that is, decreasing yields are obtained, since each producer must consider the optimal dose for its cultivation. Likewise, the pruning variable does not present significance with the dependent variable, indicating indifference towards the use of this technique.

REFERENCES

H. Ayuntamiento de Martínez de la Torre. (2019). *Martínez de la Torre 2018-2021*. Obtenido de https://www.martinezdelatorre.gob.mx/municipio/historia/

Agroproductores. (2020). *agroproductores*. Obtenido de http://agroproductores.com/category/cultivos/citricos/

Instituto Nacional de Estadística y Geografía (INEGI). (20 de febrero de 2020). Obtenido de https://www.inegi.org.mx/datos/

Instituto Nacional de Estadistica y Geografía. (Noviembre de 2020). *Encuesta Nacional Agropecuaria*. Obtenido de https://www.inegi.org.mx/contenidos/programas/ena/2017/doc/ena2017_pres.pdf

Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO). (11 de Noviembre de 2020). Subsectores agrícolas. Obtenido de http://www.fao.org/rural-employment/agricultural-sub-sectors/es/

Pérez, C. (2007). *Econometría básica Técnicas y herramientas*. Pearson Educación, S.A.,Madrid.

SIAP. (11 de Noviembre de 2020). siap.gob.mx/gobmx. Obtenido de http://infosiap.siap.gob.mx/gobmx/datosAbiertos_a.php

Vivero, J. B., & Álvarez, R. V. (11 de Noviembre de 2020). Introducción a la agronomía. *EDIMEC*, 127. Obtenido de http://www.dspace.uce.edu.ec/bitstream/25000/5221/1/Introduccion%20a%20la%20agronomia.pdf

AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Determination of the point of zero charge of pineapple (*Ananas comosus* L.) peel and its application as copper adsorbent

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ABSTRACT

Objective: To determine the optimum pH at which the pineapple peel can adsorb the greatest amount of copper.

Design/methodology/approach: *Sorbent material.* The size of the pineapple peel was reduced to 0.250 mm; it was chemically modified with 0.2 M NaOH and 0.2 M $CaCl_2$. *Point of zero charge (PZC).* Six solutions were prepared with 0.5 g of sorbent in an aqueous medium (with a 3-8 pH range), they were stirred at 225 rpm for 48 h. The derivative method was used to plot the initial pH versus final pH, in order to determine the PZC. *Copper adsorption.* $CuSO_4$ solutions were prepared in 2, 4, 6, 8 10 mg/L concentrations; 0.1 g of pineapple biomass was added adjusting the pH to 5. The solutions had a contact time of 0 to 24 h.

Results: The pineapple peel had a 5.0 point of zero charge (PZC) value, which indicates that pH values higher than the PZC are required to obtain an adsorbent with a negatively charged surface and favor the copper adsorption. A 50% copper removal was obtained in all concentrations after a 1 h contact time.

Limitations on study/implications: This research had no limitations.

Findings/conclusions: The point of zero charge is a reliable parameter that allows the adsorption process to take place and provides a greater certainty to the metal adsorption process. Meanwhile, pineapple peel can be used as an adsorbent material, consequently reducing its accumulation in open dumps.

Keywords: Pineapple peel, point of zero charge, copper adsorption.

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INTRODUCTION

The climate, soil, temperature, rainfall, and altitude conditions of the humid tropics region of Mexico is suitable for pineapple (*Ananas comosus* L.) production. The states of Veracruz, Tabasco, Chiapas, and Campeche have the highest potential surface for its

Image by armennano at Pixabay

production. In Mexico, 44,182 ha of pineapple are currently grown; the total production is 1,041,161 t (SIAP, 2020). However, only 780 t are used for fresh consumption or to make juice, jams, and preserves (Vélez-Izquierdo *et al.*, 2020). This difference between production and consumption leads to an environmental and economic problem, as a consequence of the generation of a large volume of pineapple waste (including peel, crown, and pulp); all this inedible portion accounts for 41% of the fruit. Therefore, looking for alternative uses for this waste —based on effective techniques that can contribute to its reduction— is necessary.

Fruit peels —particularly from citrus fruits— have been studied as pollutant adsorbents (Romero, 2018; Pathak *et al.*, 2016). They have high metal adsorption capacity, as a result of their pectin content. Pectin contains carboxylic groups, which in its anionic form attracts cationic species such as metals (García Villegas *et al.*, 2011).

The $CaCl_2$-modified orange peel can adsorb 36.1 mg/g of Cu (II) at a pH of 4.86 (Villanueva, 2007); the peanut shell adsorbs 60% Cu (II) ions at a pH of 3 (Tapia, 2018); the pomegranate peel had a maximum adsorption of Cu (II) at a pH of 5.6-5.8 (El-Ashtoukhy *et al.*, 2008). The pH is a factor that must be taken into account in the adsorption process, as it can indicate the degree of ionization of the adsorbent surface and its interaction with the adsorbate. Based on the point of zero charge, the pH at which the adsorbent retains anions or cations and thus optimizes the removal process can be determined (Amaringo-Villa and Hormaza-Anaguano, 2013). Several studies have been made about the use of pineapple residues as metal and dye adsorbent (Urrego *et al.*, 2018). Succinic anhydride-modified pineapple peel has a maximum adsorption capacity of Cu (II) ions of 27.68 mg g^{-1} at a pH of 5.4 (Hu *et al.*, 2011). However, most of those studies do not describe the determination of the point of zero charge. Therefore, the objective of this study is to establish a parameter that enhances the adsorption process and establishes an improved adsorbent-adsorbate interaction.

MATERIALS AND METHODS

The peel was obtained from Cabezona pineapples (*Ananas comosus* L.) acquired at the municipal market of Cárdenas, Tabasco.

Physical Modification of the Pineapple Peel

Drying: the pineapple peel is washed with plenty of water, cut into small fractions of approximately 5 cm, dried in the sun for 3 days, and dehydrated at 60 °C for 5 h in a drying oven. Crushing and sieving: once the material is dry, it is crushed until a small particle is obtained, which is then sieved through a #40 mesh (upper part) and a #60 mesh (lower part).

Chemical Modification of Pineapple Peel with $CaCl_2$

10 g of the dry material are added to 250 mL of a 0.2 M NaOH solution; the mixture is stirred for 2 hours, then filtered and washed until the excess NaOH is eliminated. Afterwards, it is dried at 40 °C for 2 h. Once dry, it is added to 250 mL of a 0.2 M $CaCl_2$ solution, adjusting the pH to 5, and stirred at a steady 200 rpm for 24 h. It is filtered and washed with deionized water and dried at 60 °C for 6 h.

Determination of the Point of Zero Charge

Six solutions were prepared in 100-mL Erlenmeyer flasks. Fifty mL of distilled water were added to each flask and their pH was adjusted with standard NaOH and 0.1 M HCl solutions, in a 3-8 pH range. Pineapple biomass (0.5 g) at room temperature was added to each solution and it was constantly stirred at 225 rpm for 48 h. At the end of this process, the pH value was measured. The initial pH versus the final pH was plotted and the PZC was determined by the derivative method. The pH readings were taken with a Hanna H198103 Checker pH meter.

Adsorption Study

Five $CuCl_2$ solutions were prepared at different concentrations (2, 4, 6, 8 and 10 mg/L). Adsorbent (0.1 g) was added to each solution and the pH was adjusted to 5. The mixtures were placed in contact time for 24 h. A sample was taken every hour and its Cu (II) removal percentage was determined, using the following equation:

$$\%Removal = \frac{Co - Ceq}{Co} \times 100\%$$

Where: *Co*=initial concentration; *Ceq*=balanced concentration.

Copper Identification by Scanning Electron Microscopy Coupled with Elemental Analysis

It was carried out in a JEOL JSM 610 LA model Scanning Electron Microscope (SEM-EDX).

RESULTS AND DISCUSSION

Determination of the Point of Zero Charge (PZC)

The PZC is defined as the pH value corresponding to a balance point of (external and internal) charges on the adsorbent material (Sposito, 1998). pH values higher than PZC indicate that the surface is negatively charged, while pH values lower than PZC mean that the surface is positively charged (Amaringo and Hormaza, 2013). The PZC corresponds to the point where the curve of the final pH —versus the initial pH— crosses the diagonal. Figure 1 shows the graph of the initial pH versus final pH. The pineapple peel had a 5.0 PCZ value; therefore, pH values higher than PZC will favor uptake during the metal ions adsorption in aqueous media.

Copper Adsorption

In order to demonstrate the application of the point of zero charge in the adsorption process, the removal of different concentrations of Cu (II) from aqueous solutions with a contact time of 24 h was studied. The particle size, temperature, and stirring speed remained constant. Figure 2 shows the maximum adsorption reached during the study. During the first hour, a maximum percentage of 66 was reached for the concentration of

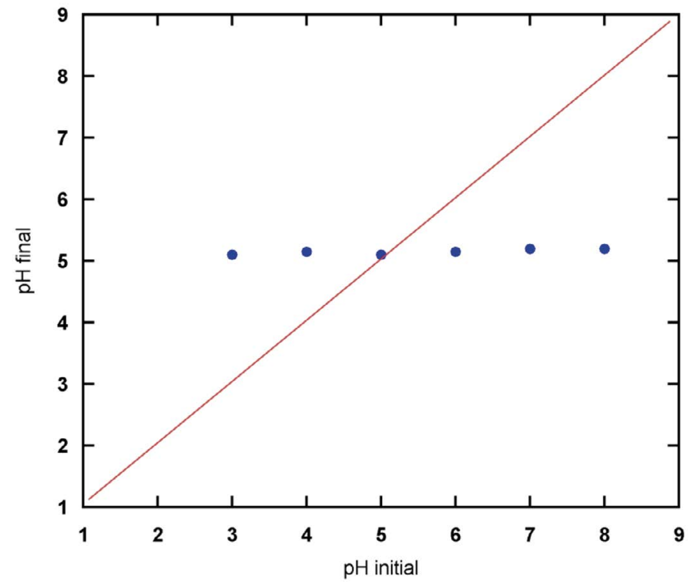

Figure 1. Point of zero charge of pineapple peel.

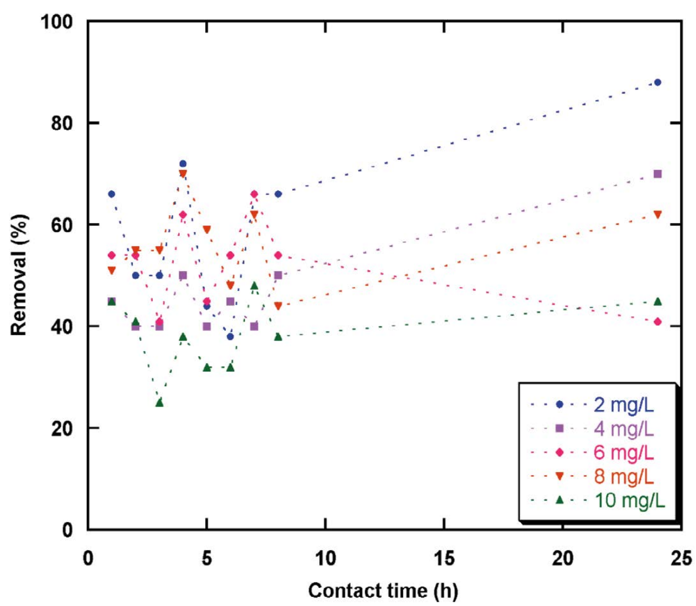

Figure 2. Removal percentage of Cu (II) at different contact times.

2 mg/L; from the 2^{nd} to the 7^{th} hour, there was a variation in the removal percentage, as a result of the balancing of the loads; at the 8^{th} hour, the removal percentage was similar to the 1st hour. The maximum adsorption percentage is reached for all concentrations after 24 hours (Table 1).

Figure 3 shows the SEM image of the surface of the pineapple peel after it had adsorbed the copper ions —which can be seen as shiny particles lodged within the cavities of the adsorbent. The EDX elemental analysis confirms that its composition includes copper.

Table 1. Maximum removal percentage.

Time (h)	% R 2mg L^{-1}	%R 4 mg L^{-1}	%R 6 mg L^{-1}	%R 8 mg L^{-1}	%R 10 mg L^{-1}
1	66	45	54	51	45
2	50	40	54	55	41
3	50	10	41	55	25
4	72	50	62	70	38
5	44	40	45	59	32
6	38	45	54	48	32
7	66	40	66	62	48
8	66	50	54	44	38
24	88	70	41	62	45

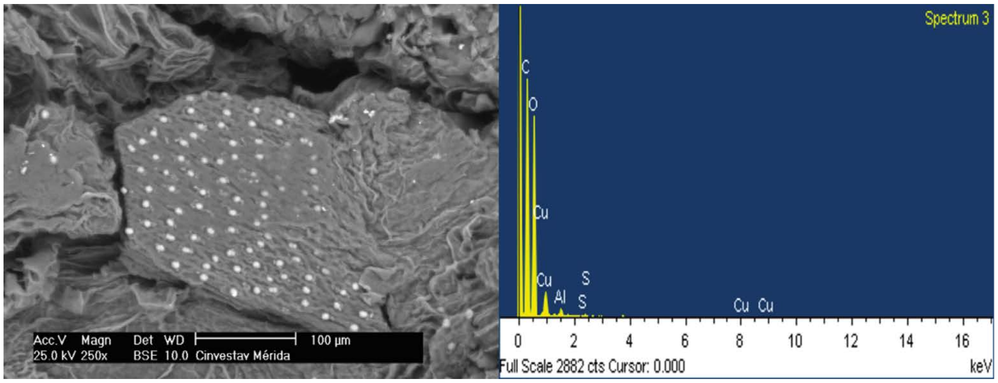

Figure 3. SEM image and elemental analysis of copper adsorption on pineapple peel.

CONCLUSIONS

Using the derivative method to determine the point of zero charge is a simple and fast procedure to establish the distribution of the charges on the surface of an adsorbent material. The $CaCl_2$-modified pineapple peel can adsorb up to 50% of Cu (II) ions dispersed in water at a pH of 5. The results of this study prove the potential of agricultural waste as metal adsorbents and consequently their capacity to avoid the negative environmental impact generated by its final disposal in open dumps.

REFERENCES

Amaringo Villa, F. A. & Hormaza Anaguano, A. (2013). Determinación del punto de carga cero y punto isoeléctrico de dos residuos agrícolas y su aplicación en la remoción de colorantes. *Revista de Investigación Agraria y Ambiental* 4(2):27-36. https://hemeroteca.unad.edu.co/index.php/riaa/article/view/982/963

El-Ashtoukhy, E.S.Z., Amin, N.K. & Abdelwahab, O. (2008). Removal of lead (II) and copper (II) from aqueous solution using pomegranate peel as a new adsorbent. *Desalination* 223(1-3):162-173. https://doi.org/10.1016/j.desal.2007.01.206

García Villegas, V.R., Yipmantin Ojeda, A.G., Guzmán Lezama, E. G., Pumachagua Huertas, R. & Maldonado García H.J. (2011). Estudio de la cinética de bioadsorción de iones plomo en pectina reticulada proveniente de cáscara de cítricos. *Revista de la Sociedad Química del Perú* 77(3):173-181. https://doi.org/10.5377/torreon.v8i23.9534

Hu, X., Zhao, M., Song, G. & Huang, H. (2011). Modification of pineapple peel fibre with succinic anhydride for Cu^{2+}, Cd^{2+} and Pb^{2+} removal from aqueous solutions. *Environmental Technology* 32(7): 739-746. https://doi.org/10.1080/09593330.2010.510853

Pathak, P.D., Mandavgane, S. A., & Kulkarni, B.D. (2016). Characterizing fruit and vegetable peels as bioadsorbents. *Current Science* 110(11): 2114-2123. https://doi.org/10.18520/cs/v110/i11/2114-2123

Romero Cano, L.A., (2018). Preparación y caracterización de materiales adsorbentes a partir de cáscara de frutas para su uso en la remoción de metales y aplicación a procesos ambientales. Tesis profesional. Universidad de Granada. Centro de Investigación y Desarrollo Tecnológico en Electroquímica S.C. Santiago de Querétaro, Qro., México. https://cideteq.repositorioinstitucional.mx/jspui/handle/1021/259

SIAP. 2020. Sistema de Información Agropecuaria y Pesquera. Cierre de la producción agrícola por estado. SIAP-SAGARPA. https://www.siap.gob.mx/agricultura-producción-anual

Sposito, G. (1998). On Points of Zero Charge. *Environmental Science & Technology* 32(19):2815-2819. https://doi.org/10.1021/es9802347

Tapia, P., Pavez, O., Garrido, N., Sepúlveda, B. (2018). Remoción de iones cobre y níquel con cáscara de maní. *HOLOS* 34(3): 57-69. https://doi.org/10.15628/holos.2018.7064

Urrego Durango, V.L., Vásquez-Noreña, P.A. & Barrera Zapata, R. (2018). Uso de cáscara de piña como adsorbente de rojo 40 (típico de la industria alimentaria). *Revista Colombiana de Investigaciones Agroindustriales* 5(1): 87-95. http://dx.doi.org/10.23850/24220582.1362

Vélez-Izquierdo, A., Espinosa-García, J.A., Uresti-Gil, J., Jolalpa-Barrera, J.L., Rangel-Quintos, J., & Uresti-Duran, D. (2020). Estudio técnico-económico para identificar áreas con potencial para producir piña en el trópico húmedo de México. *Revista Mexicana Ciencias Agrícolas* 11(7): 1619-1632. https://doi.org/10.29312/remexca.v11i7.2594

Villanueva, C., (2007). Biosorción de cobre (II) por biomasa pretratada de cáscara de *Citrus sinesis* (Naranja), *Citrus limonium* (limón) y *Opuntia ficus* (plameta de nopal). Tesis de licenciatura de Químico. Universidad Nacional del Mayor de San Marcos, Facultad de Química e Ingeniería Química, Lima-Perú. https://hdl.handle.net/20.500.12672/2112

AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Suspension of irrigation during the maturation phase of sugarcane (*Saccharum* spp.) cultivation

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ABSTRACT

Objective: To evaluate if there is an increase in the concentration of sugars in sugarcane grinding stalks as a result of controlled water stress.

Design/Methodology/Approach: An experimental plot on cultivar MEX 69-290 was established in 2nd ratoon cycle, on a mollic Gleysol soil in the supply area of the Pujiltic sugar mill. Five treatments in irrigation suspension were established: T1=15 days; T2=30 days; T3=45 days; T4=60 days, and T5=75 days of suspension. The treatments were distributed in the field in a gradient plot design, following the slope of the land, with four repetitions within each irrigation strip.

Results: The results show that suspending irrigation between 45 and 60 days before harvest increases the quality of the juices as well as the yields of the grinding stalks. In addition, natural precipitation plus irrigation water do not satisfy the water needs of the crop in the area under the scheme followed by the farmers.

Study Limitations/Implications: Irrigation rotation.

Findings/Conclusions: The authors recommend providing the necessary amount of auxiliary irrigation to satisfy the water demand of the crop during the growth cycle but suspending irrigation at the beginning of the ripening and maturity stage.

Keywords: juice quality, water stress, stalk yield, irrigation, sucrose.

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INTRODUCTION

In its last fifteen harvests and within its 16,869 ha of supply surface, the Ingenio Pujiltic sugar mill has reached an average sugarcane sucrose level of 14.9% and an average yield of 101.54 t ha^{-1} of grinding stalk, currently ranking fourth at the national level (Cañeros, 2021). An average annual rainfall of 1,006 mm is recorded in the sugar mill's supply area; this does not satisfy the water needs of the crop, which requires around 1,500 mm per cycle, so producers usually apply two to three relief irrigations. A common practice is to irrigate

up to a month before the beginning of the harvest, which turns out to be inconvenient since this last irrigation can dilute the sucrose stored in the stalk. This occurs because when the plant receives more water, it is stimulated to begin a new growth spurt and thus the quality of the juices decreases with respect to the following parameters: °Brix, sucrose, humidity, reducing sugars, and purity (Salgado *et al.*, 2012). Different studies show that continuous water stress affects the rate of water uptake, biomass accumulation, structural growth, and sucrose storage in the sugarcane stalk (Singels *et al.*, 2010). This study was therefore conducted in order to find a water management method that achieves optimal effects. The objective of this study was to evaluate the effect of the controlled suspension of irrigation during the ripening stage on the stalk yield and juice quality of the MEX 69-290 cultivar, cultivated on a mollic Gleysol soil.

MATERIALS AND METHODS

The study area. Located in the supply area of the Ingenio Pujiltic sugar mill, along coordinates UTM 561817 X−1801347 Y on km 46+000 of the Tuxtla Gutiérrez-Venustiano Carranza federal highway in Chiapas, at an altitude of 625 m above sea level. The climate is subhumid warm with summer rains with an average annual temperature of 25 °C and average annual rainfall of 1,006 mm (CONAGUA, 2012). The soil where the experiment was established is a mollic Gleysol cultivated with the MEX 69-290 variety, second ratoon crop (Salgado *et al.*, 2008).

Treatments and experimental design. Five irrigation suspension treatments were tested with four repetitions during the last 75 days of the crop's maturation phase (Figure 1). Each treatment received several irrigations throughout the growth cycle, initiating suspension on the scheduled dates: T1 suspension at 15 days, T2 suspension at 30 days, T3 suspension at 45 days, T4 suspension at 60 days, and T5 suspension at 75 days before harvest.

Agronomic management. The following were conducted: reseeding, cultivation with hooks, manual and chemical weed control, and fertilization at a dose of 140N-80P-120 NPK at 75 days, using triple 17+urea+KCl as sources (Salgado *et al.*, 2008).

Soil water content. It was determined by the gravimetric method (NOM-2000). Sampling began on 11/15/2011 using a stainless steel auger. Three preliminary samples

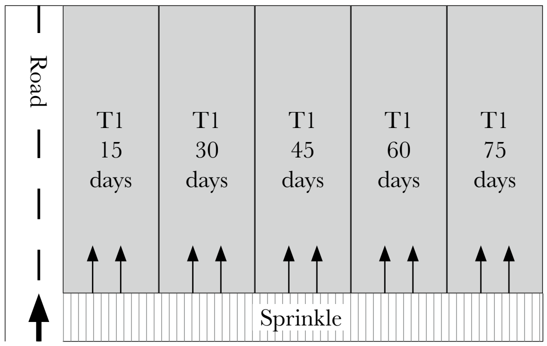

Figure 1. Distribution of Irrigation Suspension Treatments in sugar cane at Pujiltic sugar mill. The water in the plots runs through the sprinkle, from the left to the right.

were taken from a depth of 0 to 30 cm and 30 to 60 cm, with three repetitions in each plot. At the beginning of the treatments, the samples were taken from 0 to 30 cm for each treatment, with three repetitions. The wet weight was recorded in the field with a semi-analytical scale and the samples were taken to the LASPA laboratory at the Tabasco Campus where they were dried in an oven at 105 °C for 24 h for subsequent analysis.

Water retention curves. The soil samples were dried at room temperature, ground, sieved at 0.5 mm, and then taken to the Soil Physics Laboratory of Colegio de Postgraduados (Montecillo Campus) to calculate their moisture retention curves and to determine the soil moisture constants: moisture at field capacity (FC=1/3 atm), critical moisture (CM=5 atm), and permanent wilting point (PWP=15 atm).

Irrigation depth. The amount of water applied to the crop was determined based on the rainfall recorded by the Ingenio Pujiltic weather station, added to the water applied through surface irrigation, which was calculated using a triangular pourer. The flow rate was calculated according to the experimental formula developed by King (1962):

$$Q=C*H*2.47$$

where: Q=flow rate (L/s), C=experimental coefficient (constant value for right angles = 1.34), H=head height (m).

The irrigation depth was calculated with the following formula:

$$Lr=(Q*t)/S$$

where: Lr=irrigation depth, Q=flow rate (L/s), t=time (h), S=surface (ha).

Experimental yield. In each treatment, 10 linear m were measured in two furrows and the number of stalks per primary shoot was counted. In each row, five complete stalks were randomly sampled and their total weight was recorded, followed immediately by the weight of the grinding stalk. These data were used to calculate the biomass yield as well as the grinding stalk yield (t ha^{-1}).

Climatological data. The evaporation data (mm), minimum and maximum temperatures (°C), and precipitation data (mm) were taken from the records of the automated weather station of the Ingenio Pujiltic mill. The irrigation requirement was determined using the method by Blaney and Criddle (1950) and the irrigation requirement schedule was subsequently developed.

Maturity control. A record of the degrees Brix was made every 15 days starting at 9.5 months of the crop's growth. The juice samples were taken in the middle part of the stalk using a stainless steel punch. In each treatment, a °Brix reading was made with five repetitions using an ATAGO® brand manual refractometer.

Juice quality. It comprises degrees Brix, sucrose, moisture, reducing sugars, and purity. The juice sampling was carried out three days before harvest in 10 stalks per treatment, with four repetitions. The quality of the juices was determined in the Ingenio Pujiltic laboratory using the method of Section 8-10.

Statistical analysis. The data were analyzed using a completely randomized design and Tukey's multiple comparison ($p \leq 0.05$) was used with the SAS software.

RESULTS AND DISCUSSION

Water balance. The plot was scheduled to be harvested on April 25, 2012. During its growth cycle, it received 1,049 mm of rainfall (Table 1), 219.0 mm of water from three supplementary irrigations during the months of November to January (Table 2), and had 20.11 to 40.60 mm of surface water depth on average in the suspended irrigation treatments. The total sum of irrigation and precipitation fluctuates between 1,288.11, and 1,308.60 mm depending on the suspended irrigation treatments (Table 2), which does not satisfy the 1,500 mm water requirement that sugarcane needs for normal growth; the Blaney and Criddle (1950) method estimates a 1,303.6 mm water requirement for this plot.

The irrigations applied on the months of June, August, and September exceed the water needs of the crop (Table 1), which is why the crop was stressed by excess water during the growth stage. In contrast, irrigation is required during the months of October to February to prevent the crop from being stressed by drought. To increase water efficiency, 8 irrigations should be applied during this period with a surface depth of 40.0 mm each, which means watering every 18 days. In this way, water would be retained by the soil and absorbed better by the crop.

The rainy season took place from May to October 2011 and accumulated a total of 880 mm; in this period, the Blaney and Criddle (1950) method indicates an excess of water for the crop (Table 1). However, from November to December 2011, it rained 24.3 mm, which is insufficient and affects the final stage of sugarcane growth, therefore making it necessary to apply supplementary irrigation (Salgado *et al.*, 2003). Figure 2 shows the water content data at FC (50.7%), CM (31.2%), and PWP (25%). This soil retains 19.5% of usable water.

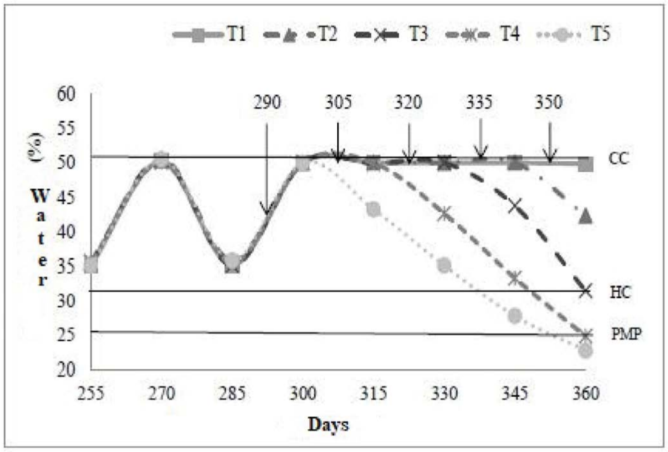

Figure 2. Content of water (%) in the soil cultivated with the MEX 69-290 cv., in a molic Gleysol during the growth stage. Plot of Mr. Hipólito Pedrero Alegría at Pujiltic sugar mill, Chiapas, Mexico.

Table 1. Irrigation schedule for the Cuatro Caminos sugarcane module, from the Pujiltic sugar mill, Chiapas, design based on the Method of Blaney y Criddle (1950).

Month	Period (month)	Temp (°C)	$\frac{t+17.8}{21.8}$	P (%)	f (cm)	Kt	f*Kt	Kc	Et	Et'	P	Pe	Rr
									(mm)				
Jan	1	15.5	1.528	7.90	12.06	0.72	8.7112	0.30	26.134	28.459	1.8	1.988	26.471
Feb	1	16.1	1.555	7.34	11.41	0.74	8.452	0.35	29.583	32.216	2.6	2.340	29.876
Mar	1	19.4	1.706	8.44	14.39	0.84	12.145	0.50	60.726	66.131	8.1	7.290	58.841
Apr	1	22.0	1.826	8.47	15.46	0.92	14.300	0.60	85.801	93.438	34.1	30.690	62.748
May	1	23.0	1.872	9.03	16.89	0.96	16.146	0.77	124.325	135.390	93.8	84.420	50.970
Jun	1	22.7	1.858	8.85	16.44	0.95	15.563	0.90	140.067	152.533	231.1	207.990	−55.457
Jul	1	22.1	1.830	9.09	16.64	0.93	15.437	0.98	151.286	164.751	154.8	139.320	25.431
Aug	1	21.6	1.807	8.86	16.01	0.91	14.609	1.02	149.010	162.272	182.7	164.430	−2.158
Sep	1	21.6	1.807	8.27	14.95	0.91	13.636	1.02	139.087	151.466	213.8	192.420	−40.954
Oct	1	20.8	1.771	8.23	14.57	0.89	12.931	0.98	126.728	138.007	102.2	91.980	46.027
Nov	1	18.4	1.661	7.71	12.79	0.81	10.397	0.90	93.577	101.906	20.5	18.450	83.456
Dec	1	16.2	1.560	7.82	12.19	0.74	9.070	0.78	70.745	77.041	3.8	3.420	73.621
Total	12				173.8		151.397			1303.6	1049.3		358.872

Soil moisture data starting at 255 days indicate an increase in soil moisture above FC due to the last application of supplementary irrigation.

Effect on degrees Brix and soil moisture. Irrigation suspension and the corresponding measurements of soil moisture began 290 days after sprouting (Table 4 and Figure 4). T5 started at 290 days when the soil moisture was between FC and CM. In this treatment, 23 °Brix were recorded in the stalk, the ripening and maturing process was interrupted, and it was observed that the crop matured slowly, reaching 24 °Brix at 335 days (Figure 3), affected by the decrease in water content to CM as reported by Inman-Bamber *et al.* (2012). Since the water content decreased to PWP, 25 °Brix were recorded at the time of harvest. Before harvesting, water stress was observed in the plant as well as small cracks in the soil. It is not advisable to suspend irrigation 75 days before harvest.

T4 began at 305 days, when the soil still had excess moisture; FC was reached at 317 days. This excess in moisture lasting 15 days interrupted the maturation process (Figure 3). As the soil moisture decreased to PWP, the MEX 69-290 cultivar showed signs of a water deficit that affected the ripening and maturing process (Salgado et al., 2012), reaching 24.5 °Brix at harvest. Mollic Gleysol soil can maintain moisture between FC and CM from one irrigation during 32 days in early March, and for 30 days after mid-March, so watering every 15 days was detrimental to the crop.

T3 was applied at 320 days when the moisture content was higher than FC. After 335 days, the soil moisture decreased until reaching CM at the time of harvest. Due to the high soil moisture, the ripening and maturing process was interrupted (Singels *et al.*, 2010) reaching only 23 °Brix at the time of harvest (Figure 3).

T2 was applied at 335 days, with accumulated moisture from previous irrigations higher than FC. After 350 days, soil moisture began to decrease, staying close to FC at harvest time. Excess water content interrupted the ripening and maturing of the MEX 69-290 cultivar (Taiz and Zeiger, 2004), achieving only 22 °Brix at time of harvest (Figure 3). T1 was applied at 350 days, but the soil maintained moisture above FC due to the previous irrigations, which caused the stalks to not ripen (Pimentel, 2004; Taiz and Zeiger, 2004), reaching only 23 °Brix at the time of harvest (Figure 3). This was moderately favored by an increase in temperature in the month of April (Table 1).

Table 2. Water balance in sugarcane cultivation in a plot in the supply area of Pujiltic sugar mill, Chiapas.

Phenologic stage	Irrigation Dates	Time (h)	Water spending (L/s)	Irrigation sheet (mm)	Total sheet (mm)	Irrigation requirement (mm)	Deficit (−) or Excess (+)
Growth	10/11/2011	24	43.65	75.43		83.46	+8.03
	10/12/2011	24	39.46	68.19	219.05	73.62	+5.43
	10/01/2012	24	43.65	75.43		26.47	−48.96
Seasoning and Maturity	11/02/2012	9	52.66	20.11	20.11	26.46	+6.35
	26/02/2012	10	19.20	11.35	31.46	26.46	−5.00
	12/03/2012	9	31.76	16.81	36.92	51.85	+14.93
	27/03/2012	12	22.15	15.64	35.75	51.85	+16.10
	11/04/2012	8	43.55	20.49	40.60	52.88	+12.28

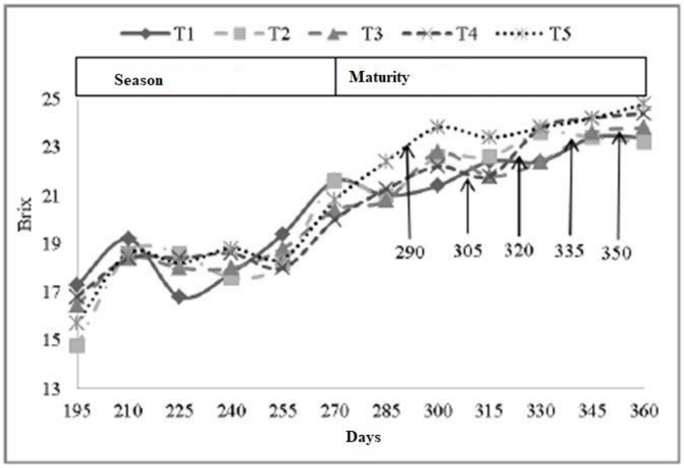

Figure 3. Evolution of Brix on cv. MEX 69-290 grown in a plot on a Gleysol molic soil, at Pujiltic sugar mil, Chiapas.

Effect on juice quality and yield of the grinding stalk. The effects on the juice quality and yields of the MEX 69-290 cultivar are presented in Table 3. No significant differences were observed for degrees Brix, sucrose, moisture, fiber, or cane yield. Due to the impact that some of these variables have on the price of sugarcane, an interpretation of the observed trends has been put forth.

T5 suspended irrigation at 75 days before harvest and presented a maturity index of 6.9, indicative that the cane is ripe with reducing sugars close to zero. Purity was high and the juice sucrose content was 12.8% with 13.2 °Brix, which are considered low compared to other treatments. The estimated yield was 109 t ha^{-1} (Table 3). This could be due to excess water stress when suspending the water supply 75 days before harvest and due to stress from excess water during the growth stage (Viator *et al.*, 2012); therefore, suspending water for 75 days is not recommended for the Ingenio Pujiltic area.

T4 suspended irrigation at 60 days before harvest and showed a maturity index of 7 with reducers close to zero (Table 3); the previous irrigation possibly caused this value

Table 3. Juice quality and yield of sugarcane mill stem under controlled water stress treatments at the Pujiltic sugar mill, Chiapas.

Treat. days without irrigation	Weight (kg)	Brix	Saccharose (%)	Purity (%)	Humidity (%)	Reducing sugars (%)	Fiber (%)	Maturity index	Yield (t/ ha)
1 (15)	12.95 a	15.15 a	12.86 a	85.06 a	70.28 a	0.32 a	11.80 a	5.98 a	112.06 a
2 (30)	11.05 a	14.81 a	13.03 a	88.14 a	69.78 a	0.22 a	11.95 a	8.45 a	109.11 a
3 (45)	12.33 a	15.92 a	13.20 a	88.03 a	70.05 a	0.22 a	12.27 a	8.65 a	125.99 a
4 (60)	12.65 a	15.66 a	12.90 a	83.57 a	71.02 a	0.25 a	12.76 a	7.38 a	123.79 a
5 (75)	11.75 a	14.81 a	12.81 a	86.58 a	70.08 a	0.28 a	11.31 a	6.94 a	109.73 a
Average	12.15	15.26	12.95	85.27	70.23	0.26	12.01	7.47	116.12
C.V. (%)	10.61	8.18	6.60	5.47	1.43	21.28	7.04	20.07	15.04
F Prob.	0.29	0.63	0.96	0.53	0.50	0.12	0.23	0.13	0.51
MSD	2.90	2.82	1.93	10.52	2.27	0.12	1.91	3.38	39.37

to be less than T3. Purity was high, however, with juice sucrose of 12.9% and 15.7 °Brix. The crop did not show water stress and the loss of soil moisture was beneficial for the accumulation of sucrose (McCormick *et al.*, 2008). The stalk yield was 123 t ha^{-1}, higher than the T5 yield. Cane yield in T4 could be increased if it is drained every 18 furrows to avoid excess moisture during the growth stage. In addition, the water need of the crop must be satisfied (Singels *et al.*, 2010) since the required volume of water is not provided (1,500 mm), although the irrigation depth must be smaller. The sugarcane crop should only be watered in the growth stage followed by suspended irrigation for the crop to ripen and mature.

In T3, irrigation was suspended 45 days before harvest, reached a maturity index of 8, and showed the highest quality among the treatments despite the effect of the previous irrigations that reduced the degrees Brix (Table 3). The warm temperatures of March and April (Table 1) and the soil with CM favored ripening and maturing (Inman-Bamber *et al.*, 2012), resulting in the yield of 125 t ha^{-1} that turned out to be the highest. The irrigation program aplied in Cuatro Caminos area should be reviewed to adjust the irrigation volume, interval, and depth, in addition to surface drainage (Silva *et al.*, 2008). Irrigation at up to 45 days before harvest is recommended for this area of Ingenio Pujiltic.

In T2, irrigation was suspended 30 days before harvest, obtaining a maturity index of 8, and the sugarcane was considered mature with reducers close to zero. The excess of soil moisture in the final stage of maturation of the MEX 69-290 cultivar induced new growth (Lingle *et al.*, 2010), and although it is not reflected in the juice quality, it is manifested in the decrease in yield to 109 t ha^{-1} with regard to T4 and T3. For this reason, it is not advisable to water 30 days before harvest in this area of Ingenio Pujiltic.

T1 suspended irrigation 15 days before harvest, presented the lowest maturity index with 5, high purity, 12.9% juice sucrose, and 15.2 °Brix. The experimental yield was 112 t ha^{-1}. Excess rainfall during the growing season and the lack of a drainage system were limitations to achieving higher yield and quality of sugarcane juice (Lingle *et al.*, 2010). To achieve increases in sugarcane yields, it is necessary to establish a surface drainage system with drains every 18 furrows. It is not advisable to water 15 days before harvest because the moist soil makes the tasks difficult, in addition to the undesirable effects on ripening and maturation.

CONCLUSIONS

Using the method by Blaney and Criddle (1950), the irrigation requirement for the Cuatro Caminos module of the Ingenio Pujiltic sugar mill was estimated at 1,303.6 mm, a value that is lower than the 1,500 mm requirement generally established for the cultivation of sugarcane; natural precipitation plus irrigation water does not satisfy the water needs of the crop. Therefore, the authors recommend providing the necessary supplementary irrigations to satisfy the water needs of the sugarcane crop according to the irrigation schedule. In addition, the authors recommend establishing a surface drainage system and suspending irrigation between 45 and 60 days before harvest to increase the yield of the grinding stalk and to improve the quality of sugarcane juices.

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REFERENCES

Blaney, H. F., & Criddle, W. D. 1950. Determining Water Requirements in Irrigated Areas from Climatological Irrigation Data. Technical Paper No. 96, US Department of Agriculture, Soil Conservation Service, Washington, D.C. 48 p.

Cañeros. 2021. Boletín técnico de la Unión Nacional de Cañeros A.C.- CNPR: Info-zafra 2020-2021. www.caneros.org.mx. Fecha de consulta 24/06/2021.

CONAGUA. 2012. Normales climatológicas por estado. https://smn.conagua.gob.mx/es/informacion-climatologica-por-estado?estado=chis. Fecha de consulta 15/08/2012.

Inman-Bamber, N.G., Lakshmanan, P., & Park, S. 2012. Sugarcane for water-limited environments: Theoretical assessment of suitable traits. *Field Crops Research*, 134: 95-104.

King, W. H. 1962. Manual de hidráulica, para la resolución de problemas de hidráulica. Unión Tipográfica Editorial Hispano-Americana. México. 536 p.

Lingle, S. E., Johnson, R. M., Tew, T. L., & Viator, R. P. 2010. Changes in juice quality and sugarcane yield with recurrent selection for sucrose. *Field Crops Research*, 118: 152-157.

McCormick, A. J., Cramer, M. D., & Watt, D. A. 2008. Culm sucrose accumulation promotes physiological decline of mature leaves in ripening sugarcane. *Field Crops Research*, 108: 250-258.

NOM.2000. Que establece las especificaciones de fertilidad, salinidad y clasificación de suelos. Estudios, muestreo y análisis. NOM-021-RECNAT. DOF. 85 p.

Pimentel, C. 2004. La relación de la planta con el agua. Seropédica: Ed. 191 p.

Salgado G. S., D. J. Palma-López, J. Zavala-Cruz, L. C. Lagunes-Espinoza., M. Castelán-Estrada., C. F. Ortiz-García., J. F. Juárez-López., J. A. Rincón-Ramírez y E. Hernández-Nataren. 2008. Programa sustentable de fertilización para el ingenio Pujiltic, Chiapas, México. *Terra-Latinoamericana*, 26(4): 361-373.

Salgado, G. S., Núñez, E. R., Peña, C. J. J., Etchevers, B. J. D., Palma, L. D. J. & Soto, H. M. R. 2003. Manejo de la fertilización en el rendimiento, calidad del jugo y actividad de invertasas en caña de azúcar. *Interciencia*, 28(10): 476-480.

Salgado, G.S., Lagunes, E. L.C., Ortiz, G. C. F., Bucio, A. L., & Aranda, I. E. M. 2012. Caña de azúcar: producción sustentable. Colegio de Postgraduados-Mundi Prensa. México. 493 p.

Silva, M. A, Silva, J. A. G., Enciso, J., Sharma V., & Jifon, J. 2008. Yield components as indicators of drought tolerance of sugarcane. *Sci. Agric*., 65: 620-627.

Singels, A. M., van den Berg, Smit, M. A., Jones, M. R., & van Antwerpen, R. 2010. Modelling water uptake, growth and sucrose accumulation of sugarcane subjected to water stress. *Field Crops Research*, 117: 59-69.

Taiz, L.; Zeiger, E. 2004. Fisiología Vegetal. 3. ed. Porto Alegre: Artmed. 719 p.

Viator, R. P., White Jr. P.M., Hale, A. J., & Waguespack, H.L. 2012. Screening for tolerance to periodic flooding for cane grown for sucrose and bioenergy. *Biomass and Bioenergy*, 44: 56-63.

AGRO PRODUCTIVIDAD

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Morphometric characteristics and seed germination of *Dalbergia granadillo* Pittier

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ABSTRACT

Objective: To know the variability and quality of seed in a *Dalbergia granadillo* Pittier (Fabaceae) population from the Soconusco, Chiapas, Mexico.

Design/methodology/approximation: The seed was obtained from six trees during the months of February and March 2020. Two weeks after the seeds were collected, pod and seed variables were determined. Seed length (LS), width (AS), color (CS), weight of 100 seeds (g) (PS) and germination percentage (PG). The results were statistically analysed by analysis of variance using the GLM procedure and the comparison of means by Tukey ($P \leq 0.05$) with the SAS 9.0 program.

Results: There is wide variation in seed content. The pods registered from 1, 2, 3 and up to four seeds, although pods of one predominated. There are seeds of two colours, dark brown and light brown, and the latter are the most abundant; however, light colored seeds registered higher germination (96%). The weight of 100 seeds registered values of 9.6-9.7 g.

Study Limitations/implications: The species has a restricted distribution and presents a low number of individuals in the evaluated populations.

Findings/conclusions: The morphometric characteristics of the seeds allowed to identify the variability and quality, mainly in shape, size and coloration of the seeds, as well as the number of seeds per pod.

Key words: Granadillo, seed quality, weight, and germination.

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INTRODUCTION

Tropical species considered as precious woods have been subjected to various anthropic actions, such as deforestation and fragmentation of their habitat. In addition to the above, it registers low natural repopulation, and they are of slow initial growth. The level of incidence of these factors or their combination, favor vulnerability to their loss (CITES, 2013; Cervantes, 2016). *Dalbergia* sp. (Fabaceae) is a tropical species, with a registered distribution in Chiapas, Guerrero, Jalisco, Michoacán, Nayarit and Oaxaca, (Pittier, 1922; CONABIO, 2017; CITES, 2015; Guala and Döring, 2020). The species *D. granadillo* Pittier known as granadillo (Pittier, 1922), cocobolo or rosewood (Cervantes, 2016) is considered high quality wood. Its contrasting colours, ranging from reddish-dark brown, with almost black veins of its heartwood and yellow sapwood, make it very attractive and increase its preference.

Its acoustic properties have been used since pre-Hispanic times by the Aztecs for the elaboration of musical instruments (Herrera-Castro *et al.*, 2019), and currently in certain regions of Mexico (Suárez-Islas *et al*., 2020), it is used mainly to make studio and concert guitars (Guridi and García-López, 1996). It is also used to make handicrafts, kitchen utensils, castanets, marimba keys and decoration in general (Niembro, 1990).

It registers high natural durability when exposed in the soil compared to other tropical species (Colín-Urieta *et al*., 2019).

In the Soconusco, Chiapas, Mexico, the habitat destruction has been the action with the greatest impact over the *D. granadillo* (Díaz-Gallegos *et al*., 2010) populations, and at present it has been classified as an endangered species, both in NOM-059-SEMARNAT-(2010), as in the appendices of the Convention on International Trade of Species (CITES, 2016 a). An unquantified risk is that the immoderate logging of the habitat has caused the accelerated loss of its genetic diversity. This reduces the possibilities of generating knowledge about the germination process of its seed that facilitates the design of multiplication and reinsertion strategies. Based on the above, it was proposed to know the variability and quality of seed in a population of *Dalbergia granadillo* Pittier (Fabaceae) from the Soconusco, Chiapas, Mexico.

MATERIALS AND METHODS

Location of the study area and biological material

The trees from which the seeds were obtained are located in "La Rioja" Community, in Cacaohatán, Chiapas, Mexico (14° 58' 37" LN and 92° 16' 15" LO), at an altitude of 480 m. The climate belongs to the warm humid group Af (w") i g, with 4720 mm of annual precipitation and an average temperature of 25.4 °C (INEGI, 2017). The soils have a volcanic origin and belong to the mollic Andosol group. They are characterized by the strong phosphate binding but are easy to grow and have good rooting and water storage characteristics (FAO, 2008). The soil of the place presents the following physicochemical characteristics: sand and loam texture (82.5% sand, 12.3% silt, 5.2% clay, 3.7% organic matter) (Walkley-Black), 0.12 ds m^{-1} electrical conductivity, pH 5.71, 0.13% N (Kjeldhal), 4.0 ppm P (colorimetry), 18.5 ppm K^{++} (atomic spectrophotometry), 59 ppm Ca^{++} (atomic spectrophotometry), 9.3 ppm Mg^{++}, 16.8 ppm Na^{++}, y 5 Meq 100 g^{-1} of cation exchange capacity.

Collection date and number of trees

Fruits were collected during February and March 2020 from six trees that constitute the population. Pods were collected from each tree and stored in labelled paper bags. The characterization was carried out in the Laboratory of Forest Biotechnology and Biofertilizers of the Faculty of Agricultural Sciences, Huehuetán, Chiapas (15° 19' N and 92° 44' O) where the pods with and without seeds were separated. The pods with seed were grouped according to the number of seeds and kept at room temperature in the laboratory (23.2 °C) in a ziploc® plastic bag.

At 15 days after its collection, basic initial quality tests were determined using the established rules of the International Seed Testing Association (ISTA, 2010), such as morphometric and germination variables (Table 1).

Table 1. Variables of pods and seeds of *D. granadillo* Pittier collected in the Soconusco, Chiapas, Mexico.

Variable	Description
Number of pods per tree	The number of pods with seed of each tree was quantified.
Pod length	It was measured with a digital vernier (Caliper Brand, Stainless Hardened, USA) with a precision of 0.1 mm.
Seeds per pod	The number of seeds per pod in each tree was counted.
Number and colour of seeds per tree	The number of seeds per tree was recorded and separated for germination by colour; dark brown and light brown.
Weight of 100 seeds	100 seeds (g) were weighed with four repetitions on a digital scale (Ohaus Brand, SocutPro Model SP401 USA).
Number of seeds per kg	It was determined by the formula (Number of seeds in the sample / Weight of the sample) * 100. (ISTA, 2010).
Germination	It was measured in percentage (%) according to the Testa colour through the paper germination method (ISTA, 2010) the first germination count was made after 5 days and the last one on day 20.

Statistical analysis

The mean results of the variables in the induction phase were plotted with the Sigma Plot (V. 11.0) program from Jandel Scientific. The data of the variables in the multiplication stage were analysed with the SAS Program for Windows Ver. 8.1 (1999-2000) and the comparisons of means between treatments with Tukey ($P \leq 0.05$).

RESULTS AND DISCUSSION

Number of pods and seeds per pod

The trees show a difference in the number of pods and the number of seeds per pod. The values fluctuated between 65 to 102 pods among the trees and with 1, 2, 3 and up to 4 seeds (Table 2). The immature fruit has a green colour and resembles a leaf.

The number of pods with one seed dominated in all trees, and it appears to be a characteristic of the species. Tree 4, with the lowest number of pods, presented the highest percentage of pods with one seed, while trees 1, 2, 6 and 12, with the highest number of pods, represented on average 25% fewer pods with one seed. In trees 2, 6 and 12, the pods with two seeds fluctuated in percentage from 20 to 23% and only tree 1 registered 40%. In the case of pods with three seeds in most trees, it represented between 3 and 7% of the total;

Table 2. Number of pods and seeds per pod of *D. granadillo* Pittier trees.

Tree and total number of pods	Number of seeds per pod			
	1 (%)	2 (%)	3 (%)	4 (%)
1 (102 pods)	56 (54.9)	40 (39.3)	5 (4.9)	1(0.98)
2 (84 pods)	55 (65.4)	23 (27.3)	6 (7.3)	
4 (65 pods)	56 (86.1)	6 (9.2)	3 (4.7)	
5 (78 pods)	58 (74.3)	14 (18.0)	5 (6.4)	1(1.3)
6 (84 pods)	49 (58.4)	20 (23.8)	15 (17.8)	
12 (88 pods)	60 (68.2)	20 (22.7)	7 (7.9)	1 (1.2)

however, it increased to 15% in tree 6. According to CITES, (2016) these data coincide with those found for *Dalbergia calycina* Benth.

Seed length and width

The size of the seeds was greater in three trees 1, 2 and 4 (Figure 1) and they do not present a relationship between the total number of pods and the percentages of seeds per pod. It is important to mention that the variation in the size of the seed plays a significant role in the germination process. This indicates that heavier seeds may be more appropriate for their multiplication (Seltmann *et al.*, 2007).

The length and width values of the seeds did not coincide with the *Dalbergia tucurensis* Donn species, where the average value in the seeds was 1.0 cm long and 0.5 cm wide (CITES, 2016b). Changes in seed size also occur in accessions of various tropical forest species and the changes are contrasting, as in *Cedrela odorata* L. where Alderete-Chávez *et al.* (2005) cite differences in size based on length and width of the seeds with values of 2.60 cm and 3.64 mm, respectively.

The size and weight of the seed are closely related to its reserve content, which varies between species. They are also related to the establishment, the growth of the seedlings and when the reserves are high, greater persistence of the species is achieved in a given site (Fenner and Thompson, 2005; Luna *et al.*, 2018), it is also relevant to harvest the seed with optimal humidity to favour its benefit, conservation (Sánchez *et al.*, 2010).

Weight of 100 seeds

The average weight of one hundred seeds per tree (W100S) was in a range of 8.8 and 9.2 g, and the trees that expressed the highest values were 5 and 6 (Figure 2). The differences in the size of the seeds, which induce weight differences, occur in other tropical species, such as *Cedrela odorata* L. (Arce-Córdoba *et al.*, 2018).

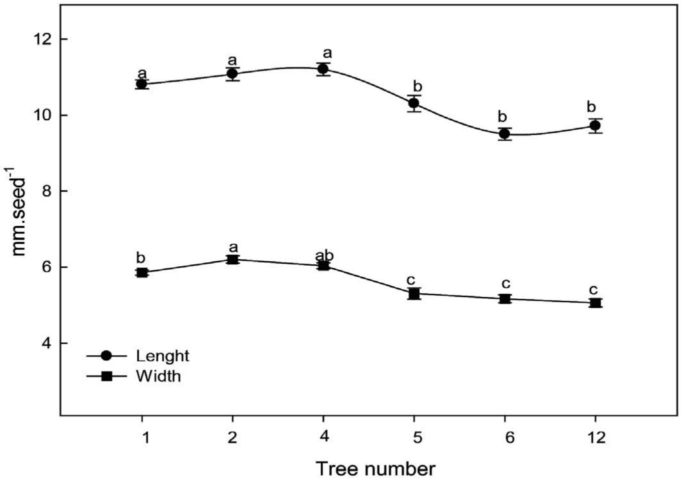

Figure 1. Length and width of *D. granadillo* Pittier seeds from six trees located in Soconusco Chiapas, Mexico. The values are means of 35 repetitions ± standard error and the letters that are not equal indicate statistical difference (Tukey p≤0.05).

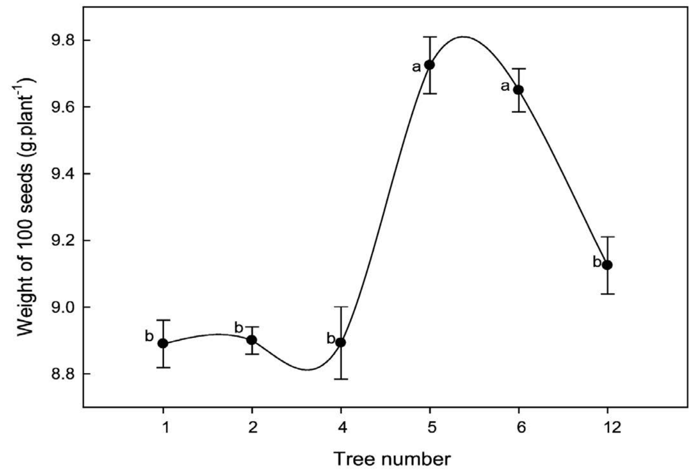

Figure 2. Weight of 100 seeds per tree of *D. granadillo* collected in the Soconusco region, Chiapas Mexico. The values are means of 35 repetitions ± standard error and the letters that are not equal indicate statistical difference (Tukey p≤0.05). CV = 1.7%.

The variation in the weight of 100 seeds can be influenced by genetic factors in interaction with the environment, and in this regard, Mendizábal-Hernández *et al.* (2012; 2013) mention that the differences may also be due to pollen viability and fertility, as well as stigmatic receptivity.

In other species, such as *C. odorata* L., Arce-Córdova *et al.* (2018) and *Roseodendron donnell-smithii* Miranda, Agustín-Sandoval *et al.* (2017) variations in weight of 100 seeds between collections in the same region of Soconusco, Chiapas are consigned. It is important to highlight that the seeds of greater size and greater weight present the highest quality in attributes for germination and vigour, therefore, full, healthy and mature seeds are stored better than those that do not reach maturity (Doria, 2010).

The storage of nutrients in the cotyledons has a direct influence on the initial growth of the seedlings (Soriano *et al.*, 2011; Bewley *et al.*, 2013; Soriano *et al.*, 2013) and in the dry tropical forest It has been observed that the species that produce larger seeds produce larger seedlings (Soriano *et al.*, 2011), survival in the field (Khurana and Singh, 2004) and in general, an increase in the content of aerial and root biomass (Velázquez- Rosas *et al.*, 2017).

The establishment and survival of the seedlings depends greatly on the content of reserves stored in the seeds (weight), in such a way that seeds with a lower weight express a low level of vigour and, consequently, a decrease in the probability of their establishment (Rubio *et al.*, 2011). However, those seeds of greater weight are also more susceptible to consumption by local fauna with respect to those of lower weight, although the potential for dispersal (zoocoria) may be greater in the former (Fenner and Thompson, 2005).

Number and colour of seeds per tree

All the trees presented seeds of two colours (light and dark brown), some in the same pod. In trees number 1, 2, 6 and 12, dark brown seeds dominated and in trees 4 and 5, the proportion of the two seed colours was similar (Figure 3).

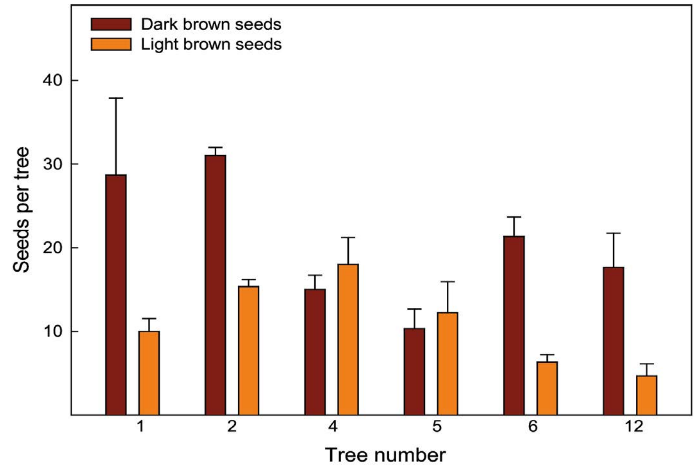

Figure 3. Number and colour of seeds per tree of *D. granadillo* collected in the Soconusco, Chiapas, Mexico.

In this regard, Doria (2010) refers to the importance of seed colour for germination, highlighting that the difference can be an adaptive characteristic for the species as it can germinate in a wider range of environmental conditions.

In the present case, the dark and light brown colours presented a contrasting difference in germination. Authors such as Debeajun *et al.* (2000) mention that the colour of the seed is positively correlated with restriction to germination, due to the phenolic components in the seed coat.

Todd and Vadkin (1996) mention that the seeds with darker colours germinate little due to the pigments of the Testa, while the seeds with lighter colours, in addition to having less pigments, absorb less heat, they can be kept at a temperature closer to optimal and germinate in greater quantity.

In addition, dark brown seeds are more prone to phytosanitary problems. They are attacked by insects of the Bruchidae family, and *Ctenocolum salvini* that deposit their larvae in the young fruits to complete their development and destroy the interior of the seed. Fungi such as *Alternaria* sp. and *Aspergillus* sp. have been reported in the seed of *Dalbergia retusa* Hemsl which is possibly the same that happens with *Dalberia granadillo* Pittier (CATIE, 2000; Cordero & Boshier, 2003).

According to Baskin and Baskin, (2001) the variation of sizes in seeds, colours and shapes is controlled by the environment, genetics and their interaction. Among the environmental factors, nutrients, light, shade, time of year, defoliation, temperature and humidity intervene, as well as the position in the fruit. Such variation is related to differences in the requirements for germination and the breaking of dormancy.

Germination

The highest germination (90 and 96%) was observed in the light brown seeds in trees number 2 and 12 (Figure 4). However, in the rest of the trees the same trend was presented, that is, greater germination in the light brown seeds. The dark brown seeds, which were the dominant ones in the seed population, presented the lowest germination percentage.

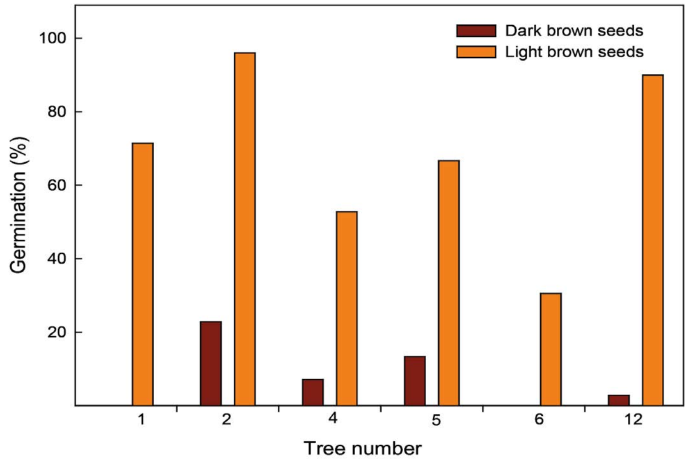

Figure 4. Seed germination by colour of the testa of *Dalbergia granadillo* Pittier collected in the Soconusco Chiapas, Mexico.

The variation in the colour of the seed may be due to the content and distribution of pigments such as anthocyanins, which are present in some Fabaceae seeds as well as glycosides, flavonoids and condensed tannins (Lobova *et al*., 2003). This is what can happen with *D. granadillo* Pittier with regarding the amount and distribution of flavonoids that can affect the colour of the cover, as it happens with other tropical species.

In the case of the *Dalbergia* genus, in *D. retusa* Hemsl. the seeds present high germination percentage (80-90%), and when extracting the seeds from the pod, Knoblauch (2001) cites a 2.14-fold increase in germination, improving the viability value.

In other species such as *C. odorata* L. without separating seeds by Testa colour, high germination values similar to those found in this study have been recorded (Arce-Córdova *et al*., 2018). However, in Mahogany (*Swietenia macrophylla* King), red cedar (*Cedrela odorata* L.) and oak or maculís (*Tabebuia rosea* Bertol) contrasting germination percentages of 76%, 54% and 37% respectively are cited (Quinto *et al*., 2008).

While *D. congestiflora* Pittier, *P. acatlense* Benth and *M. benthamii* J.F. Macbr. which are species of the same family (Fabaceae), showed germination percentages similar to *D. granadillo* Pittier with 90% germination (González *et al*., 2019). Other authors such as Rojas and Torres (2014) point out the importance of moderate irrigation in greenhouse conditions in *C. odorata* L. plantations for seed production, which under these conditions can achieve up to 80% germination when applying irrigation.

CONCLUSIONS

There are variations in number of pods, seeds per pod, seed size and weight of 100 seeds among the *D. granadillo* Pittier tree population. Pods with a dark brown seed predominate in all trees. The germination of the seeds was differential to the colour of the Testa. The highest percentage was registered in light brown seeds and they represent the smallest number of the total (96%).

REFERENCES

Agustín-Sandoval W.G., Espinosa-Zaragoza S., Avendaño-Arrazate C.H., Reyes-Reyes A. L., Ramírez-González S.I., López-Báez O., Andrade-Rodríguez M., Rangel-Zaragoza J.L. (2017). Calidad de semillas de primavera (*Roseodendron donnell-smithii* Miranda syn *Tabebuia donnell-smithii* Rose). *Agroproductividad, 10* (3): 81- 86. Recuperado a partir de https://www.revista-agroproductividad.org/index.php/agroproductividad/article/view/974

Alderete-Chávez, Ángel; de la Cruz Landero, Nancy; González de la Torre, Juan Elio (2005). Variación en semillas de *Cedrela odorata* L. procedentes de los estados de Campeche y Tabasco, México. *Foresta Veracruzana, 7* (2): 41-44. https://www.redalyc.org/pdf/497/49770207.pdf

Arce-Córdova, D. Espinosa-Zaragoza, S., Aguirre-Medina, J.F., Wong-Villarreal, A., Avendaño-Arrazate, C.H., Cadena-Iñiguez, J. (2018). Características morfométricas y germinación de semillas de *Cedrela odorata* L. *Agroproductividad, 11* (3): 82- 89. Recuperado a partir de https://revista-agroproductividad.org/index.php/agroproductividad/article/view/221

Baskin, C. C., and J. M. Baskin. (2001). Seeds. Ecology, Biogeography and Evolution of Dormancy and Germination. Academic Press. San Diego. 666 p.

Bewley, J. D., Bradford, K., Hilhorst, M. and Nonogaki, H. (2013). Seeds Physiology of Development, Germination and Dormancy. 3rd. ed. Springer. New York, USA. 445 p.

CATIE. (Centro Agronómico Tropical de Investigación y Enseñanza). (2000). Manejo de semillas de 100 especies forestales de América Latina. Turrialba, Costa Rica. 204 pág.,

Cervantes M. A. (2016). La conservación del granadillo en México: una carrera contra el tiempo. CONABIO. Biodiversitas. 128:6-11

CITES. (Convención sobre el comercio internacional de especies amenazadas de fauna y flora silvestres) (2013). CoP16 Prop. 61. *Dalbergia retusa* y *Dalbergia granadillo*, Inclusion in Appendix II. Belize. Disponible en: https://cites.org/sites/default/files/esp/cop/16/prop/S-CoP16-Prop-61.pdf

CITES. (Convención sobre el comercio internacional de especies amenazadas de fauna y flora silvestres). (2015). PC22 Doc. 22.4: Evaluación del riesgo de las especies maderables del género *Dalbergia* en México", Secretaría de la CITES, Ginebra.

CITES. (Convención sobre el comercio internacional de especies amenazadas de fauna y flora silvestres). (2016a). Guía de identificación de las maderas tropicales protegidas por la Convención sobre el Comercio International de Especies Amenazadas de Fauna y Flora Silvestres (en línea). Disponible en: file:///E:/DALBERGIA/ART.%20AGROPRODUCTIVIDAD/INFORME%20T%C3%89CNICO%20DEL%20PROYECTO%20LABORATORIO%20FORENSE%20IDENTIFICACI%C3%93N%20MADERAS.pdf

CITES. (Convención sobre el comercio internacional de especies amenazadas de fauna y flora silvestres). (2016b). Informe técnico del proyecto laboratorio forense identificación maderas por la Convención sobre el Comercio International de Especies Amenazadas de Fauna y Flora Silvestres (en línea). Disponible en: http://www.itto.int/files/user/cites/guatemala/INFORME%20T%C3%89CNICO%20DEL%20PROYECTO%20LABORATORIO%20FORENSE%20IDENTIFICACI%C3%93N%20MADERAS.pdf

Colín-Urieta, S., Carrillo-Parra, A., Rutiaga-Quiñones, J.G., López-Albarran, P., Gabriel-Parra, R. y Corral-Rivas, J.J. (2019). Assessing the natural durability of different tropical timbers in soil-bed tests. *Maderas, Ciencia y tecnología 21*(2): 231 – 238. https://www.redalyc.org/jatsRepo/485/48559145006/html/index.html

CONABIO. (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad). (2017). Granadillo (*Dalbergia granadillo*), Enciclovida, Biodiversidad Mexicana, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México, Disponible en: https://enciclovida.mx/especies/186583-dalbergia-granadillo

Cordero, J. and Boshier, D. (eds.) (2003). Árboles de Centroamérica: Un manual para extensionistas. Turrialba, CR, OFI/CATIE. 1079 p.

Debeajun, I., L. Kloosterziel M., and M. Koorneef. (2000). Influence of the testa on seed dormancy, germination, and longevity in *Arabidopsis*. *Plant Physiology 122*(2) 403–414. doi: 10.1104/pp.122.2.403.

Díaz-Gallegos, J. R., J. F. Mas & A. Velázquez. (2010). Trends of tropical deforestation in Southeast Mexico. *Singapore Journal of Tropical Geography 31*(2):180-196. DOI: 10.1111/j.1467-9493.2010.00396.x

Doria J. (2010). Generalidades sobre las semillas: su producción, conservación y almacenamiento. *Cultivos Tropicales, 31*(1): 74-85 Disponible en: http://scielo.sld.cu/pdf/ctr/v31n1/ctr11110.pdf

FAO. (Organización de las Naciones Unidas para Alimentación y la Agricultura). (2008). Base referencial mundial del recurso suelo e Informes sobre recursos mundiales de suelos. Roma Italia 103p.

Fenner, M., and Thompson, K. (2005). The Ecology of Seed. Cambridge University Press. New York. 249 p.

González Vélez G., Agustina R. Andrés-Hernández, Valdez-Eleuterio G, Álvarez-Quiroz N., Martínez-Moreno D., Sombra P. Rivas-Arancibia. (2019). Germinación de semillas de seis especies arbóreas maderables de una selva baja caducifolia en Puebla, México. *Revista Agrociencia 54*: 227-240. Disponible en: file:///E:/DALBERGIA/Germinacion%20de%20seis%20especies.pdf

Guala G, Döring M (2020). *Dalbergia granadillo* Pittier Integrated Taxonomic Information System (ITIS). National Museum of Natural History, Smithsonian Institution. Checklist dataset https://doi.org/10.15468/rjarmt accessed via GBIF.org on 2021-06-27.

Guridi, G. L. I. y García-López, Y. A. (1996). Las maderas en los instrumentos musicales de cuerda de Paracho, Michoacán, México. Morelia: Editorial Universitaria, Universidad Michoacana de San Nicolás de Hidalgo. Facultad de Ingeniera en Tecnología de la Madera. 45p

Herrera-Castro, M., Quintanar-Isaías, A., Orduña-Bustamante, F., Olmedo-Vera; B. y Jaramillo-Pérez, A.T. (2019). Identificación de la madera y análisis acústico de tres teponaztlis, instrumentos musicales aztecas originales. *Madera y Bosques 25*(1): 1-17. doi: 10.21829/myb.2019.2511690

INEGI. (Instituto Nacional de Estadística, Geografía e Informática). (2017). Anuario estadístico y geográfico de Chiapas. https://www.datatur.sectur.gob.mx

ISTA. (International Seed Testing Association). 2010. Reglas del ISTA. Septiembre de 2010. Publicado en línea en: http://www.analisisdesemillas.com.ar/index.php?option=com_content&task=view&id=15&Itemid=31

Knoblauch, B. (2001). Estudio ecológico, silvícola y de utilización del Granadillo (*Dalbergia tucurensis* J.D. Smith) en bosques latifoliados de Honduras. Proyecto especial presentado como requisito parcial para optar al título de Ingeniero Agrónomo en el Grado Académico de Licenciatura, Carrera de Desarrollo Socio-económico y Ambiente, El Zamorano, Honduras. 44 p.

Khurana, E., and Singh, J. S. (2004). Germination and seedling growth of five tree species from tropical dry forest in relation to water stress: impact of seed size. *Journal of Tropical Ecology 20*(04):385-396. Doi: 10.1017/S026646740400135X

Lobova, A., L. Mori A., S. F. Blanchard, H. Peckham, and C. Dominique P. (2003). Cercopia as food resource for bats in French Guiana and the significance of fruits structure in seed dispersal and longevity. *American Journal of Botany 90*(3): 388-403. doi: 10.3732/ajb.90.3.388.

Luna, C., Rodríguez, O., Enríquez, V., Ruíz, L., García, A. y Campos, A. (2018). Frutos y semillas de *Bursera simplex* Rzed. & Calderon en diferentes sitios y estructura arbórea. *Revista Mexicana de Ciencias Forestales, 9* (48): 92-114. https://cienciasforestales.inifap.gob.mx/index.php/forestales/article/view/188

Mendizábal-Hernández, L. Del C., J.J. Hernández-Viveros, J. Alba-Landa, E. Ramírez-García, y H. Cruz-Jiménez. (2012). Potencial y eficiencia de producción de semillas de *Cedrela odorata* L. *Foresta Veracruzana 14*(2):31-36. https://www.redalyc.org/pdf/497/49728290005.pdf

Mendizábal-Hernández, L. Del C., J.J. Hernández-Viveros, y J. Alba-Landa. (2013). Estudio de conos y semillas de *Cedrela odorata* L. en una generación parental y una generación filial. *Foresta Veracruzana 15*(1):45-51. https://www.redalyc.org/pdf/497/49728291006.pdf

Niembro, A. (1990). Semillas de árboles y arbustos: Ontogenia y estructura. Editorial Limusa, Mexico. 285p:

Pittier, H. (1922). On the species of Dalbergia of México and Central América. *Journal of the Washington Academy of Sciences, 12* (3 February): 54-64. Retrieved from: Stable URL: https://www.jstor.org/stable/24532566

Quinto, L., Martínez-Hernández P. A., Pimentel-Bribiesca L., Rodríguez-Trejo D. A. (2008). Alternativas para mejorar la germinación de semillas de tres árboles tropicales. *Revista Chapingo Serie Ciencias Forestales y del Ambiente 15*(1): 23-28. https://www.redalyc.org/pdf/629/62916073003.pdf

Rojas, R. y Torres, C. (2014). Árboles del Valle Central de Costa Rica: reproducción cedro amargo (*Cedrela odorata* L.). *Revista Forestal Mesoamericana 11*(26): 25-27. file:///C:/Users/JF%20Aguirre%20Medina/Downloads/Dialnet-ArbolesDelValleCentralDeCostaRica-5123216.pdf

Rubio, L., Romero, R., Rojas, Z., Durán, D. y Gutierrez, G. (2011). Variación del tamaño de frutos y semillas en siete especies de encino (Quercus, Fagaceae). *Polibotánica 32*: 135-151. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S1405-27682011000200008

Sánchez, S., Jurado, Y., Pando, M., Flores, R. y Muro, P. (2010). Estrategias germinativas de las semillas en ambientes áridos. *Revista Chapingo Serie Zonas Áridas. 9* (1): 35-38. https://www.redalyc.org/pdf/4555/455545062006.pdf

SEMARNAT (Secretaría del Medio Ambiente y Recursos Naturales). (2002). Norma Oficial Mexicana NOM-059-SEMARNAT-2001, Protección Ambiental Especies Nativas de México de Flora y Fauna Silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio, lista de especies en riesgo. Diario Oficial de la Federación, México, D.F., México.

Seltmann, P., Leyer, I., Renison, D. & Hensen, I. (2007). Variation of seed mass and its effects on germination in *Polylepis australis*: implications for seed collection. *New Forests 33*(2): 171-181. DOI: 10.1007/s11056-006-9021-8

Soriano D., Orozco-Segovia, A., Márquez-Guzmán, J., Kitajima, K., Gamboa-de Buen, A. and Huante, P. (2011). Seed reserve composition in 19 tree species of a tropical deciduous forest in Mexico and its relationship to seed germination and seedling growth. *Annals of Botany 107*, (6): 939-951. Doi: 10.1093/aob/mcr041

Soriano, D., Huante, P., Buen, A., & Orozco-Segovia, A. (2013). Seed reserve translocation and early seedling growth of eight tree species in a tropical deciduous forest in Mexico. *Plant Ecology, 214*(11), 1361-1375. Retrieved July 5, 2021, from http://www.jstor.org/stable/24553704

Suárez-Islas A., Capulín-Grande J., Mateo-Sánchez J. J. (2020). Performance of *Dalbergia* palo-escrito Rzed. & Guridi-Gómez, a valuable timber tree, in a coffee plantation in Hidalgo, Mexico. *Bois et Forêts des Tropiques, 344*: 47-57. Doi: https://doi.org/10.19182/bft2020.344.a31899

Todd, J., and L. O. Vadkin. (1996). Duplications that suppress and deletions that restore expression from a Chalcone syntrase multigene family. *Plant Cell. 8*(4): 687-699. doi: 10.1105/tpc.8.4.687.

Velázquez-Rosas, N., Ruiz-Guerra, B., Sánchez-Coronado, M. E., Gamboa-de Buen, A. and Orozco-Segovia, A. (2017). Morphological variation in fruits and seeds of *Ceiba aesculifolia* and its relationship with germination and seedling biomass. *Botanical Sciences, 95*: 81-91. Doi: 10.17129/botsci.636

AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Ecophysiology and nutrition of cabezona pineapple (*Ananas comosus* L. Merril) in Chontalpa, Tabasco, Mexico

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ABSTRACT

Objective: To improve the nutrition of the pineapple (*Ananas comosus*) cultivation using fertilizers.

Design/Methodology/Approach: The Sistema Integrado para Recomendar Dosis de Fertilizantes (SIRDF) established the fertilization doses for pineapple in the Cutanic Acrisol (Endoclayic, Hyperdystric, Ferric) (ACct(ncehdfr)) —N(230kg)-P(183kg)-K(300kg)— and Cutanic Acrisol (Endoclayic, Ferric) (ACct(ncefr)) —N(253kg)-P(138kg)-K(360kg)— soil sub-units. The SIRDF doses were compared with the control dose (producer): N(85kg), P(85kg), and K(85kg).

Results: The fruits harvested from the ACct(ncehdfr) soil to which the SIRDF dose was applied were larger (cm, without the crown), heavier (kg, with and without the crown), and also had higher °Brix values compared with control. This was not the case for the crown, which was heavier when the producer dose was applied. Meanwhile, the produce harvested from the ACct(ncefr) soil to which the SIRDF dose was applied included taller plants (cm), larger fruits (cm, with crown), larger crowns (cm), wider fruits (cm, circumference), heavier fruits (kg, with and without crown), and higher °Brix values; on the contrary, the crowns were heavier (kg) in control.

Study Limitations/Implications: Yield and fruit quality observations are affected by the quality of the *Cabezona* pineapple vegetable materials, agronomic management, and the attack of citrus mealybugs.

Findings/Conclusions: The fruits produced using the SIRDF doses had lower °Brix than the Cayena Lisa and MD pineapples. A 56-58 t ha^{-1} volume of fruit can be produced. This study proves that the doses established by the SIRDF had positive results for the improvement of the *Cabezona* pineapple production in Tabasco, Mexico.

Key words: *Cabezona* pineapple, fertilizer dose, acid soils.

INTRODUCTION

Although fertilizers have been used for two centuries, and their use is based on the chemical nutrition of plants, they have had a great impact in the increase of production

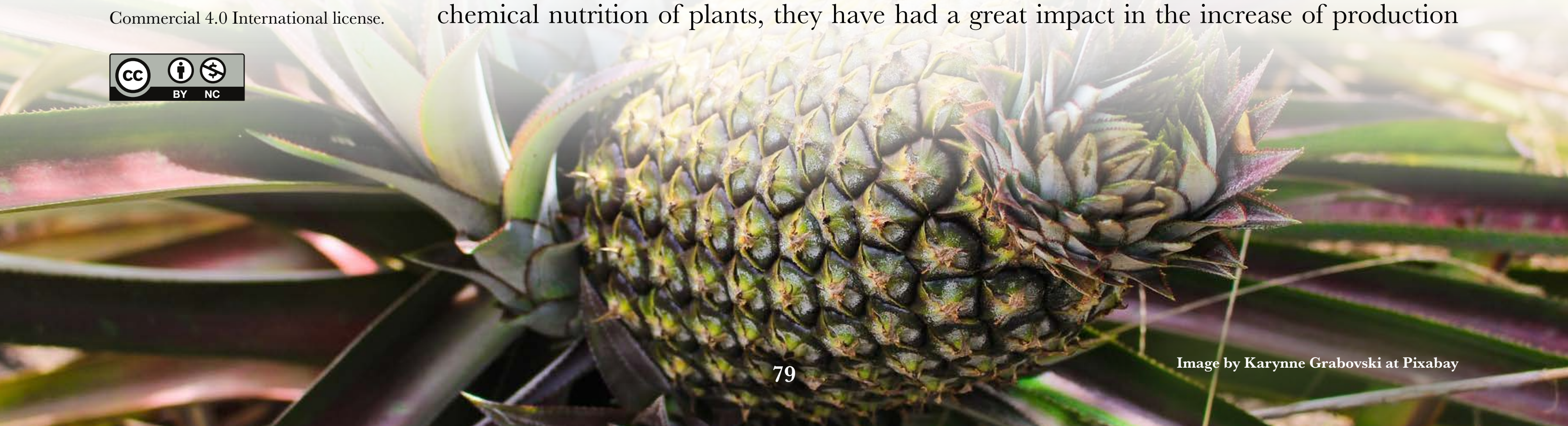

Image by Karynne Grabovski at Pixabay

and the quality of food. Additionally, this enhanced production has led to an increase in the return on invested capital rate for the production systems (Finck, 1992). All agricultural production systems (short-, medium-, and long-term) must use fertilizers in order to maintain crop yield, particularly, when the whole plant is removed from the production system (Salgado and Núñez, 2012). Pineapple crops (*Ananas comosus*) are not the exception, particularly in Mexico, where these crops are only grown in acid soils. These soils are characterized by their high phosphorous fixation, zinc and boron deficiency, low ammonium and nitrates formation, low calcium, magnesium, and potassium content, and high aluminum saturation (Pastrana *et al.*, 1998; Salgado *et al.*, 2007). These fertility limiting conditions impact the pineapple yield and quality.

Fertilizer doses can be applied to pineapple crops; however, they are only applied to the MD2 and the Cayena Lisa cultivars. These cultivars are grown for commercial purposes (supermarkets, exportation), while the *Cabezona* pineapple is sold only in regional markets. The Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) has developed technologies for the main or most important cultivars. However, it has not established a definitive dose; instead, it recommends applying two or three times the N12-P8-K12-Mg4 mix (25 g per plant on the soil) and 15 applications of 2.5% DAP (18-46-00) (on the leaves) to the MD2 cultivar (Uriza, 2011).

In Tabasco, Mexico, a definitive dose has not been established. Only the doses generated by SIRDF in 2010 —as a result of the study carried out by the Colegio de Postgraduados, Campus Tabasco— are available. Appropriately applying the dose of each nutriment is necessary for the nutrition of the *Cabezona* pineapple; currently, the producers nurture the crops based on their own experience (Salgado *et al.*, 2010; Salgado *et al.*, 2017a). Therefore, this study validated two fertilization doses for two soils sub-units of the savannah of Humanguillo.

MATERIALS AND METHODS

Experimental Plots Location

Two simultaneous experiments were carried out, using the vegetative material known as *hijuelos* or *gallos* (basal shoots); these experiments were carried out in two communities of Huimanguillo, Tabasco: Ejido La Esperanza and Salvador Neme Castillo. These communities are located in La Chontalpa, the second most important region after the Pedregales area. The first plot was established at the UTM X 431955 and Y 1980750 coordinates, at a 24 masl altitude. The second plot was located at the UTM X 434073 and Y 1979811 coordinates, at a 27 masl altitude. Both plots were established on soils were *Cabezona* pineapple had been grown for more than 10 years.

Experimental Plots

Two fertilization treatments were established in each community: T1, the dose of the producer and T2, the dose established by SIRDF (Salgado *et al.*, 2010). Both treatments were established using a completely randomized design with three repetitions.

In La Esperanza, the experiment was carried out in a 60×65 m (3900 m^2) plot, owned by Mr. Candelario Gómez Torres. The planting distance was 130 cm between rows and

25 cm between plants, which generated a population density of 11,999 plants. In Salvador Neme Castillo, the experiment was carried out in a 70×75 m (5250 m^2) plot, owned by Mr. Edilberto de la Cruz Osorio. The planting distance was 130 cm between rows and 25 cm between plants, which generated a population density of 16,152 plants.

Soil Sampling

Samples were taken from both plantations. Six sub-samples were taken from each experimental plot, using a stainless-steel earth sampler: three samples were taken from the space between the plants and the other three were taken from the alleys. These samples were placed in a bucket. Afterwards, the six samples were homogenized and a 1.5 kg sample was taken. The sampling depth was 0-30 cm. The samplings were dried in the shade for 20 days, and then they were ground and filtered with 0.5- and 2-mm sieves. Once sieved, they were sent to the soil laboratory of the Colegio de Postgraduados – Campus Montecillo, where their physical and chemical properties were determined.

Fertilizer Doses Application

Pineapple plants have high soil nutrient demands. This condition explains the sharp yield drop in plots that have been sown for many years without agronomical management or the use of fertilizers (Montilla *et al.*, 1997). SIRF recommends the following fertilizer doses for pineapple crops (Salgado *et al.*, 2010; Salgado *et al.*, 2017a):

N230kg - P183kg - K300kg. La Esperanza.
N253kg - P138kg - K360kg. Salvador Neme Castillo.

These doses were those recommended for the Cutanic Acrisol (Endoclayic, Hyperdystric, Ferric) and Cutanic Acrisol (Endoclayic, Ferric) soil sub-units in La Esperanza and Salvador Neme Castillo, respectively (Salgado-García *et al.*, 2017b). The DAP, UREA, and KCI sources were used to apply the fertilizer doses. The mixes were prepared after their ingredients were weighted. The producer applies a 500 kg dose of triple 17 (*i.e.*, 85 kg of N, 85 kg of P, and 85 kg of K). Once the dose was obtained, it was applied to the crop.

Plant (Height) and Fruit (Length and Diameter) Measurement

The plants that showed optimal harvesting conditions were measured. Thirty plants (10 small, 10 medium, and 10 large fruits) were measured. These plants were chosen according to the harvesting characteristics established by the producer (size, color, and texture of the fruit). Once the plants were selected and cut, the following measurements were taken: height of the plant, diameter and length of the fruit (with and without crown), and height of the crown.

Plant and Fruit Weight and °Brix

Once the 30 selected pineapples were measured, they were weighted in a common 50-kg scale. We weighted the complete plant (with and without fruit), the fruit (with and without crown), and, finally, the crown. A steel bodkin juice extractor was used to obtain samples in order to determine the ripeness of the fruit. The bodkin was introduced into each selected pineapple; the juice was extracted and it was placed on a handheld refractometer to measure the °Brix.

Statistical Analysis

The 9.3 SAS System statistical package software was used to carry out a completely randomized design ANOVA and a Tukey comparison test for all the variables.

RESULT AND DISCUSSIONS

Analysis of the Macro and Microelements of the Soils

The soils where the experiments were carried out had a strongly acid pH (≤5.0), zero salinity effects, a low exchange capacity, high organic matter content, rich nitrogen content, medium phosphorus content, low potassium, calcium, and magnesium content, and marginal to deficient micronutrient (iron, copper, zinc, and manganese) content (Table 1 and 2) (NOM- 021, 2001).

Agronomic Characteristics of Pineapple Cultivation in Cutanic Acrisol (Endoclayic, Hyperdystric, Ferric) Soil (ACct(ncehdfr))

According to the variance analysis and a Tukey comparison test, there were no significative differences in the agronomic variables evaluated for *Cabezona* pineapples: plant height, fresh plant weight, height of the fruit with crown, crown height, and fruit circumference (diameter) (Table 3). These variables had 6.64-26.33 coefficients of variation. The average plant height was similar —with no significative difference— to the height recorded by García *et al.* (2006) for this cultivar grown in the hillsides of Monagas and Sucre, Venezuela.

The fruit and the crown account for 39.74 and 60.26% of the total height, respectively. These results are similar to the findings about this cultivar reported by García *et al.* (2006). In general terms, the circumference (diameter) of this barrel-shaped fruit was greater than the 13.23 cm diameter reported by García *et al.* (2006), who also reported 10.58 °Brix for total soluble solids, a higher °Brix value than the one reported for the SIRDF dose.

Meanwhile, there were significative differences for the following variables: height of the fruit without crown, total weight of the fruit with crown or whole fruit, weight of the fruit without crown, crown weight, and °Brix. These variables showed 10.33-27.43% coefficients of variation.

Based on this difference, the fruit (with and without crown) was heavier with the SIRDF dose, while the crown was heavier with the producer dose. In the case of both doses, their results were higher than those founds by García *et al.* (2006), who recorded 2.28, 2.03, and 0.25 kg for each of these variables. Likewise, the fruit accounts for 87.24% of the total weight; this figure is similar to the 89.03% reported by García *et al.* (2006). Meanwhile, the crown accounts for 12.76% of the total fruit weight. In general terms, approximately 53 and 60.75 t ha^{-1} of *Cabezona* pineapple —fresh fruit without crown and fruit with crown— can be produced in Cutanic Acrisol (Endoclayic, Hyperdystric, Ferric) soil; Pérez-Romero *et al.*

Table 1. Chemical and physical analysis of ACct(ncehdfr)) and ACct(ncefr) soils.

Identification	pH 1:2 * H20	CE 1:5 H_2O mmhos/ cm dS m^{-1}	OM (%) Walkley -Black	CEC →	TN (%) estimate	P Olsen ppm	K ← meq/100g (cmoles+ Kg^{-1})	Ca	Mg	Bouyoucos texture			Textural Classification
								NH4OAc 1 N pH 7		Sand ←	Silt (%)	Clay →	
*Soil ACct(ncehdfr)	4.40	0.10	3.40	0.80	0.17	10.86	0.04	0.61	0.15	58.40	12.84	29.00	sandy loam
Soil ACct(ncefr)	4.40	0.10	4.07	0.82	0.20	11.48	0.05	0.56	0.20	67.60	5.64	27.00	sandy loam

* Published data (Murillo-Hernández, 2019).

Table 2. Chemical analysis (microelements and acidity) of ACct(ncehdfr)) and ACct(ncefr) soils.

Identification	Fe	Cu	Zn	Mn	Acidity	H	Al
	DTPA				KCl 1N		
	ppm				meq/100g (cmoles+Kg^{-1})		
*Soil ACct(ncehdfr)	52.82	0.59	0.22	2.42	1.37	0.73	0.63
Soil ACct(ncefr)	33.30	0.46	0.07	0.68	1.39	0.79	0.60

* Published data (Murillo-Hernández, 2019).

(2020) report a similar yield. The SIRDF dose seems to have had a positive impact in the fresh fruit yield in these savannah soils located in Huimanguillo, Tabasco.

Agronomic Characteristics of Pineapple Cultivation in Cutanic Acrisol (Endoclayic, Ferric) Soil (ACct(ncefr))

Based on the results presented in Table 4 and according to the variance analysis and the Tukey comparison test (0.05), there were significative differences in the following variables: plant height, height of the fruit with crown, crown height, fruit circumference (diameter), weight of the whole fruit with crown, weight of the fruit without crown, crown weight, and °Brix. These variables had a 6.18-19.60% coefficient of variation. The weight of the plant and height of the fruit without crown variables did not show significative differences. In general terms, the coefficient of variation had a 28.67-11.15% fluctuation —variations in the measurement of variables that are usually expected in every fieldwork.

The tallest plants (126.65 cm) were obtained with the application of the SIRDF dose; these results match the findings of García *et al.* (2006) who published that *Cabezona* pineapples achieve an average height of approximately 125 cm and an average weight of approximately 2.83 kg. The fruits to which the producer's dose was applied had bigger crowns and consequently were taller than those to which the SIRDF dose was applied. Without the crown, the fruit had an average height of 17.13 cm (35% of the fruit's total height). These results match the findings of García *et al.* (2006). The biggest crowns were obtained with the dose applied by the producer based on his experience; therefore, this dose had a greater effect on the size of the crown than on the size of the fruit; additionally, the crown accounts for 64% of the total fruit height.

The longest circumference (diameter) was obtained with the SIRDF dose. This means that these *Cabezona* pineapples had a 16.34 cm diameter. This result is greater than the results obtained by García *et al.* (2006), who recorded hillside-grown pineapples with a 13.23 diameter.

The highest weight for whole fruits (fruit with crown) was obtained applying the dose established by SIRDF. That result was higher than the findings of García *et al.* (2006) who recorded a 2.28 kg weight. Likewise, the application of the SIRDF dose resulted in the heaviest fruits without crown; this accounts for 86% of the full weight of the whole fruit (fruit with crown). These results are higher than the findings of García *et al.* (2006) who recorded 2.03 kg results.

The crown was heavier and bigger when the producer's dose was used. Likewise, it accounts for 14% of the total fruit weight. In general terms, the fruit weight percentage was lower than the percentages recorded by García *et al.* (2006), who determined that the fruit without crown accounts for 89% of the weight of the whole fruit (fruit with crown). The whole fruits and the fruits without crowns had yields of 67.25 and 58.25 t ha^{-1} in Cutanic Acrisol (Endoclayic, Ferric) soil, respectively. These results were higher than those obtained in Cutanic Acrisol (Endoclayic, Hyperdystric, Ferric) soil.

The dose applied with the SIRDF methodology resulted in higher total soluble soils (°Brix) than the producer dose. These results are lower than those found by García *et al.* (2006), who recorded 10.58 °Brix for *Cabezona* pineapples grown in the hillsides of the

Table 3. Agronomic characteristics of pineapple cultivation in ACct(ncehdfr) soil.

Dose	Plant height (cm)	Plant weight (kg)	Fruit height with crown (cm)	Fruit height without crown (cm)	Crown height (cm)	Fruit circumference (cm)	Whole fruit weight (kg)	Fruit weight (kg)	Crown Weight (kg)	°Brix
SIRDF	126.30a	3.27a	44.67a	18.63a	26.03a	47.93a	2.43a	2.12a	0.31b	7.59a
Producer	127.63a	3.28a	43.05a	16.22b	26.83a	46.63a	2.03b	1.66b	0.37a	7.12b
Means	126.9667	3.274833	43.85833	17.425	26.43333	47.28333	2.231917	1.891	0.340917	7.357
C.V.	6.923672	26.33437	11.31117	13.24043	16.94119	6.626745	16.03889	17.89573	27.43219	10.33258
Prob. of F.	0.5593	0.9489	0.2121	0.0002*	0.4919	0.1137	0.0001**	0.0001**	0.0279	0.0193
DMS	4.5467	0.446	2.5658	1.1933	2.3161	1.6206	0.1851	0.175	0.0484	0.3932

Note: The same letter in each column means that there are no significative differences. * Significative difference. ** Highly significative difference.

Table 4. Agronomic characteristics of pineapple cultivation in Ej. Salvador Neme Castillo, Huimanguillo, Tabasco.

Dosis	Plant height (cm)	Plant weight (kg)	Fruit height with crown (cm)	Fruit height without crown (cm)	Crown height (cm)	Fruit circumference (cm)	Whole fruit weight (kg)	Fruit weight (kg)	Crown Weight (kg)	°Brix
SIRDF	126.65a	2.89a	46.28b	17.45a	28.83b	51.36a	2.69a	2.33a	0.36b	7.05a
Producer	114.67b	2.78a	50.13a	16.80a	33.33a	49.83b	2.52b	2.00b	0.52a	6.10b
Means	120.66	2.833583	48.20833	17.125	31.08333	50.595	2.607867	2.164933	0.442933	6.572667
C.V.	6.18	28.66887	12.78418	11.15346	19.59564	3.203106	9.857873	11.23706	18.12409	9.518168
Prob. of F.	0.0001**	0.6214	0.0188*	0.1929	0.0059*	0.0006*	0.0127*	0.0001**	0.0001**	0.0001**
DMS	3.8536	0.4202	3.1876	0.9879	3.1503	0.8382	0.133	0.1258	0.0415	0.3236

Note: The same letter in each column means that there are no significative differences. * Significative difference. ** Highly significative difference.

States of Monagas and Sucre, Venezuela. Therefore, this genetic material is more acid that the Cayena Lisa and MD2 materials which are edible or 100% market-oriented.

CONCLUSIONS

There were significative differences in the following characteristics of *Cabezona* pineapples grown in Cutanic Acrisol (Endoclayic, Hyperdystric, Ferric) (ACct(ncehdfr)) soil compared to control: height of the fruit without crown, total fruit weight, weight of the fruit without crown, crown weight, and °Brix. There were significative differences in Cutanic Acrisol (Endoclayic, Ferric) soils for the following variables: plant height, height of the fruit with crown, crown height, fruit circumference, total fruit weight, weight of the fruit without crown, crown weight, and °Brix. Up to 56-58 t ha^{-1} of fresh fruit can be produced. Fruits produced with the SIRDF dose had lower °Brix than Cayena Lisa and MD cultivars.

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REFERENCES

Finck, A. (1985). Fertilizantes y fertilización: Fundamentos y métodos para la fertilización de los cultivos. Editorial REVERTÉ, S.A. España. 439 p.

García, M., Salcedo, F., Barrios, R., y Carrera, L. (2006). La piña "pilón" o cabezona cultivada en las laderas de los estados de Monagas y Sucre. *Revista de difusión de tecnología agrícola, pecuaria, pesquera y acuícola*. INIA Divulga 7: 27-29.

Montilla, B.I., Fernández, S., Alcalá, De M.D., y Gallardo, M. 1(997). El cultivo de la piña en Venezuela. Maracay, Ven. FONAIAP-CIAEL. IICA/CREA/PROCIANDINO/FRUTHEX. 155 p.

Murillo-Hernández, F.E., Córdova-Sánchez, S., Salgado-García, S., Bolio-López, G.I., De la Cruz-Burelo, P., y Sánchez-Gutiérrez, F. (2019). Determinación nutrimental de piña criolla (*Ananas comosus* L. Merril) en la sub-región de la Chontalpa Tabasco, México. *Agroproductividad 12*(7): 31-34. DOI https://doi.org/10.32854/agrop.v0i0.1472

Norma oficial mexicana 021- secretaria de Medio Ambiente y Recursos Naturales (NOM-021-RECNAT. 2001). Que establece las especificaciones de fertilidad, salinidad y clasificación de suelos. Estudios, muestreo y análisis. Secretaría de Medio Ambiente y Recursos Naturales. Norma Oficial Mexicana NOM-021-RECNAT-2001. 75 p.

Pastrana. L.A., Rodríguez, C.M., León, A.I.E., y Ramírez, D.G. (1995). Manual de producción de naranjo en suelos ácidos de Tabasco. INIFAP-CIRGOC-CAEHUI-ISPROTAB. Villahermosa, Tabasco. 30 p.

Pérez-Romero, J., Salgado-García, S., Córdova-Sánchez, S., del Carmen Lagunes-Espinoza, L., & Ortiz-García, C. F. (2020). Evaluación de parámetros de crecimiento en piña "cabezona" (*Ananas comosus* (L.) Merr.) en respuesta a la fertilización NPK. *AgroProductividad 13*(8): 3-9. DOI https://doi.org/10.32854/agrop.vi.1797

Salgado, G.S., y Núñez, E.R. (2012). Manejo de fertilizantes y abonos orgánicos. Editorial-Colegio de Postgraduados-Mundi Prensa, México, D.F. 146 p.

Salgado, G.S., Palma, L.D.J., Zavala, C.J., Ortiz, G.C.F., Lagunés, E.L.C., Castelán, E.M., Guerrero, P.A., Ortiz, C.A.I., and Córdova, S.S. (2017a). Integrated system for recommending fertilization rates in pineapple (*Ananas comosus* (L.) Merr.) crop. *Acta Agronomica 66*(4): 566-573. http://dx.doi.org/10.15446/acag.v66n4.62257

Salgado, G.S., Palma, L.D.J., Zabala, C.J., Lagunes, E.L.C., Castelán, E.M., Ortiz, G.C.F., Ventura, U.F., Marín, A.Á., Moreno, C.E., y Rincón, R.J.A. (2007). Sistema integrado para recomendar dosis de fertilizantes en las plantaciones de cítricos de Tabasco, México. Colegio de Postgraduados - Campus Tabasco. H Cárdenas, Tabasco. 89 p.

Salgado, G.S., Palma-López, D.J., Zabala-Cruz, J., Ortiz, L.H., Castalan-Estrada, M., Lagunes-Espinoza, L.C., Guerrero-Peña, A. y Córdova-Sánchez, S. (2010). Sistema integral para recomendar dosis de fertilizantes (SIRDF): en la zona piñera de Huimanguillo, Tabasco. Colegio de Postgraduados - Campus Tabasco, H. Cárdenas, Tabasco, México. 81 p.

Salgado-García, S., Palma-López, D.J., Zavala-Cruz, J., Ortiz-García, C.F., Lagunes-Espinoza, L.C., Ortiz-Ceballos, A.I., Córdova-Sánchez, S. y Salgado-Velázquez, S. (2017b). Los suelos ácidos de la sabana de Huimanguillo, Tabasco, México. *Agroproductividad, 10*(12): 16-21.

Uriza, Á.D.E. (2011). Paquete Tecnológico Piña MD2 (*Ananas comosus* var. *comosus*): Establecimiento y mantenimiento. Centro de Investigación Regional Golfo Centro. Campo Experimental Cotaxtla / Papaloapan (INIFAP). Isla, Veracruz. 16 p.

AGRO PRODUCTIVIDAD

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Reproductive aspects of the female jaguar (*Panthera onca* L.)

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ABSTRACT

Objective: To describe the reproductive characteristics of female jaguars and assisted reproduction techniques.

Methodology: A literature review about reproductive characteristics and assisted reproduction techniques was done to document information on this subject.

Results: Jaguars are the largest feline native to America; females sizes range from 1.57 to 2.19 m in length and weigh 45 to 82 kg. Jaguar females are ready for mating at about 2 years old and have an estrous cycle every 37 days. The reproductive behaviour patterns of the jaguar in captivity are grouped into four categories: 1) sexual, 2) affiliative, 3) agonists and 4) individual. The gestation period lasts between 91 to 111 days, and usually give birth to one to four young. For assisted reproduction, artificial insemination contributes to the genetic distribution or variability of germplasm.

Limitations on study: The destruction of forests and jungles, habitat fragmentation, indiscriminate hunting, the lack of prey and food sources and the conflict with ranchers put Jaguars on the brink of extinction. Also, there is few information about their reproductive characteristics, which is why in Mexico, it is imperative to generate this information, mainly for free-living jaguars.

Conclusions: The reproductive characteristics and assisted reproductive techniques in the female jaguar were documented. However, work is needed on assisted reproduction techniques in this species, which will contribute to the conservation of oocytes or embryos. It is advisable to design protocols to control and induce ovulation in the female *Panthera onca*.

Keywords: reproduction; feline; female; preservation.

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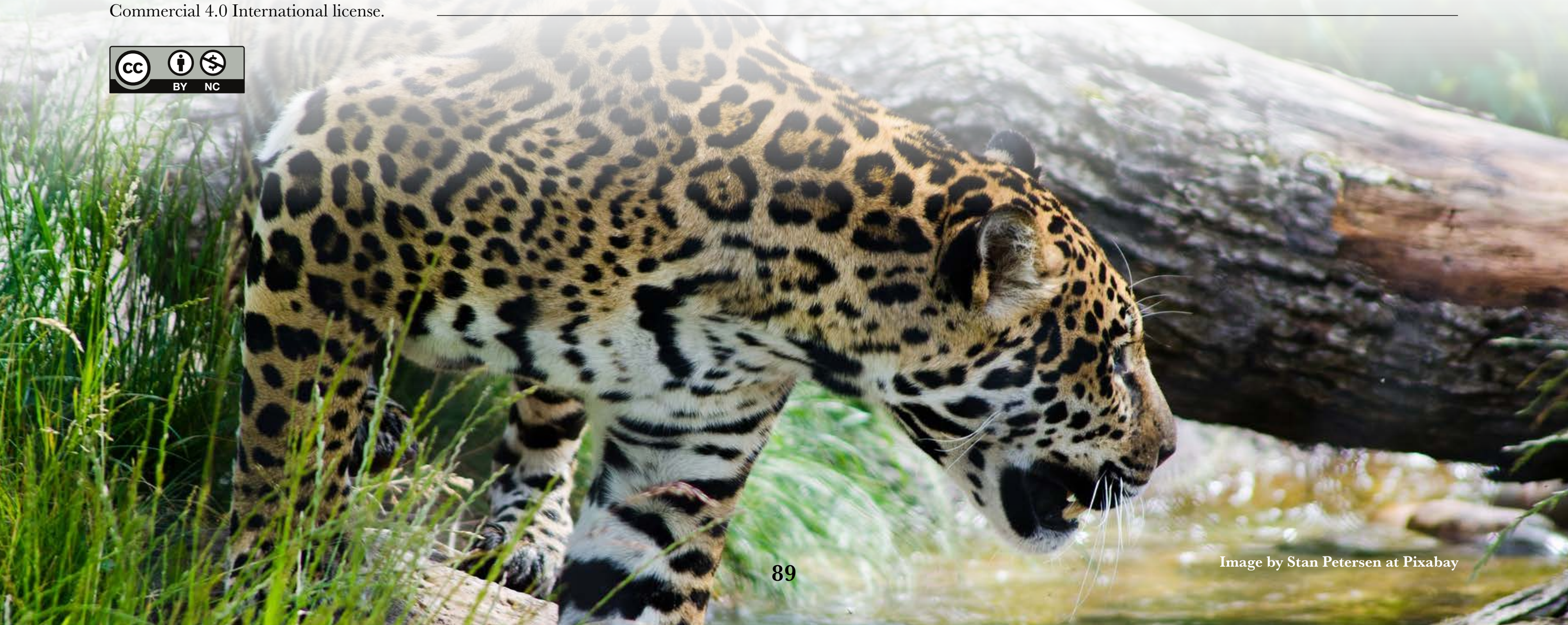

Image by Stan Petersen at Pixabay

DESCRIPTION

The jaguar is the largest feline in the Americas (Emmons, 1987; Chávez *et al.*, 2005). Its size varies geographically. Brazil is the country where the largest specimens have been found, with a body length, without the tail, up to 1.85 m (Nowak and Paradiso, 1983); its body is robust, with short and muscled forelimbs and hind limbs. Females measure between 1.57 and 2.19 m length and weigh 45 to 82 kg (Leopold, 1965; Seymour, 1989). The skin coloration varies from pale yellow to reddish brown, the belly color and inner part of the legs is pale, almost white or totally white. To the naked eye spots can be observed called rosettes on the body, of variable size, which have small spots in the center (Yescas and Ramírez, 2013). There is another phenotype with black or blackish-brown fur in which the rosettes are barely noticeable (Hoogesteijn and Mondolfi, 1993; Chávez *et al.*, 2005). Historically, it had a wide distribution, from southern United States to Argentina (Hall, 1981; Ceballos and Oliva, 2005). In Mexico, it distributes in tropical and subtropical regions, from the United States border, through Sonora and Tamaulipas to Chiapas and the Yucatan Peninsula (Figure 1; CONANP, 2009; Ceballos *et al.*, 2011; Yescas and Ramirez, 2013).

REPRODUCTION

Reproductive tract anatomy

The structures that form the female reproductive system are: ovaries, oviducts, uterus, vagina and vulva (Figure 2). The ovaries are surrounded by the ovarian pouch formed by

Figure 1. Current jaguar distribution according to CONABIO records (2010). Taken from http://www.conabio.gob.mx/informacion/gis/layouts/panoncadcgw.png. Consultation of use of license at https://creativecommons.org/licenses/by-nc/2.5/mx/legalcode

the mesosalpinx and mesovarium. As in other carnivore species, the ovaries (Figure 2) are located caudal to the kidneys, in a dorsal position into the abdominal cavity (Mayor and Lopez, 2010). The oviducts are tubular structures of sinuous trajectory included in the mesosalpinx, with considerable peristaltic activity during estrus. The oviducts transport oocytes to the uterine horns for their subsequent implantation in the uterus (Barrueta, 2012). The uterus is bicornuate and gestation can take place in both horns, the body of the uterus is reduced, the cervix has a thick muscular wall that isolates the uterus from the outside. The vagina is longer than the vaginal vestibule, shared by the genital and urinary tracts. The vulva is formed by two lips that join at the dorsal and ventral vulvar commissures. The clitoris has a rudimentary glans and is centrally located within the clitoral fossa (Mayor and Lopez, 2010).

Puberty

Puberty and sexual maturity of the female occurs at between 12 and 24 months (Wildt *et al.*, 1998), although Henderson (2010) indicates it is reached at 36 months of age. These differences may be due to factors such as daylight hours (latitude), longitude and even prey availability.

Estrous cycle

There is only limited information concerning the estrous cycle of wild felids and especially of the jaguar. One of the most studied females of the Felidae family is the domestic cat, which is sometimes used as a reference. However, it should be considered that this animal is in a different environment than wild felines, in addition to the physiological characteristics of each species, and even among individuals of the same species (Brown *et al.*, 2006).

In jaguars, studies have been conducted to evaluate the hormonal profiles (estradiol and progesterone) of estrous cycles and puberty by collecting fecal samples and found that the cycle length was 38.28±2.52 days, ranging from 25 to 44 days, while sexual maturity was reached within 22 months (Wildt *et al.*, 1998; Viau *et al.*, 2020), the estrous cycle is divided

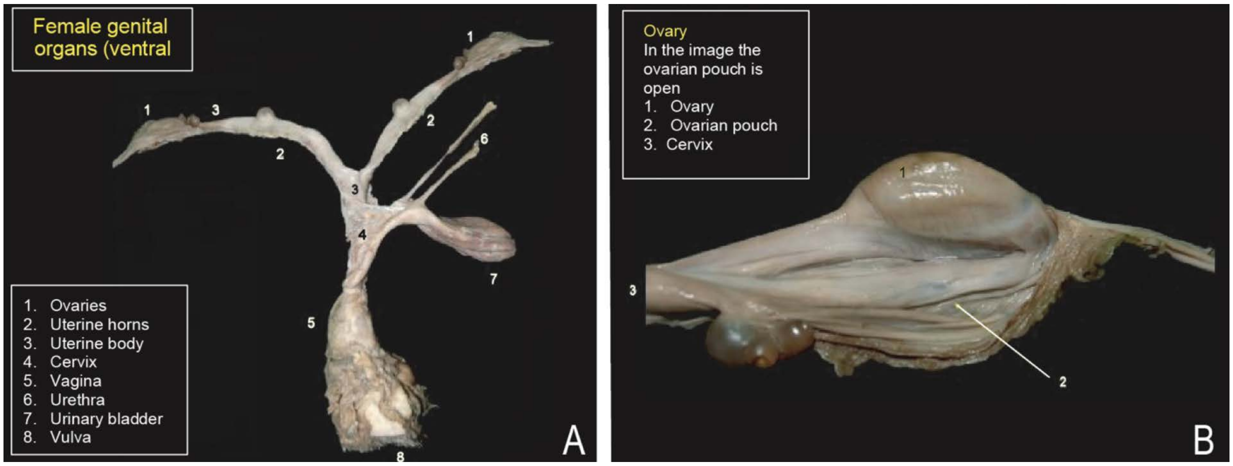

Figure 2. A: Reproductive apparatus of the female jaguar. B: Structure of the ovary of the female jaguar. With permission from Mayor and López (2010). Atlas of Anatomy of wild species of the Peruvian Amazon.

into five stages called proestrus, estrus, diestrus, interestrus and anestrus, with characteristic events as listed below. Proestrus: vocalizations, friction of the female on inanimate objects to attract males without accepting mating. Estrus: mating is accepted. The observable signs are vocalizations in the form of grunts, calling the male, rubbing against inanimate objects, lordosis, rolling on the ground, the female allows the male to sniff the vulva and accommodates it in order to perform the mating (Yescas and Ramírez, 2013). It has been reported that the mean estrous duration of 194 hormonally measured estrous periods in seven females was 6.5±0.3 d (Barnes *et al.*, 2016). Oestrus: in this phase, fertilization is likely to occur, corpora lutea will develop and there will be progesterone secretion to maintain gestation. However, there are females that ovulate, but the oocytes are not fertilized, and present pseudocyesis, which triggers a behavior like that of the pregnant female, caused by the production of progesterone. Interestrus: also known as sexual rest period, in this stage the female does not attract males and it is very common that the signs of estrus disappear. After this period the female resumes sexual activity or goes into anestrus if the reproductive season ends. Anestrus: this occurs during the non-breeding season and is characterized by the absence of ovarian activity and male acceptance (Stornelli, 2007; Barrueta, 2012).

Currently, studies have been conducted to evaluate the hormonal profiles (estradiol and progesterone) of estrous cycles and puberty, by collecting fecal samples and report that the cycle duration was of 38.28±2.52 days, with a range of 25 to 44 days, while sexual maturity was reached at 22 months (Viau *et al.*, 2020).

Reproductive behavior

The behavioral patterns of jaguars in captivity can be grouped into four categories 1) sexual, 2) affiliative, 3) agonistic and 4) individual (Yescas and Ramírez, 2013). Among the sexual ones: vocalization, mating attempts, naso-genital contact, naso-anal contact, genital preening and urine sniffing are the most characteristic activities, it is believed that the reproductive behavior of females in the wild is similar.

Naso-genital contact. The male approaches the female, performing an exploration and sniffs the vulva. Naso-anal contact. It consists of putting the nose of one of them in contact with the anus of the other, although according to Yescas and Ramírez (2013) it is difficult to identify whether the male sniffs the vulva or the anus.

Urine and excreta sniffing. In wild species, it is common for biological fluids (urine, excreta, sweat or saliva) to contain pheromones that induce ethological and/or endocrine responses in animals of the same species (Figure 3).

In reproductive aspects, males can identify if the female is in estrus, depending on the pheromones present in the urine and feces. Pheromone production in the female is cyclical and related to estrogen production (Rigau *et al.*, 2008). Vocalization. It is characterized by loud "grunting" sounds to call the males, who respond in the same way; between grunts and other vocalizations, copulation takes place.

Attempted mating. When the female is receptive, she rubs herself against objects, rolls on the ground and allows mating attempts. If the female is not receptive, she rejects the male in an aggressive manner or otherwise does not accommodate herself adequately for penetration. Full mating.

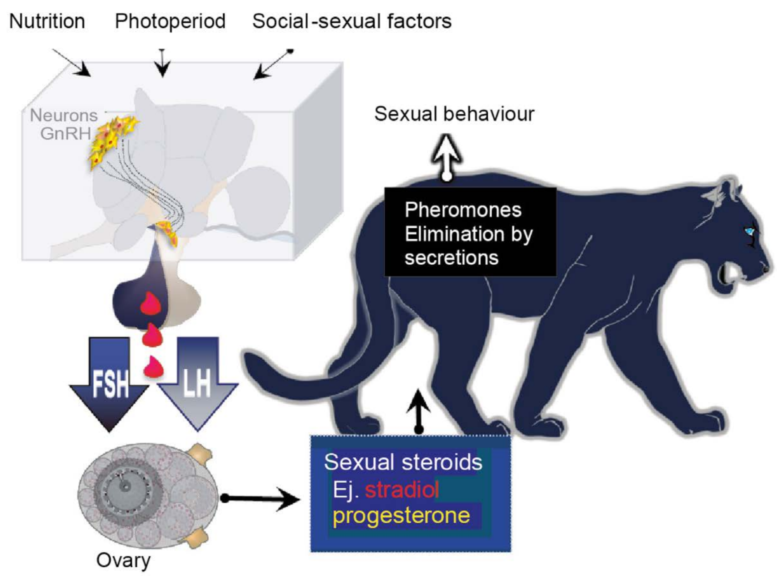

Figure 3. Environmental factors influence on jaguar reproductive behavior. Steroids stimulate the production of pheromones and in the case of the female, secretion is cyclical (Rigau *et al*., 2008).

The female lies down, the male arrives and presses with his head or forelimbs on the female's abdomen (it is believed that this is to accommodate her for copulation), begins to move his tail, then bites the nape of the female's neck and penetrates her for a period of 7 to 22 seconds and ejaculates. They can copulate several times in a day (Yescas and Ramírez, 2013).

Reproductive process

Reproductive particularities

Ovarian activity in females of the Felidae family is variable, although their reproduction is highly dependent on the photoperiod. They are seasonal polyestrous with induced ovulation. However, some individuals of the domestic cats (*Felis catus*), nebulous panther (*Neofelis nebulosa*), lioness (*Panthera leo*), leopard (*Panthera pardus*) and tigrina (*Leopardus tigrinus*) have been reported to present cases of spontaneous ovulation, a phenomenon that varies among species and individuals of the same species (Brown, 2011). Another characteristic reported in females of this family is the inhibition of ovarian activity when integrated into groups in captivity; it is reported that the socio-sexual environment is important for estrus onset, as is the case of the cheetah (*Acinonyx jubatus*; Brown *et al*., 1996). In free-living female jaguars, little is known regarding their reproductive behavior; most information is derived from animals in captivity (Hoogesteijn and Mondolfi, 1993), such as lioness (*Panthera leo*; Schaller, 1972) and leopards (*Panthera pardus*; Bailey, 1993). Leuchtenberger *et al*. (2009) pointed out that female jaguars are polyestrous; however, mating is not limited to a single season. This depends on several factors including geographic space, photoperiod, temperature, food availability and social-sexual environment (Feldman and Nelson, 1996). In regard to the photoperiod, Seymour (1989) indicated that in South America, births occur in June, August, November and December, in an average of 100 days of gestation.

It is assumed that mating occurs from April to October. On the other hand, in Mexico, the most recent information of the reproductive season of free-living jaguars mentions that mating occurs in December and January, with births occurring between February and April (Aranda, 1990). However, Leopold (1965) reported that jaguars give birth from July to September and mate from April to July.

Considering geographic location, jaguar mating in Mexico (northern hemisphere) occurs mainly in winter (short days) and in South America (southern hemisphere) in autumn and spring. Therefore, it is believed that the jaguar's reproductive season is determined by day length, so that it is inhibited when day length is longer (more daylight hours) and females have seasonal anestrus and estrus in the short-day season (fewer daylight hours). It seems that as in other mammals, circadian rhythms regulate the neuroendocrine and physiological changes presented by felines in extreme climates, such as hormonal cycles, metabolic rhythms and annual reproduction when they inhabit places with marked seasonality. So, melatonin would also have an important effect on ovarian activity (Wood *et al.*, 2015), whose secretion increases in darkness hours, coincides with the estrus onset (Yamauchi and Matsuura, 2009) and the onset of sexual activity. In tropical areas where there is not such marked seasonality, it seems that reproduction occurs at any time, since light and humidity remain constant. Another factor that may influence the onset of the reproductive season is the food and/or prey availability in extreme climates, where the seasons are well differentiated.

Fertilization, gestation and birth

Although the physiological changes during estrus, fertilization, gestation and birth are already known in domestic animals, information for felines is scarce and contradictory, especially in the case of the jaguar. After insemination, the fertilized oocytes become implanted in the uterine horns and gestation is established with a duration of 91 to 111 days. Female jaguars give birth to up to four cubs, although births of one to two cubs are more common. The cubs are born weighing between 600 and 900 g. The average interval between births is of two years (Ceballos and Oliva, 2005; Ceballos, 2010).

Lactation

The survival of the offspring depends on the mother's ability to produce milk and to obtain prey during rearing. Most female cats have four or five pairs of teats (Barrueta, 2012), although the female margay has only two (Henderson, 2010). From the sixth week, jaguar cubs follow their mothers to learn the secrets of hunting and are weaned at approximately three months, beginning to eat meat, although the lactation period can last from five to six months. During this period, estrous cycles are inhibited (postpartum anestrus). The calves remain with their mother for about 18 months, after which they separate in search of their own territory (Ceballos, 2010).

Hormonal studies

There are large "gaps" in the understanding of reproductive physiology of the jaguar, so it is necessary to generate information and improve the knowledge of the species to ensure its reproductive success. The analysis of biological samples to characterize over time their hormonal profiles [levels of cortisol, luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone, estradiol and prolactin], would allow a better understanding of the annual reproductive activity (reproduction vs. anestrus) of the jaguar (Barrueta, 2012). There are large differences in the levels of reproductive hormones among felids. Thus, for example, it is known that the concentration of progesterone in puma is higher than in domestic cat (Genaro *et al.*, 2007), estradiol is higher in female ocelot than in domestic cat; while, in jaguar, margay, oncilla, Geoffroy's cat, puma and jaguarundi, they are similar and cortisol levels are higher in pumas than in jaguarundis (Genaro *et al.*, 2007).

Assisted reproduction techniques

Oestrus induction and ovulation. In wild females, estrus induction is an alternative that could help to increase the chances of gestation, even females could be artificially inseminated with fresh or frozen semen (Micheletti *et al.*, 2015). This strategy requires the exogenous administration of hormones such as gonadotropin-releasing hormone (GnRh), LH, FSH or gonadotropins, alone or in combination, which modify the endocrine environment and induce ovulation. Although these hormones have been used in free-living animals, the differences that exist between species in relation to the reproductive cycle should be taken into account, for example, spontaneous ovulation in the case of margay and induced ovulation in the case of the female ocelot and tigrina, as in these cases (spontaneous ovulation and induced ovulation), prostaglandins are not an option for wild animals in captivity or in the wild. This is because the action of prostaglandins is limited until day 40 post-ovulation, and they cannot lyse the corpus luteum during the diestrous (Pelican *et al.*, 2006). In pumas and jaguars, porcine FSH has been used to induce follicular development and progestogens to inhibit follicular activity in tigrina (Morato *et al.*, 2000; Pelican *et al.*, 2006).

Oocyte retrieval for in vitro culture. It is a technique that represents the possibility of preserving frozen female gametes as well as pieces of ovarian tissue, follicles, or mature or immature oocytes (Pukazhenthi *et al.*, 2006, Lermen *et al.*, 2009). This technique has allowed to recover ovarian tissue from tigrina, puma and jaguars post mortem (Wiedemann *et al.*, 2013; Baldassare *et al.*, 2015), in the last two species, also, antral follicles (40-90 μm) were isolated. Laparoscopic aspiration has been used to recover oocytes from jaguar (Morato *et al.*, 2000), tigrina, ocelot (Swanson, 2006) and puma (Baldassare *et al.*, 2015).

In vitro embryo production. This technique consists of oocyte extraction, *in vivo* or *in vitro* maturation, oocyte selection, *in vitro* fertilization and embryo development with the aim of obtaining healthy offspring. An important characteristic to consider is the adjustment of the pharmacological protocols to be used for each species. For example, in the case of wild felines, *in vitro* fertilization has been successfully performed in jaguar, ocelot and tigrina (Swanson and Brown, 2004). In jaguar females, FSH/LH was used and an average of 25 follicles/female were obtained, with more than 80% of good quality oocytes; however, the fertilization rate was less than 25% (Morato *et *al*., 2000). In contrast, in ocelot and tigrina treated with eCG/hCG gonadotropins, an average of 10 follicles and between seven and nine good quality oocytes/female were obtained (Swanson *et al*., 2002).

Embryo cryopreservation. In recent years, great advances have been made in embryo cryopreservation, using slow freezing or conventional vitrification. Protocols developed for domestic species appear to be suitable for embryo cryopreservation in wild felids; ocelot offspring have been obtained using this technique (Swanson and Brown, 2004).

Generation, establishment and preservation of primary somatic cell cultures and cloning. These procedures are routinely used in domestic animals; however, there is little or no use in free-living animals, especially in felines. In addition, cloning for animal conservation is often questioned because of the risk of reducing genetic variability. However, cloning allows to know the principles of nuclear reprogramming, the conservation of cells and tissues for different purposes (reproductive, therapeutic, etc.), clones can also be used in experimental studies where it is necessary to avoid genetic variation.

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CONCLUSIONS

There are no reports of oocyte and embryo cryopreservation in female *Panthera onca*. Assisted reproductive techniques should focus on ovulation induction, control protocols and gamete cryopreservation.

REFERENCES

Aranda, J.M. (1990). *El jaguar (Panthera onca) en la Reserva de Calakmul, México: morfometría, hábitos alimentarios y densidad de población*. [Tesis de Maestría. Universidad Nacional de Costa Rica. Heredia, Costa Rica].

Baldassare, H., Carelli, J.B., Requena, L.A., Rodrigues, M.G., Ferreira, S., Salomão, J., & Jorge Neto, P.N. (2015). Efficient recovery of oocytes from onça parda (*Puma concolor*) by laparo662 Insights from animal reproduction scopic ovum pick-up of gonadotropin-stimulated females. *Animal Reproduction, 12* (3), 717.

Barnes, A.S., Teare, A.J., Staaden, S., Metrione L., & Penfold L.M. (2016). Characterization and manipulation of reproductive cycles in the jaguar (*Panthera onca*). *General and Comparative Endocrinology, 225*, 95–103.

Barrueta, A.F.M. (2012). Reproducción de felinos silvestres. http://exzooticos.blogspot.mx/

Brown, J.L. (2011). Female reproductive cycles of wild felids. *Animal Reproduction Science*, 124, 155–162.

Brown, J.L., Graham, L.H., Wielebnowski, N., Swanson, W.F., Wild, D.E., & Howard, J.G. (2006). Female reproductive cycles of wild female felids. *Theriogenology, 166*(1), 25–36.

Brown, J.L., Wildt, D.E., Wielebnowski, N., Goodrowe, K.L., Graham, L.H., Wells, S., & Howard, J.G. (1996). Reproductive activity in captive female cheetahs (*Acinonyx jubatus*) assessed by faecal stereoids. *Journal of Reproduction and Fertility, 106*, 337–346.

Ceballos, G., & Oliva, G. (2005). *Los mamíferos silvestres de México*. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Fondo de Cultura Económica, México D. F., México.

Ceballos, G. (2010). El Jaguar. Señor de las selvas. En G. Ceballos, R. List, R. Medellín, C. Bonacic, y J. Pacheco. (Eds.). *Los felinos de América. Cazadores Sorprendentes* (pp. 47-95). México, D. F., México, editorial Grupo Cars.

Ceballos, G., Chávez, C., List, R., Zarza H., & Medellín, R.A. (2011). *Jaguar Conservation and Management in México: Case Studies and Perspectives*. México. Alianza WWF/Telcel-Universidad Nacional Autónoma de México.

CONABIO. (2010). *Panthera onca (jaguar)*. Distribución conocida. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. México. Datos obtenidos del Sistema Nacional de Información sobre Biodiversidad (SNIB), registros comprendidos entre los años de 1878 y 1998 (registros no continuos).

Chávez, T.J.C., Aranda, M., & Ceballos, G. (2005). Jaguar, tigre En G. Ceballos y O. Giselle (Coordinadores). *Los mamíferos silvestres de México*. (pp. 367–370) CONABIO.

CONANP. (2009). *Programa de Acción para la Conservación de Especies: Jaguar (PACE: Jaguar)*. Comisión Nacional de Áreas Naturales Protegidas.

Emmons, L. (1987). Comparative feeding ecology of felids in a neotropical rainforest. Behavior. *Ecology and Sociobiology, 20*, 271–283.

Feldman, E.C., & Nelson, R.W. (1996). Canine and feline endocrinology and reproduction. Philadelphia: W. B. Saunders Co.

Genaro, G., Moraes, W., Silva, J.C.R., Adania, C.H., & Franci C.R. (2007). Plasma hormones in neotropical and domestic cats undergoing routine manipulations. *Research in Veterinary Science, 82* (2), 263–270.

Hall, E.R. (1981). The Mammals of North America. Nueva York, U.S.A. John Wiley and Sons.

Henderson, C. (2010). *Mammals, Amphibians, and Reptiles of Costa Rica: A Field Guide*. Texas, U.S.A. University of Texas Press.

Hoogesteijn, R., & Mondolfi, E. (1993). *The Jaguar*. Armitano Editores. C.A.

Leopold, A.S. (1965). *Fauna Silvestre de México: aves y mamíferos de caza*. Ediciones del Instituto Mexicano de Recursos Naturales Renovables.

Lermen, D., Blömeke, B., & Browne, R. (2009). Cryobanking of viable biomaterials: implementation of new strategies for conservation purposes. *Molecular Ecology, 18*, 1030–1033.

Leuchtenberge, C., Crawwshaw, P., Mourão, G., & Lehn, C.R. (2009). Courtship behavior by Jaguars in the Pantanal of Mato Grosso do Sul. *Natureza & Conservação, 7*(1), 218–222.

Mayor, P., & López, C. (2010). *Atlas de Anatomía de especies silvestres de la Amazonía*. http://atlasanatomiaamazonia.uab.cat

Micheletti, T., Brown, J.L., Walker, S.L., Cubas, Z.S., Furtado, P.V., Putman, S.B., & Moreira, N. (2015). The use of altrenogest to avoid hyperestrogenism after eCG-hCG ovulation induction in southern tigrina (*Leopardus guttulus*). *Theriogenology, 84*, 575–582.

Morato, R.G., Crichton, E.G., Paz, R.C.R., Zuge, R.M., Moura, C.A., Nunos, A.V.L., & Loskutoff, N.M. (2000). Ovarian stimulation and successful *in vitro* fertilization in the jaguar (*Panthera onca*). Theriogenology, 53, 339.

Nowak, R., and Paradiso, J.L. (Eds). (1983). *Walker's Mammals of the world*. Baltimore, USA. Johns Hopkins University Press.

Pelican, K.M., Wildt, D.E., Pukazhenthi, B., & Howard, J. (2006). Ovarian control for assisted reproduction in the domestic cat and wild felids. *Theriogenology, 66*, 37–48.

Pukazhenthi, B., Comizzoli, P., Travis, A.J., & Wildt, D.E. (2006). Applications of emerging technologies to the study and conservation of threatened and endangered species. *Reproduction, Fertility and Development, 18*, 77–90.

Rigau, I.T., Mora I.F., Font, J.G., Rivera, A.M.M., & Rodríguez, G.J.E. (2008). *Reproducción. Papel de las feromonas en la reproducción de los conejos*. Cunicultura 55–58.

Schaller, G. (1972). *The Serengeti Lion: A Study in Predator-Prey Relations*. Chicago, USA. University of Chicago Press.

Seymour, K.L. (1989). *Panthera onca*. *Mammalian Species, 340*, 1–9.

Stornelli, M. (2007). Evaluación de semen en el gato doméstico: análisis de rutina y metodologías especiales felino. *Revista Brasileira de Reprodução Animal, Belo Horizonte, 1* (1), 135–140.

Swanson, W.F. (2006). Application of assisted reproduction for population management in felids: the potential and reality for conservation of small cats. *Theriogenology, 66*, 49–58.

Swanson, W.F., & Brown, J.L. (2004). International training programs in reproductive sciences for conservation of Latin American felids. *Animal Reproduction Science*, 82–83, 21–34.

Swanson, W.F., Paz, R.C.R., Morais, R.N., Gomes, M.L.F., Moraes, W., & Adania, C.H. (2002). Influence of species and diet on efficiency of *in vitro* fertilization in two endangered Brazilian felids the ocelot (*Leopardus pardalis*) and tigrina (*Leopardus tigrinus*). *Theriogenology, 57*, 593.

Viau, P., Cattaruzzi, R. D., Sobral, G., Siqueira Martins, G., Gonçalves Morato, R., & Alvarenga de Oliveira, C. (2020). Puberty and oestral cycle length in captive female jaguars *Panthera onca*. *Conservation Physiology, 8*, 1–10.

Wiedemann, C., Zahmel, J., & Jewgenow, K. (2013). Short-term culture of ovarian cortex pieces to assess the cryopreservation outcome in wild felids for genome conservation. *BMC Veterinary Research 9*: 37.

Wildt, D.E., Brown, J.L., & Swason, W.F. (1998). Reproduction in cats. En E. Knobil, J. Neill (eds.). *Encyclopedia of reproduction*. (pp. 497-510). New York, USA. Academic Press.

Wood, S H., Christian, H.C., Miedzinska, K., Saer, B.R.C., Johnson, M., Paton, B., & Loudon, A.S.I. (2015). *Binary Switching of Calendar Cells in the Pituitary Defines the Phase of the Circannual Cycle in Mammals. Current Biology 25*, 2651–2662.

Yamauchi, K., & Matsuura, Y. (2009). Endocrinology of Sika Deer. En D.R. McCullough, S. Takatsuki, K. Kaji (eds) *Sika Deer* (pp. 83–99). Tokyo, Japan: Springer.

Yescas, L. A., & Ramírez, S.F. (2013). Etología Reproductiva del Jaguar en Cautiverio en el Parque de Conservación de Vida Silvestre Yaguar Xoo. En M.A. Aquino, V. De la Cruz, M.A. Briones-Salas, V.A. Sánchez, M.A. Huerta (Eds.). *El Jaguar en Oaxaca* (pp. 11-129). Oaxaca de Juárez, México. Gobierno del Estado de Oaxaca. Secretaría de las Culturas y las Artes de Oaxaca.

AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Use of agricultural technology, yields and economic profitability of small-scale producers (*Coffea arabica* L.)

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ABSTRACT

Objective: The objective of this study was to determine the relationship between use of agricultural technology and levels of crop yield and profitability of coffee farmers (*Coffea arabica* L.) en Puebal and Oaxaca, Mexico.

Design/Methodology/Approach: The field work was carried out in the Mazateca region of Oaxaca and the Cuetzalan region of Puebla, in 2019. The data were gathered by applying a survey, using a statistical sample, with 95% confidence and accuracy of 6% of the average coffee yields.

Results: The profitability (BCR) was 0.90, which means that for each invested peso, 0.10 pesos were lost, that is, 10% of the investment. For Puebla farmers, the RBC was 1.0 and for Oaxaca producers, 0.81. The BCR of the producers that sold as parchment coffee was 1.16 for those from Cuetzalan and 1.04 for those from the Mazateca region. The group of producers that have high TUI have a different average yield and profitability than the average of the groups of producers that have medium and low TUI. The explanatory variables of yield and profitability are the TUI, the size of the PU, and human capital.

Study Limitations/Implications: This study, in a next stage, could benefit from estimating the rate of technology adoption and the training needs of coffee farmers.

Conclusions: Due to the positive effect of TUI and HC on yields and profitability, these can be part of public policy interventions to improve coffee growing.

Keywords: profitability, yields, coffee producers, use of technology.

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INTRODUCTION

In Mexico, the coffee industry (*Coffea arabica* L.) is very important in social, environmental and economic terms; it is estimated that 515,000 producers participate in the production, of which 60% cultivate a hectare or less, 85% of the producers are indigenous people, located in 15 states (CEDRSSA, 2019). However, Chiapas, Veracruz, Puebla and Oaxaca contribute 87% of the surface harvested and 90% of the national production (SIAP, 2020).

Image by ExplorerBob at Pixabay

Compared to the environmental importance, Contreras-Hernández (2010) documented the environmental benefits of shade coffee: carbon capture, soil protection, recharge of water tables, and protection of biodiversity.

The primary production of coffee in Mexico is in crisis since 1992 and until today, period when there was a mean annual growth rate of −4.27%, explained by the fall in international prices and those paid to the producer; the latter were 41% lower in real terms in the period from 2015 to 2019, compared to the period of 1995 to 1999 (SIAP, 2020; Organización Internacional del Café, 2020).

Due to the fall in prices, the surface harvested also decreased, which went from 746,148 to 629,300 ha (reduction of 16%). The production decreased from 1.98 to 0.90 million tons of cherry coffee (a reduction of 48%). The fall in production is explained by the reduction in the unitary yields, which went from 2.65 ton/ha to 1.43 ton/ha, which represented a fall of 46% (SIAP, 2020). Under this scenario it is relevant to analyze the relationship between the use of technology, the yields and the profitability.

Use of agricultural technology, production and profitability

Technology, understood as a set of techniques that allow the practical use of scientific knowledge (Burgelman *et al.*, 2008), means the practical application of scientific knowledge to create something new or to transform something that already exists. Productivity is the capacity of the production factors (land, work and capital) to generate goods and services in order to obtain benefits (Valbuena *et al.*, 2018). In the agriculture and livestock sector, productivity is related to higher yields per surface unit. In this sense, Wambua *et al.* (2019) found that with greater use of agricultural technology, there is greater productivity and higher profitability of productive units.

Productivity is the main driver of the development process and represents half of the differences in the *per capita* gross domestic product among countries (Fuglie *et al.*, 2020). The use of agricultural technology is positively related with higher profitability, mainly through greater production per surface unit and lower production costs (Espinosa-García *et al.*, 2016). Therefore, researching the way that the use of technology generates greater productivity and profitability is a relevant task to improve the income of coffee producers. The hypothesis is that with higher technological level, there is higher yield and economic profitability. Based on this, the relationship between the use of agricultural technology in coffee production (*Coffea arabica*) and the levels of production, yields, and producers' profitability was determined with the purpose of generating information that contributes to improve the public interventions for coffee producers of Puebla and Oaxaca, Mexico.

MATERIALS AND METHODS

This study was conducted in the Mazateca region of Oaxaca and the Cuetzalan region of Puebla, Mexico. The two regions are characterized by their dependency on coffee production as source of income and by their high socioeconomic and environmental vulnerability (Monterroso *et al.*, 2014). Cuetzalan is located in the northeastern region of Puebla, where coffee production is the most important economic activity for small-scale producers (Rivadeneyra and Ramírez, 2006). The poverty levels in the eight municipalities that make up the region of Cuetzalan range from 70.2% to 93%, which is why its income was below the minimum welfare line. The Mazateca region is located on the northeastern part of the state of Oaxaca; coffee production is the main source of income for the population, and has poverty levels between 74 and 98% (CONEVAL, 2020).

The data were obtained through the application of a survey, using a statistical sample, with 95% confidence and accuracy of 6% of the mean of cherry coffee yields. The sample was n=192 producers. The distribution between both regions was proportional to the number of producers in each region. Applying the questionnaires was carried out in the first trimester of 2019. The information obtained was verified by key informants (technicians, technical coordinators, professors from the communities, and representative producers of social organizations). The questionnaire applied was made up of seven sections: social demographics, practices and technology, agriculture of the coffee crop, costs and income, sales prices, impacts and responses to the coffee crisis, climate events, and risk perception.

Data analysis

The relevant variables were the Benefit-Cost Ratio (BCR), the Technology Use Index (TUI), the Technical Assistance Index (TAI), the Human Capital (HC), the yields per hectare of cherry coffee, the production costs, and the income. The costs and the benefits were calculated for the BRC. The first are integrated by the costs of installation (fixed, variable and process). The benefits are the sales value, price received for the amount sold, whether cherry or parchment coffee.

The technological process that producers practice was made up of seven technological components: establishment of plant nursery, application of compost, fertilizer, pest/disease control, pruning, shade regulation, and grain processing. The TUI value for each producer resulted from dividing the number of technological components applied to the crop by the total technological components.

Four aspects were considered for the TAI, where producers received technical assistance services at least once during the productive cycle; elaboration and use of composts, fertilization, pest and disease control, and pruning. The producers who received technical assistance services in the four components were assigned a value of 1.0. For its part, the human capital was calcualted as the average of two components; years of schooling and number of training courses in coffee in the last three years. The weighing was 50% for each component.

Analysis method: Benefit-Cost Ratio (BCR)

To estimate the BCR, production costs were calculated, made up of installation costs (plantation) and operation costs. The first included soil preparation, cost of the plant material and workforce. The operation costs are paid each year and integrated by five concepts: fertilization, pest and disease control, weeding, pruning, harvest and transport. In addition, for producers that also conducted grain processing it also included the cost of depulping, washing, drying and cost of transport to the selling place. The benefit or income was the result of the amount sold multiplied by the market price. An Analysis of Variance (ANOVA) was used to identify the probable difference of the means of yield between groups of producers with different level of technology use. In order to explain the behavior of the unitary yield and of profitability, a multiple regression analysis was used, estimated by ordinary mean squares.

RESULTS AND DISCUSSION

The coffee producers were 57 years old on average, and had six years of schooling, equivalent to finished primary school. The number of members of a household was 3.6, nearly four members in Oaxaca and three in Puebla. These sociodemographic data are similar to those reported by INEGI (2020). The producers had much experience in both regions: 30 years in Cuetzalan and 24 years in the Mazateca. The average size of the plantations was 1.3 ha. In the region in Puebla, the plots were larger (1.6 ha) than in the region in Oaxaca (1.0 ha) (Table 1). In this regard, Espinoza-García *et al.* (2016) reported that the predominant size in the coffee producing regions is of less than 2 ha per producer.

The calculated household income was $47 thousand MX pesos annually; $54 thousand in the Cuetzalan region and $41 thousand in the Mazateca. Converted into *per capita* income, it was $1645.0 pesos in Cuetzalan and $1063.0 in the Mazateca. CONEVAL (2020) reported the value of the food basket for March 2019 in $1,110.00 pesos and of the food and non-food basket in $2012.0 pesos.

These define the extreme poverty line from income and the poverty line from income, respectively. This reveals that, on average, all producers in the sample were considered as poor. Apodaca-González *et al.* (2020) reported poverty levels from income equivalent to those found in this study for households of coffee producers in Puebla.

The use of agricultural technology by coffee producers was generally low (0.60), higher for Cuetzalan (0.71) and lower for the Mazateca region (0.50). The access to technical assistance services was also low, with a value of 0.39. The producers from Cuetzalan had greater access (0.49) than producers from the Mazateca region (0.30), which could explain in part the lower yields per hectare in the Mazateca (1316 kg ha^{-1}) compared to Cuetzalan (1805 kg ha^{-1}). These results are consistent with official statistics (SIAP, 2020) and with

Table 1. Sociodemographic and technological characteristics of coffee producers.

Variable	General		Cuetzalan		Oaxaca	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Age (years)	57.1	13.8	60.7	11.7	54.0	14.8
Human capital	0.55	0.29	0.62	0.37	0.44	0.36
Members	3.6	1.8	3.2	1.5	3.8	2.0
Experience (years)	26.5	10.7	29.6	15.3	23.9	13.7
Size (ha)	1.31	0.7	1.6	1.2	1.0	0.8
Per capita income ($)	1332.6	730.8	1645.0	833.3	1062.8	431.6
I.U.T	0.60	0.22	0.71	0.26	0.50	0.20
I.A.T.	0.39	0.21	0.51	0.19	0.28	0.17
Yields (kg ha^{-1})	1542.5	619.2	1804.8	410.4	1315.8	268.0

what was reported by Canet *et al.* (2016), who showed a decrease in the yields, both in Puebla and in Oaxaca since 1990 until today.

Costs and profitability of coffee production

The production cost of cherry coffee is integrated by installation costs and operation costs. Within the operation costs, the cost of workforce represented 70%, which shows the social importance of this activity. From the total of workforce between, between 30 and 60% was family members, depending on the size of the plantation. In general, the costs were higher for Cuetzalan, derived from the greater use of technological components. The general profitability (BCR) was 0.90, which means that for each peso invested, 0.10 pesos were lost; that is, 10% of the investment. For producers in Puebla, the BCR was 1.0 and for those in Oaxaca, 0.81 (Table 2). In previous studies, negative profitability was reported for small-scale producers in Hawaii (Woodill *et al.*, 2014) and for producers in Brazil (Turco *et al.*, 2017), while Espinosa-García *et al.* (2016) reported for Mexico that yields under 4.5 tons per hectare generate negative profitability.

The producers, once the cherry coffee production has been harvested, have the options of selling the coffee as cherry or processing it (depulping, washing and drying). Of the producers, 65% processed and sold parchment coffee, the rest sold it as cherry. The BCR of the producers that sold parchment was 1.16 for those in Cuetzalan and 1.04 for those in the Mazateca. The increase in profitability that producers who sold parchment obtained is the result of the added value from processing.

Table 2. Costs and income of coffee production in Oaxaca and Puebla, Mexico.

Instalatión costs ($)	Average	Standard deviation	Mean Puebla	Standard deviation	Mean Oaxaca	Standard deviation
Tillage costs	3789.8	3109.2	4483.1	3669.0	3190.8	2388.8
Coffee plant	15159.4	12436.7	17932.6	14675.9	12763.1	9555.2
Labour costs	6316.4	5182.0	7471.9	6115.0	5318.0	3981.3
Instalation	25265.6	20727.8	29887.6	24459.8	21271.8	15925.3
Operation costs						
Costs of fertilizer	1574.3	2423.1	2583.7	2670.4	702.0	1784.5
Pests and diseases	1232.0	2424.9	2090.6	3152.6	490.1	1112.2
Cleaning	1779.5	1254.0	2083.7	1519.7	1516.7	895.3
Pruning	430.9	663.1	672.6	839.1	222.0	347.5
Harvest	6887.4	5172.1	7290.7	6063.7	6538.9	4255.2
Transportation	909.9	1327.7	974.4	1563.5	854.2	1088.8
Total operation costs	12814.0	13264.9	15695.7	15809.0	10324.0	9483.5
Fixed costs	2127.8	1748.8	2441.1	1960.8	1857.1	1500.1
Production costs	14911.5	10083.0	18089.7	11641.3	12165.3	7551.6
Income (cherry)	13443.20	8672.71	18124.2	8299.91	9898.65	4891.21
BCR (cherry)	0.90		1.0		0.81	

Level of use of technology and yields

The level of use of technology in the coffee production process is a factor that is fostered to improve the unitary yields, and in the case of fixed prices, also to improve the profitability. The analysis of variance revealed that the level of use of technology, represented by the "low" and "medium" Technological Index (TUI) belong to the same group, which means that the group of producers with "low" TI and "medium" TI have a mean of coffee yield (ton/ha) that is statistically equal. Instead, "low" TUI and "high" TUI have different means (Table 3). Similarly, the "medium" and "high" TUIs are significant, which is why they are in different groups, and their means are different. The behavior of the groups with "low" and "medium" level of use of technology also have an equal profitability mean (BCR). Meanwhile, the "low" and "high", and "medium" and "high" groups also have different means. These results are consistent with what is reported by Wambua *et al.* (2019) for coffee producers in Kenya and by Afolami *et al.* (2015) for producers in Nigeria.

Explicative model of the yields and the profitability

The coffee yield (t ha^{-1}) and the profitability (BCR), according to the results presented, are related variables. The results from the statistical model show that the TI and the size of the PU are the most important explicative variables both of the yield and of the profitability (Table 4). In the case of the yields, the "region" variable is very important and significant ($p>0.05$). In the case of the explanation of the profitability, the "schooling" variable is the third most important. The size of the plot of the PU, the TUI and the HC are variables that are susceptible of being modified to improve the yields and the profitability, especially the last two. The positive effect of agricultural technology on yield and profitability was reported by Wambua *et al.* (2019) in a study in Kenya. Xu *et al.* (2009) reported, in a study in Zambia, that schooling had a positive effect on the profitability, and Bravo-Monroy *et al.* (2016) found that with larger size of the population the net profit of coffee producers in Colombia will be affected positively.

Table 3. Difference of the means of yield (ton/ha) and profitability (BCR) according to the level of use of technology.

Yield (kg/ha)		Means difference	E.T	Significance
IT Low	Medium	**−92.51**	**71.93**	**0.439**
	Higth	−468.06*	66.97	0.000
IT Medium	Low	**92.51**	**71.93**	**0.439**
	Higth	−375.55*	61.21	0.000
Profitability (cherry)				
IT Low	Medium	**−0.084**	**0.046**	**0.184**
	Higth	−0.404*	0.042	0.000
IT medium	Low	**0.084**	**0.046**	**0.184**
	Higth	−0.319*	0.039	0.000

Table 4. Explicative model of the yields and the profitability.

Explicative variable	Yields			Profitability		
	coefficient	Typical error	t-value	coefficient	Typical error	t-value
(Constant)	0.10	118.13	5.69	.432	.082	5.25
Region	.369	41.98	7.48	.091	.022	2.37
Members	.064	10.66	1.39	.012	.007	0.25
Age (years)	−.064	37.58	−1.29	−.059	.026	−1.17
Human capital	.134	6.26	2.36	.188	.004	3.27
Experience (years)	.109	1.49	2.12	.007	.001	0.14
Size (Ha)	.383	22.14	7.03	.576	.015	10.46
IUT	.305	79.66	5.66	.389	.056	7.14
A.T. Index	.043	82.84	0.72	−.056	.058	−0.92
Income ($)	.063	0.02	1.25	.053	.012	1.04
Adjust (R^2/R^2 ajust)	0.715/0.701			0.690/0.684		
F-statistic	49.47			47.98		
S-W (prob>z)	0.1674			0.1651		

Regarding the goodness of fit of the models, taking together the independent variables, they explain 71% of the behavior of the yields and 69% of the profitability. The values of the F statistics reject the null hypothesis that the population value of R^2 is zero. Therefore, there is a significant linear relation ($p>0.05$).

CONCLUSIONS

Coffee producers, especially those with less surface, face an adverse situation in the productive and in the economic aspects. The coffee yields in 2019 were lower than in the 1990s, and than those from the first decade of this century; as consequence, the profitability is negative in most of the sites and producers surveyed. The level of use of agricultural technology in coffee production is limited, although it shows a positive effect in improving yields and profitability. The factors that explain the behavior of the yields and of the profitability are mainly level of use of technology, size of the production unit, and human capital. These variables could be part of a public support strategy to improve the yields and the profitability of the aromatic plant.

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REFERENCES

Afolami, C.A., Obayelu, A.E., & Vaughan, I.I. (2015). Welfare impact of adoption of improved cassava varieties by rural households in South Western Nigeria. *Agricultural and Food Economics, 3*(18):1-17. https://doi.org/10.1186/s40100-015-0037-2.

Apodaca-González, C., Juárez-Sánchez, J. P., Ramírez-Valverde, B., Díaz-Ruiz, R., Rodríguez-Escobedo, F., & Vázquez-Solís, V. (2020). La alimentación familiar de pequeños productores de café y variabilidad climática en Huehuetla, Puebla, México. *Agricultura Sociedad Y Desarrollo, 17*(2):375-396. https://doi.org/10.22231/asyd.v17i2.1350.

Bravo-Monroy, L., Potts, S.G., & Tzanopoulos, J. (2016). Drivers influencing farmer decisions for adopting organic or conventional coffee management practices. *Food policy, 58*: 49-61. https://doi.org/10.1016/j.foodpol.2015.11.003.

Burgelman, R.A., Christensen, C.M., & Wheelwright, S.C. (2008). Strategic management of technology and innovation. Ed. McGraw-Hill/Irwin, 5th Edition, ISBN-13: 978-0073381541. 1280 p.

Canet, B.G., Soto V.C., Ocampo, T.P., Rivera, R,J., Navarro, H.A., Guatemala, M.G., & Villanueva R.S. (2016). La situación y tendencias de la producción de café en América Latina y El Caribe. Publicación No. IICA P311. 126 p.

Centro de Estudios para el Desarrollo Rural Sustentable y la Soberanía Alimentaria (CEDRSSA). (2019). Caracterización de los sistemas alimentarios de los pueblos indígenas de México. http://www.cedrssa.gob.mx/

Consejo Nacional de Evaluación de la Política de Desarrollo Social (CONEVAL). (2020). Evolución de las líneas de pobreza por ingresos. Disponible en: https://www.coneval.org.mx/Medicion/MP/Paginas/Lineas-de-bienestar-y-canasta-basica.aspx

Contreras-Hernández, A. (2010). Los cafetales de Veracruz y su contribución a la sustentabilidad. *Revista Estudios Agrarios, 45*, 143-citation_lastpage.

Espinosa-García, J. A., Uresti-Gil, J., Vélez-Izquierdo, A., Moctezuma-López, G., Uresti-Durán, D., Góngora-González, S. F., & Inurreta-Aguirre, H. D. (2016). Productividad y rentabilidad potencial del café (*Coffea arabica* L.) en el trópico mexicano. *Revista Mexicana de Ciencias Agrícolas, 7*(8), 2011-2024. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S2007-09342016000802011&lng=es&tlng=es.

Fuglie, K., Dhehibi, B., El Shahat, A. A. I., & AwdHassan, A. (2020). Water, Policy, and Productivity in Egyptian Agriculture. *American Journal of Agricultural Economics*. https://doi.org/10.1111/ajae.12148

Instituto Nacional de Estadística y Geografía (INEGI). (2020). Censo de Población y Vivienda. https://www.inegi.org.mx/programas/ccpv/2020/

Monterroso, A., Conde, C., Gay, C., Gómez, D., & López, J. (2014). Two methods to assess vulnerability to climate change in the Mexican agricultural sector. *Mitig Adapt Strateg Glob Change: 19*:445–461. https://doi.org/10.1007/s11027-012-9442-y

Organización Internacional del Café (OIC). (2020). Informe del mercado de café. http://www.ico.org/es/Council-20-21-c.asp

Rivadeneyra, P. J. I. & Ramírez, V. B. (2006). El comercio local del café a raíz de su crisis en Sierra Norte de Puebla. *Revista Mexicana de Agronegocios, 10*(18):1-14. https://www.redalyc.org/articulo.oa?id=14101807

Servicio de Información Agroalimentaria y Pesquera (SIAP). (2020). Siembra y Cosechas. http://infosiap.siap.gob.mx:8080/agricola_siap_gobmx/ResumenProducto.do

Turco, P.H.N., Esperancini, M.S.T., Bueno, O.D.C., & Oliveira, M.D.M. (2017). Economic profitability in conventional and irrigated coffee production systems in three municipalities in the Marilia region of Sao Paulo, Brazil. *Ciência Rural, 47*(11). http://dx.doi.org/10.1590/0103-8478cr20170170

Valbuena, D.N.J., Leal, G.M., & Urdaneta, M.A. (2018). ADN organizacional y productividad en las empresas familiares. *Desarrollo Gerencial, 10*(1):105-122. https://doi.org/10.17081/dege.10.1.2987

Wambua, D.M., Ndirangu, S.N., Njeru, L.K., & Gichimu, B.M. (2019). Effects of recommended improved crop technologies and socio-economic factors on coffee profitability among smallholder farmers in Embu County, Kenya. *African Journal of Agricultural Research, 14*(34): 1957-1966.

Woodill, A.J., Hemachandra, D., Nakamoto, S.T., & Leung, P. (2014). The economics of coffee production in Hawai 'i. *Econ, EI 25*:1-9.

Xu, Z., Guan, Z., Jayne, T.S., & Black, R. (2009). Factors influencing the profitability of fertilizer use on maize in Zambia. *Agricultural Economics, 40*(4): 437–446. https://doi.org/10.1111/j.1574-0862.2009.00384.x

AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Trichoderma harzianum in vitro mycoparasitism on *Peronospora belbahrii* in basil (*Ocimum basilicum*)

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ABSTRACT

Objective: To describe the symptomatology and to identify the mildew causal agent in basil (*Oscimum basilicum*), as well as the *Trichoderma harzianum-Peronospora belbahrii in vitro* mycoparasitic activity.

Design/methodology/approach: Samples were taken from Nufar basil cultivars that had been naturally infected by mildew and, afterwards, the causal agent was isolated in order to carry out a pathogenicity test. The *T. harzianum-P. belbahrii* parasitism stages were observed in samples from the area in which both microorganisms interact.

Results: The disease symptoms that reveal the presence of a mildew causal agent on basil plants grown in pots and soil match *Peronospora belbahrii*. Subsequently, the *Trichoderma* hyphae rolled up and penetrated and vacuolated the conidiophores and the pathogen mycelium.

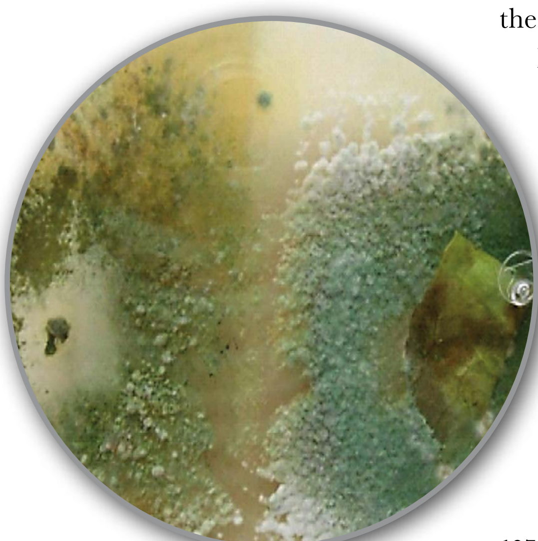
Study limitations/implications: This study was carried out using only one variety of basil.

Findings/conclusions: *T. harzianum's* capacity to parasitize *P. belbahrii in vitro* was observed after 72 h. Once the conidium of the antagonist germinated, the hyphae directed their chemotropism growth towards *P. belbahrii's* conidiophores and mycelium.

Key Words: Biological control, *Trichoderma*, Confrontation test.

INTRODUCTION

Basil (*Ocimum basilicum*) is grown throughout the world and is mostly used by the gastronomy and make up sectors, as well as for therapeutic purposes (Martínez *et al.*, 2016). In Mexico, 49,460 ha are used for its production, obtaining a 9.63 t ha^{-1} estimated yield. This crop has been traditionally grown in the following states: Baja California, Morelos, Nayarit, and Baja California Sur (SAGARPA, 2015). Fungal diseases have a major impact in basil crops. These diseases appear mainly during months with heavy rainfall, heat, and cloudy days. Therefore, producers must pay attention and monitor the crops (Briseño, 2013). Mildew (*Peronospora belbahrii*) is the main disease that affects basil worldwide (Saude *et al.*, 2013; Choi *et al.*, 2016). Belbahrii *et al.* (2005) and Thines *et al.* (2009) called this disease *Peronospora belbahrii* and they classified it in the Kingdom Chromista, Order Personosporales, and Family Peronosporaceae.



Mildew is considered a biotrophic and polycyclic parasite; the conventional alternatives for its handling include the use of tolerant varieties and the application of cyazofamid (Ranman), mandipropamid (Revus), azotrystrobin, mefenoxam, and mandipropamid (Homa *et al.*, 2014). However, this pathogen can develop resistance to fungicides, especially mefenoxam (Cohen *et al.*, 2013; Collina *et al.*, 2016). The toxic residues and the limited availability of fungicides make it difficult to exercise a chemical control of downy mildew (Gisi and Leadbeater, 2010). Therefore, we need to find different techniques that will allow producers to efficiently handle the disease. In this regard, biological control is a viable alternative to decrease the impact of *P. belbahrii* in basil plantations. Species from the *Trichoderma* genus have shown an efficient control of phytopathogenic fungi. *Trichoderma* inhabits the rhizosphere zone, protects plants from pathogens through competition, mycoparasitism, and antibiosis (Lorito *et al.*, 2010; Druzhinina *et al.*, 2011). It has shown positive responses in the control of *Alternaria alternata* (Sempere and Santamarina, 2007), *Pseudoperonospora cubensis* (Martínez *et al.*, 2011), and *Didymella bryoniae* (Martínez *et al.*, 2013).

The application of *T. harzianum* in the soil reduced *Fusarium proliferatum* in onion (*Allium cepa*) crops by 25% (Ghanbarzadeh, 2016). The seeds of different varieties of tomato (*Solanum lycopersicum*) were inoculated with *T. harzianum* to control *Pythium* spp. (Majorie *et al.*, 2016). The objective of this study was to describe the mildew symptomatology and its causal agent in basil crops, as well as the *T. harzianum in vitro* mycoparasitism on *P. belbahrii*.

MATERIALS AND METHODS

Symptomatology and identification of the mildew causal agent on basil

The pathogen used for this study was isolated from Nufar basil cultivars sowed on June 2016, which had been naturally infected. The productive areas in which that crop was grown were located in the State of Morelos, Mexico, between Puente de Ixtla and Mazatepec (km 3.5), between parallels 18° 27' and 18° 43' N and meridians 99° 11' and 98° 23' W, at 967 masl. A macroscopic description of the symptoms and typical signs of the disease in field conditions was carried out. Sprouts from infected plants were selected and sent to the Quality Herbs lab. Twenty-five subsamples were observed to develop the microscopic characterization. These structures were observed in a Nikon optical microscope, using a 40x magnification.

In order to carry out a pathogenicity test, sporangia from basil leaves infected with *P. belbahrii* were obtained. The infected leaves were immersed in cold sterile water (10 °C), and their underside was gently rubbed with a spatula. A sporangium watery suspension with a 3.7×10^5 sporangium per mL concentration was obtained. This concentration was determined using a quantitative dilution method and counting spores and sporangium with a 0,100 Mm/0,0025 Mm^2 Neubauer Improved Marienfeld camera. The solution was sprinkled on the upper side and underside of the leaves, using a 1.5-L Matabi hand sprayer. The leaves belonged to 20 Nufar basil cultivar plants; they were 45 days old and were grown in 17.5 cm (diameter) × 13.5 cm (height) × 13 cm (depth) pots. The pots contained a sterile substrate (solarization) with a 1:1:1 rate of arable land, *atocle* (sandy, humid, and fertile soil, rich in humus), and compost. They were watered until soil saturation was achieved and they were placed in 50×70 cm polyethylene transparent bags. The bags were sealed to keep a 90-100% relative moisture; the average temperature reached 32±2 °C and 22±2 °C during the day and at night, respectively (Risco *et al.*, 2018; Zhan *et al.*, 2019). Regarding their morphological identification, the dichotomous keys developed by Clements and Shear (1931) and Thines *et al.* (2009) were used to identify their genus and species, respectively. Additionally, other researches were consulted to compare the information about the symptoms, signs, and reproductive structures of the natural infection and the artificial inoculation.

In vitro mycoparasitism of *T. harzianum* on *P. belbahrii*

A Bioxon PDA (Potato-Dextrose-Agar) culture medium placed in 90mm Petri dishes was used to describe *T. harzianum's* mycoparasitism on *P. belbahrii*. A modification of the dual confrontation test described by Martínez and Solano (1994) was carried out: a 25 mm^2 fragment of basil leaves was placed in each of the 20 Petri dishes; these leaves had a 30% *P. belbahrii* infection; they were placed on the culture, 1 centimeter away from both the edge of the Petri dish and the pathogen. Afterwards, *T. harzianum* was applied (Aislamientos de la Universidad Tecnológica del Sur del Estado de Morelos, isolated in Tehuixtla, Puente de Ixtla, Morelos). Five Petri dishes in which the antagonist was not inoculated were designated as control. Once the Petri dishes were inoculated, they were placed in an incubator at 25±2 °C for 96 h. In order to observe the hyphae interaction —coiling by hyphae, penetration, vacuolation, and lysis—, three samples were taken from the contact area of both fungi per repetition (Petri dish), were placed on a microscope slide with lactophenol and were observed using a Nikon Optical Microscope, with a 40x magnification.

RESULTS AND DISCUSSION

Symptomatology and identification of the mildew causal agent on basil

Under controlled conditions, the symptoms and signs of downy mildew first appeared 18 h after the inoculation. Under both conditions (controlled and in the open fields), the symptoms and signs (Figure 1 A-B) were found on the older leaves in the middle of the plant, from where they spread to the leaves on the higher part of the plant. The leaves underwent chlorosis and had irregular stains that became dark brown and then black as the stain grew older. Cohen *et al.* (2013) and Wyenandt *et al.* (2015) pointed out that the chlorotic injuries of the leaves gradually turned necrotic. The first signs (a whitish sporulation) appeared on the underside of the leave; when the temperature started to raise above 30 °C, the sporulation turned dark brown and black (Figure 1-B). The reproductive structures changed color immediately after the first sunbeams and when there was morning dew or it had rained. Under lab conditions, Cohen and Ben-Naim (2016) determined that *P. belbahrii* started infecting the leaves just after four hours of unchecked moisture. A severe impact can be seen in the underside of the leaves (Figure 1-C). The pathogen showed hyaline and monopodial sporangiophores, emerging from the stroma located on the underside of the leaf. Dichotomously branched in an <90° angle, the upper side showed six submonopodial branches. The higher branches were curved and pointed, with curved sterigmata (one long and other short).

The zoosporangia had a slightly ovoid shape, with a round base and were chestnut brown or a darker color (Figure 1-D-E). Cohen *et al.* (2013) and Wyenandt *et al.* (2015) described dark purple and ovoid spores. The morphological description of the element isolated in the State of Morelos matched the description of *Peronospora belbahrii* made by Thines *et al.* (2009), Grabowski (2012), Bastidas *et al.* (2016), Cohen *et al.* (2017), Risco *et al.* (2018), and Zhan *et al.* (2019). According to the observation of the symptoms in plants grown in pots and in the field, the pathogenicity test showed that *Peronospora belbahrii* is the mildew causal agent of basil in the study area.

In vitro mycoparasitism of *T. harzianum* on *P. belbahrii*

The *Trichoderma* colonies placed in the PDA culture medium were originally white, but turned dark green three hours later (Figure 2-A). Their phialides were bottle-shaped: they had a small base, a swollen middle, and a narrow apex. Most of them were divided into 2-3 whorls. Their mycelium was thin and slender; their hyphae had septa with smooth walls. The conidiophore had narrow, curved branches with a main axis (Figure 2-B).

T. harzianum colonies have grey to green mycelia; the edge of their growth area is white; they have narrow conidiophores and ampulliform phialides; they have a narrow base, a swollen middle section, and a thin apex. They have subglobal, ellipsoidal conidia and elliptical chlamydosphores (Ellis, 2006; Samuels *et al.*, 2007; Chaverri *et al.*, 2015; Wang *et al.*, 2016).

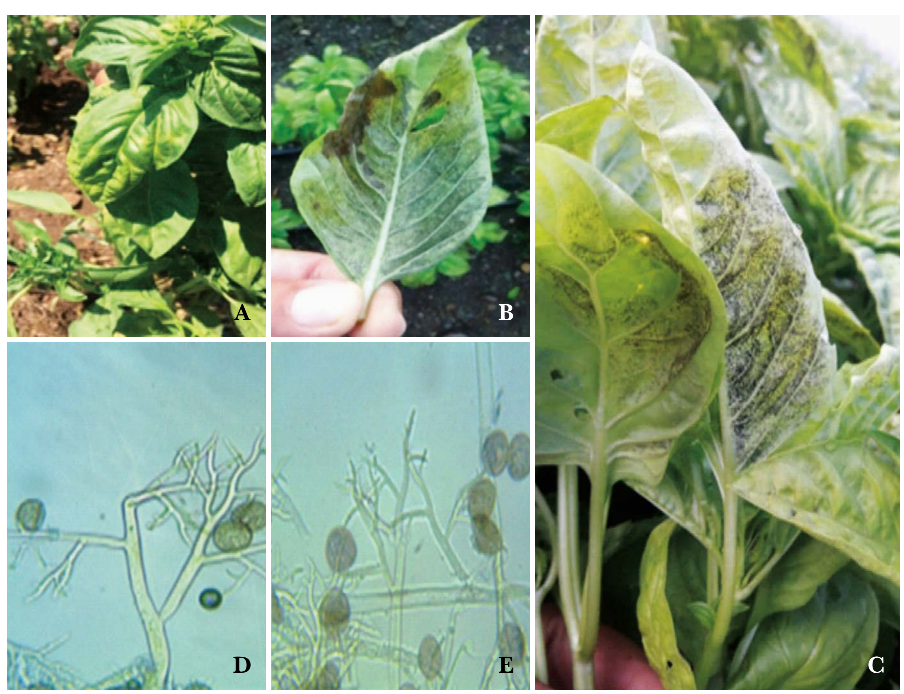


Figure 1. Signs of mildew symptoms in basil plantations (A); presence of signs on the underside (B) and upper side of the leaves (C); microscopic observations at 40x of the vegetative and reproductive structures (D) and (E).

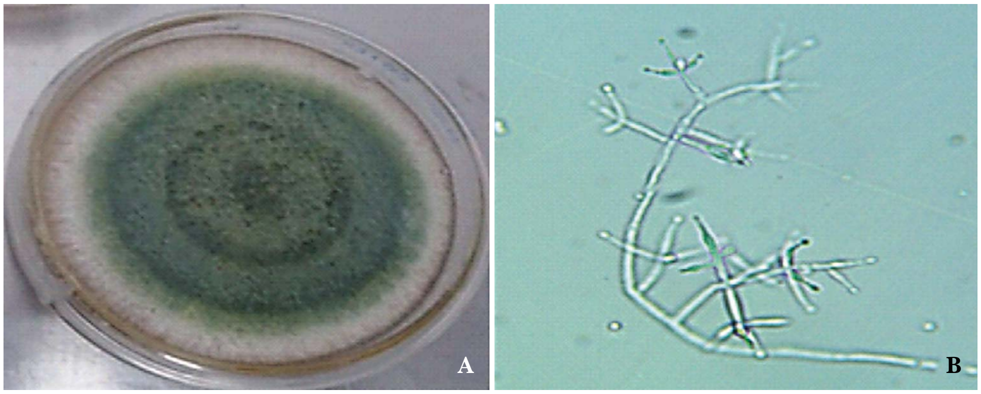


Figure 2. *Trichoderma harzianum* colony (A) and conidiophores, phialides, and conidia under the microscope (B).

Interaction with the pathogen occurred 72 hours after the dual confrontation test started; after the contact took place, *P. belhabhrii* stopped growing. After 96 hours, *Trichoderma* started to cover the pathogen (Figure 3).

Figure 4-A shows that, once *T. harzianum's* conidia germinated, the hyphae grew towards *Peronspora belbahrii's* conidiophores, sliding over its surface without penetrating it (chemotrophic growth-search). Under positive chemotropism conditions, *Trichoderma* grows directly towards the chemical stimulus released by the pathogen (*i.e.*, the first host location phase). Chet and Inbar (1994) proved that *Trichoderma* can detect the pathogen from a distance and that its hyphae grow towards it. Subsequently, the hyphae of *T. harzianum* started to wound around the conidiophores and hyphae of *P. belbahrii* (Figure 4-B); they developed hook- and appressorium-like structures to hold on to them. Finally, *Trichoderma* penetrated *P. belbahrii's* hyphae (Figure 4-C) and vacuolation took place (Figure 4-C).

When the penetration process takes place, the antagonist produces extracellular lytic enzymes —mainly, chitinases, glucanases, and proteinases—, which deteriorate the cell

Figure 3. *T. harzianum-P. belbahrii in vitro* interaction at 96 hours.

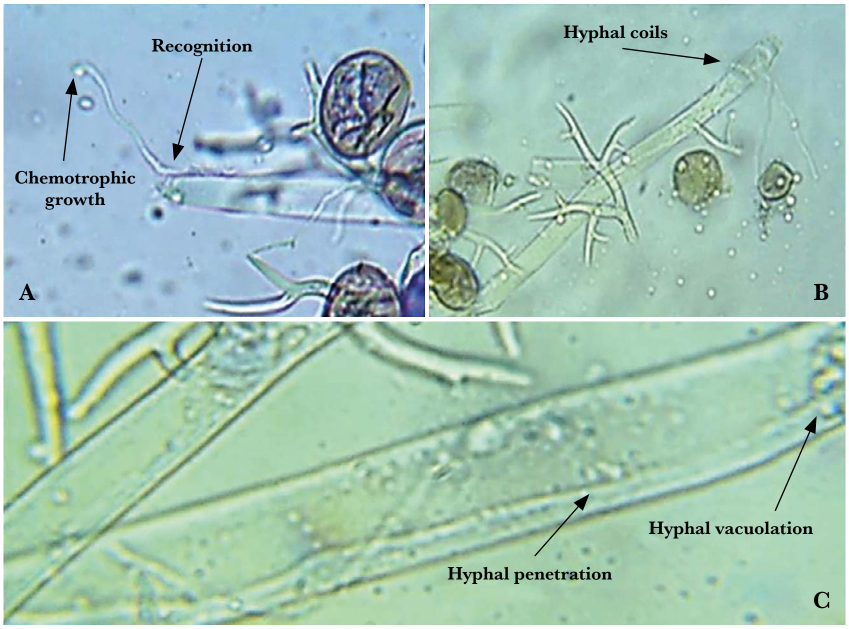


Figure 4. Pictures taken with a 40x optical microscope. The following types of *T. harzianum-Peronospora belbahrii* hyphal interaction can be seen: chemotrophic growth and search (A); coiling by hyphae (B); penetration and vacuolation (C).

walls of the host and allow the hyphae of the antagonist to penetrate the host (Haram *et al*., 1996). Martroudi *et al*. (2009) have also described the antagonism process or mycoparasitic activity of *Trichoderma* spp. against *Sclerotinia sclerotiorum* (Lib.) de Bary. Wang *et al*. (2016) reported that the mycelia of *T. harzianum* wound around the mycelia of *Lentinula edodes* (Berk) Pegle, causing the cells to start a lysis process.

CONCLUSIONS

The pathogenicity test showed that *Personospora belbahrii* is the mildew causal agent on basil in the study area. Under controlled conditions, the *T. harzianum* isolation evaluated in this study shows promising signs for the control of *Personospora belbahrii* in basil. Four types of hyphal interaction showed signs of parasitism: chemotrophic growth and search; coiling by hyphae, penetration, and vacuolation; reduction of mycelial growth; and development of sporangia.

REFERENCES

Bastidas, M.R., Picazo, L.S., Amador, B.M., Garibay, A.N., Barragan, H.L. & Montie, L.G.H. (2016). First report of *Peronospora belbahrii* on sweet basil in Baja California Sur, Mexico. *Journal of Phytopathology 164*, 122–124.

Belbahri, L., Calmin, G., Lefort, F. & Pawlowski, J. (2007). Phylogenetic analysis and real time PCR detection of a new *Peronospora* species responsible for downy mildew disease of sweet basil and sage. *Acta Horticulturae 747*, 401–408.

Briseño, S., Aguilar, M. & Villegas, J. (2013). El cultivo de albahaca. Centro de Investigaciones Biológicas del Noreste, S.C. Instituto Politécnico Nacional No. 195 Col. Playa Palo de Santa Rita Sur. La Paz, Baja California Sur. México. 33. https://silo.tips/download/el-cultivo-de-la-albahaca

Chaverri, P., Branco, R.F., Jaklitsch, W., Gazis, R., Degenkolb, T. & Samuels, G.J. (2015). Systematics of the *Trichoderma harzianum* species complex and the reidentification of commercial biocontrol strains. *Mycologia 107*(3), 558–590.

Chet, I. & Inbar J. (1994). Biological control of fungal pathogens. *Applied Biochemistry and Biotechnology 48*, 37–43.

Choi, Y.J., Choi, I.Y., Lee, K.J. & Shin, H.D. (2016). Primer informe de mildiu causado por *Peronospora belbahrii* en albahaca dulce (*Ocimum basilicum*) en Corea. Plant Disease 100, 2335. doi:org/10.1094/PDIS-05-16-0771-PDN

Clements, F.E. & Shear, C.L. (1931). The genera of Fungi. Carnegie state Institution of Washington and United State Department of Agriculture. New York and London: Hafner Publishing Company.

Cohen, Y. & Ben, N.Y. (2016). El abanico nocturno suprime las epidemias de mildiu en la albahaca dulce. *PLoS One. 11* (5), e0155330. doi: 10.1371/journal.pone.0155330.

Cohen, Y., Ben-Naim, Y., Falach, L. & Rubin, A.E. (2017). Epidemiology of basil downy mildew. *Phytopatology 107*(10), 1149–1160.

Cohen, Y., Vaknin, M., Ben, N. Y & Rubin, A.E. (2013). Light suppresses sporulation and epidemics of *Peronospora belbahrii*. *Plos One 8*(11), e81282. doi: 10.1371/journal.pone.0081282

Cohen, Y., Vaknin, M., Ben-Naim, Y., Rubin, A. E. & Galperin, M. (2013). First report of the occurrence and resistance to mefenoxam of *Peronospora belbahrii*, causal agent of downy mildew of basil (*Ocimum basilicum*) in Israel. *Plant Disease 97*, 692. doi:10.1094/PDIS-12-12-1126-PDN

Collina, M., Merighi, M., Turan, C., Pirondi, A., Minuto, G. & Brunelli, A. (2016). First report of resistance of *Peronospora belbahrii*, causal agent of downy mildew of basil, to mefenoxam in Italy. *Plant Disease 100*, 1787. doi:10.1094/PDIS-02-16-0237-PDN

Druzhinina, I.S., Seidl, S.V., Herrera, E.A, Horwitz, B.A., Kenerley, C.M. & Monte, E. (2011). *Trichoderma*: the genomics of opportunistic success. *Nature Reviews Microbiology 9*, 749–759.

Ellis, D. (2006). *Trichoderma*. The University of Adelaide Australia. Mycology [Online]. http://www.mycology.adelaide.edu.au/Fungal_Descriptions/Hyphomycetes (hyaline)/Trichoderma

Ghanbarzadeh, B., Safaie, N., Mohammadi, G.E., Rezaee, D.Y. & Khelghatibana, F. (2016). Biological control of *Fusarium* basal rot of onion using *Trichoderma harzianum* and *Glomus mosseae*. *Journal of Crop Protection 5*(3), 359–368.

Grabowski, M. (2012). Basil downy mildew *Peronospora belbahrii*. Regents of the University of Minnesota. University of Minnesota Extension.

Haram, S., Schickler, H.L. & Chet, I. (1996). Molecular mechanisms of lictin enzymes involved in the biocontrol activity of *Trichoderma harzianum*. *Microbiology 142*, 2321–2331.

Harman, G.E. (2006). Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology 96*, 190–194.

Homa, K., Barney, W.P., War, D.L., Wyenandt, C.A. & Simon. J.E. (2014). Evaluation of fungicides for the control of *Peronospora belbahrii* on sweet basil in New Jersey. *Plant Disease 98*, 1561–1566.

Infante, D., Martínez, B.C., González, N. & Reyes, Y. (2009). Mecanismos de acción de *Trichoderma* frente a hongos fitopatógeno. *Protección Vegetal 24*(1), 14–21.

Gisi, U., & Leadbeater, A. (2010). The challenge of chemical control as part of integrated pest management. *Journal of Plant Pathology 92*(4), S11–S15.

Lorito, M., Woo, S.L., Harman, G.E. & Monte, E. (2010). Translational research on *Trichoderma*: from 'omics to the field. *Annual Review of Phytopathology 48*, 395–417.

Majorie, R.M., Chaibva, P., Takawira, M. & Njeni, P. (2016). Evaluation of *Trichoderma harzianum* in controlling damping-off (*Pythium* spp) on tomato (*Solanum lycopersicum*) seedling varieties. *Annals of Biological Research 7*(6), 6–11.

Martínez, B., González, E. & Infante, D. (2011). Nuevas evidencias de la acción antagonista de *Trichoderma asperellum* Samuels. *Protección Vegetal 26*(2), 131–132.

Martínez, B., Pérez, J., Infante, D., Duarte, Y. & Moreno, M. (2013). Antagonismo de aislamientos de *Trichoderma* spp., frente a *Didymella bryoniae*. *Protección Vegetal 28*(3), 192–198.

Martínez, G.G.A., Nicolás, S.L., Ortiz, H.Y.D., Morales, I. & Gutiérrez, H.G.F. (2016). Crecimiento y contenido de aceite en albahaca (*Ocimum basilicum* L.) bajo mallas sombra de colores. *Interciencia 41*(6), 428–432.

Martroudi, S., Zamani, M.R. & Motallebi, M. (2009). Antagonistic effects of three species of *Trichoderma* sp. on sclerotinia (*Sclerotiorum*), the causal agent of canola stem rot. *Egyptian Journal of Biology 11*, 37–44.

Nico, I., Monaco, I., Bello, G. & Alippi, H. (2005). Efecto de la adición de enmiendas orgánicas al suelo sobre la capacidad patogénica de *Rhizoctonia solani*: II Micoflora asociada y antagonismo *in vitro* de los aislados más frecuentes. *Revista de Investigaciones Agropecuarias 34*(1), 29–44.

Risco, M.A., Apaza, W.E.T. & Mattos, L.L.C. (2018). Identification of basil downy mildew and severity estimation. *Anales Científicos 79* (1), 159–167.

SAGARPA, Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. (2015). Servicio de información agroalimentaria y pesquera. Datos Abiertos. Estadística de Producción Agrícola. http://infosiap.siap.gob.mx/gobmx/datosAbiertos.php

Samuels, G.J., Chaverri, P., Farr, D.F & McCray, E.B. (2007). Trichoderma Online, Systematic Botany & Mycology Laboratory. http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm

Saude, C., Westervel, S. & Filotas, M. & McDonald, M.R. (2013). First report of downy mildew caused by *Peronospora belbahrii* on basil (*Ocimum* spp.) in Ontario. *Plant Disease 97*(9), 1248. doi: 10.1094/PDIS-01-13-0026-PDN

Sempere, F. & Santamarina, M.P. (2007). *In vitro* biocontrol analysis of *Alternaria alternata* Keissler under different environmental conditions. *Mycopathology 163*:83–90.

Thines, M., Telle, S., Ploch, S. & Runge, F. (2009). Identity of the downy mildew pathogens on sage, basil and coleus, with implications for quarantine measures. *Mycological Research 113* (5), 532–540.

Wang, G., Cao, X., Ma, X., Guo, M., Liu, C., Yan, L. & Bian, Y. (2016). Diversity and effect of *Trichoderma* spp. associated with green mold disease on *Lentinula edodes* in China. *Microbiology Open 5*(4), 709–718.

Wyenandt, C.A., Simon, J.E., Pyne, R.M., Homa, K., McGrath, M.T., Zhang S., Raid R.N., Ma L., Wick R., Guo L. & Madeiras A. (2015). Basil downy mildew (*Peronospora belbahrii*): discoveries and challenges relative to its control. *Phytopathology 105*(7), 885–894.

Zhan, G., Thompson, A., Schisler, D. & Johnson, E.T. (2019). Characterization of the infection process by *Peronospora belbahrii* on basil by scanning electron microscopy. *Heliyon 5*(1), e01117. https://doi.org/10.1016/j.heliyon.2019.e01117

Greenhouse cultivation of Serrano pepper (*Capsicum annuum* var. Acuminatum): dry hydroponics

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ABSTRACT

Objective: To produce Serrano peppers using a new cultivation technique that mixes the best of hydroponic cultivation and traditional cultivation.

Design/methodology/approach: We set up a growing system where the Serrano pepper grew from seedling to its full-fledged state, in a growbag containing sand (as support material), potassium polyacrylate (as water retention material), and a nutrient solution for vegetable gardens, under greenhouse conditions. The humidity level of the growbag is controlled by adding as much water with nutrient solution as needed. This technique has generated 100% harvestable plants (total: 20 plants); more than 90% of the water can be saved in comparison to regular and hydroponic cultivation.

Results: Once the harvest began, at least 22.857 kg of Serrano pepper were obtained in 3 m^2 of soil in a system with a pyramidal structure in which the 20 plants were placed. If we extrapolated this data, approximately 93,000 kg of Serrano pepper could be harvested from a 1 ha system.

Study Limitations/implications: This technique tries to tackle the water access limitations that may exist in some areas of Mexico. However, it does not mitigate the initial costs of a greenhouse system. Nevertheless, this technique can be reused up to 10 times without requiring maintenance.

Findings/conclusions: Experience has shown that hydroponic crops are truly profitable, despite their vast water requirements, which is precisely what prevents their global expansion. However, our modification of this method saves more than 90% of the water, using potassium polyacrylate as a retention agent and sand as a support material. Therefore, this technique could be implemented even in places where water is scarce.

Keywords: Dry hydroponics, potassium polyacrylate, Serrano pepper.

INTRODUCTION

Hydrogels are cross-linked macromolecular hydrophilic polymer chains capable of absorbing water or aqueous fluids. The most commercially successful hydrogels are superabsorbent polymers, also known as SAP (Zhouriaan-Mehr *et al.*, 2010), which generally can absorb around 200-350 times their weight in distilled water, increasing proportionally in size.

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Image by Hans Braxmeier at Pixabay

An osmosis process delivers up to 95% of the retained water to the plant; its benefits include: a notable delay in wilting under hostile conditions; a greater growth of the species; and a tendency to decrease the volume of water required to maintain a crop. Hydrogels are being used in some crops, to provide plants with the water that their development requires during dry seasons —reducing up to 75% of the water used for irrigation— and to obtain other benefits —such as increasing the productivity and quality of the crops (Anonymous, 2008). The application of hydrogels in agricultural crops was first studied in Mexico by the agronomist Sergio Rico, who realized that, if water was added to a polymer powder, the resulting gel would be capable of absorbing up to 500 times its weight. This gel could be deposited next to the root of the plants, providing them the water they need for their development (Anonymous, 2021).

Competition for natural resources (soil and water) is intensifying. Climate change reduces the resistance of production systems and contributes to the degradation of natural resources. The expansion of the agricultural frontier for food production has a high environmental impact, as a result of the deforestation of forests, contributing significantly to climate change, as well as to the loss of soil fertility and production potential. From a sustainability perspective, controlled spaces influence the enhancement of plant production, contributing to the conservation of the soil and natural resources (FAO, 2020).

The objective of this research was to evaluate the water retention capacity of hydrogel in greenhouse cultivation, seeking an alternative that reduces the use of irrigation water in horticultural crops and offering a solution to grow crops in semi-arid, arid or infertile areas, as well as generating crops in confined spaces to make the most of small areas.

MATERIALS AND METHODS

The study site is located in La Venta, Huimanguillo, Tabasco, Mexico (18° 06’ 43” N, 94° 02’ 24” W), at the Instituto Tecnológico Superior de Villa La Venta, which has a hot-humid climate, a 30 °C average temperature, and a 2000-3000 mm annual precipitation (Climatología, 2021). The dry hydroponics technique proposed in this work was developed under greenhouse conditions. Temperatures were taken inside and outside the greenhouse with high precision Hanna Checktemp®1 thermometers. Moisture was measured with a CONDUCTRONIC PC18 hygro-thermometer. A Hanna potentiometer was used to measure the pH. The nutrient solution and potassium polyacrylate were used without modifications —just as they were received from the commercial provider—; recycling materials were used to complement commercially available materials.

The structure of the hydroponics technique was modified for this research. Traditionally large amounts of water are used regardless of the variation of the method used, but emphasis is made on flooding and drainage techniques, as well as root-in-water techniques —such as Nutrient Film Technique (NFT) and Deep Water Culture (DWC), described by Gravital Agency (2021). The technique was developed in a greenhouse with a curved roof and mesh walls; this type of greenhouse has a high solar energy transmittance (Agrovit, 2021).

A 3-m^2 pyramidal structure (1.5 m wide × 2 m long) was built to place 20 growbags with one Serrano pepper plant per bag. Each growbag contained 50% of sand that had been previously mixed with potassium polyacrylate (4g of SAP with 5Kg of sand and 1Kg of gravel). The seeds were extracted from a commercially available stock and were prepared for germination in agricultural foam. Once the seedlings were obtained, they were integrated into the dry hydroponics system, as part of which they were watered with a nutrient solution for vegetable gardens, the same solution used in traditional hydroponic systems. As a reference system, 20 Serrano pepper seedlings were placed in 20-L jugs with a nutrient solution that was renewed every week (1 seedling per jug).

The first tests were carried out in the experimental area of the Universidad Popular de la Chontalpa (UPCH), where 150 Serrano pepper seeds were sown in three different agricultural foam systems (Figure 1a, b). We decided not to buy standard seeds, because ideally this project should be applied in all the socioeconomic scales in the area, which include small households and little or no investment.

The germination of the seeds was 100% successful. This allowed us to establish a seedling management protocol, because an extremely hot climate can be very aggressive if the control system is neglected.

Structure of the dry hydroponics system

The experimental design was installed in 4 spatial distribution lines, on a 1.5 m (h) × 2 m (l) × 1.5 m (w) pyramidal structure (Figure 2a). The experimental system included a total of 20 plants (Figure 2b).

Hydroponic system structure (reference system)

Recyclable materials (20-L jugs) were used for the hydroponic system. A 10-cm hole was cut on one side to introduce water and plants. They were previously disinfected with chlorine, detergent, and tap water. Subsequently, 20 L of water were poured in each jug. Twenty ml of nutrients for vegetable gardens were used per jug. A 3-L bottle cut in half —in

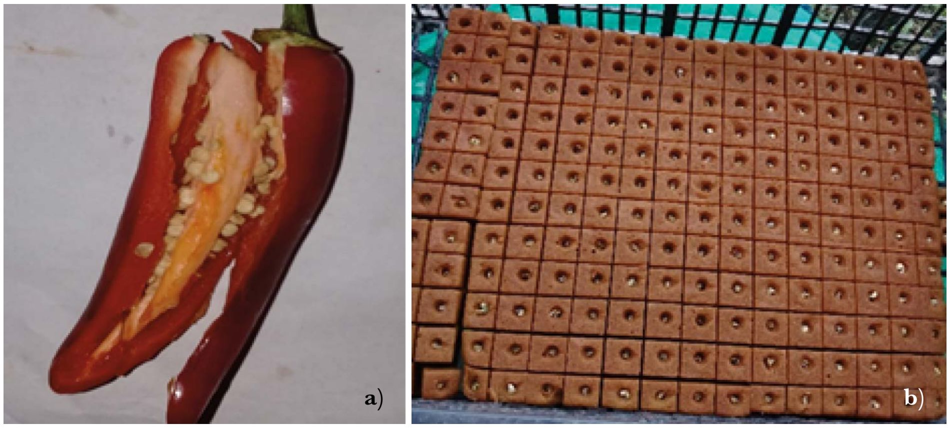

Figure 1. a) Seeds obtained from commercially-available fruits, b) Seed germination.

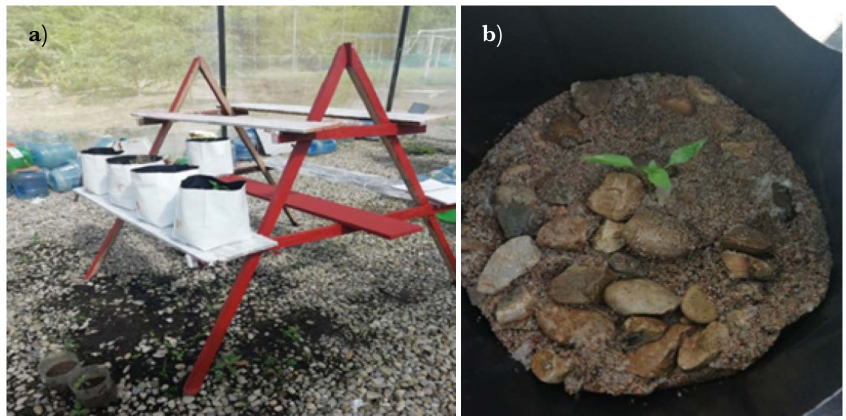

Figure 2. a) Pyramidal structure, b) Transplanted seedlings.

which a small hole was made in the lower part— was used to hold the plant. A total of 20 repetitions were carried out.

In other words, 10 3-L PET bottles were reused; they were cut in half (Figure 3a); a 2-cm hole was made in the lower part of the bottle to introduce the plant and to function as its support; finally, it was inserted in the jug that contained the abovementioned mixture (Figure 3b); the root floated on its surface.

RESULTS AND DISCUSSION

Irrigation is the most important part of the dry hydroponics technique. Table 1 shows that 20 L of the nutrient solution were added to the entire system every 4 weeks (1 L per growbag).

The results were encouraging after the end of the growing and irrigation period, up to 98% of the water was saved. Only 60 L of nutrient solution were used to grow 20 plants, while the traditional hydroponics reference system spent an average of 264 L per week for 13 weeks (total: 3440 L). One concern at this point is that, to maintain enough humidity

Figure 3. a) Section of the seedling support and b) Seedling in the support.

Table 1. Irrigation plan for the dry hydroponics system and the reference system.

Week	Dry hydroponic	Hydroponic
	Irrigation (L)	
1	20	400
2		400
3		400
4		360
5	20	360
6		320
7		300
8		260
9		200
10	20	160
11		140
12		100
13		40
	Total: 60	Total: 3440 L

for the healthy development of the plants, the average humidity measure should be 80% from the first day to the harvest. This is a major concern, since a hectare of traditional cultivation requires about 24,000 liters of water in a drip system —which is one of the most efficient systems (Horacio *et al*., 2010).

In this work, in addition to temperature and humidity, the pH of different parts of the growbag was measured. Both systems maintained a practically neutral pH of 6.73 and 6.77 for dry hydroponics and for the reference system, respectively. This is significant, because the Serrano pepper has a 5.5 and 7.5 pH range in which it can develop without any problem (Robinson, 2014). Additionally, the color, leaf size, and stem of each plant were recorded in order to determine if water control had had any kind of impact on the plants. These measurements did not show any impact, only completely healthy plants. However, the reference system had yellowish leaves before the water changes. Therefore, we concluded that water control should be carried out in shorter periods, thus causing a greater water expenditure.

Before discussing harvest yield, we must mention that the harvest fruit seemed to be ready a couple of weeks before the average harvest time. Table 2 shows that three important harvest exercises took place: 22.85 kg of Serrano pepper were obtained.

The production in the dry hydroponics system obtained the expected result: implementing potassium polyacrylate in the inert system as a water retention agent provided the plants with the humidity they required for their development and production. Additionally, 100% of the plants achieved a successful development; this is a much better result than the one obtained by the plants of the hydroponic system of reference —of which only 2 out of 20 bore fruits, at the time of harvest.

Table 2. Serrano pepper harvest record in a dry hydroponics system.

Number	HS1 harvest	HS2 harvest	HS3 harvest
	Weight (g)		
1	612	340	329
2	488	460	321
3	700	315	356
4	601	235	322
5	597	399	221
6	700	301	335
7	620	220	202
8	650	200	250
9	470	345	307
10	600	205	290
11	500	532	357
12	400	218	283
13	350	430	337
14	220	300	284
15	300	242	263
16	350	398	284
17	180	444	227
18	250	266	243
19	460	609	691
20	322	701	425
Partial	9370	7160	6327
Total	22857		

CONCLUSIONS

According to the results obtained in this greenhouse project with practically constant temperature, pH, and humidity conditions, we conclude that the use of potassium polyacrylate as a water retention agent to grow Serrano pepper, combined with this dry hydroponics technique —which does not require fertile soil, because sand and gravel can be perfectly used as support agents—, is an excellent alternative to develop an important agricultural culture in arid and semi-arid areas.

Irrigation water was saved, since only 60 L of water with nutrients for vegetable gardens were applied, providing an excellent humidity, temperature, and pH environment. As a result, 100% of the Serrano pepper plants grew perfectly healthy. We must also highlight that the Serrano pepper was chosen as test plant because it is not locally produced; it is brought from the north of the country, thus increasing its costs. This study will offer local producers an efficient technique in the near future. A 22.85 kg production of Serrano pepper was obtained, representing a competitive production quota in a market that depends on non-local production. This work aims to consolidate the dry hydroponics technique… but there is still much work to be done.

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REFERENCES

Agrovit (2021). Recuperado 4 de abril de 2021, de http://www.agrobit.com/info_tecnica/alternativos/horticultura/al_000010ho.htm#5-Invernaderos%20con%20techumbre%20curva

Científico Mexicano Sergio Rico Velasco. El Padre de la Lluvia Sólida. (s. f.). Metropolis Esceptica. Recuperado 4 de abril de 2021, de https://www.metropolisesceptica.com/

Climatología. (2021). INEGI. Recuperado 4 de abril de 2021, de https://www.inegi.org.mx/temas/climatologia/

Demin, P. E., (2014), Aportes para el mejoramiento del manejo de los sistemas de riego: métodos de riego: fundamentos, usos y adaptaciones. - 1a. ed. - San Fernando del Valle de Catamarca, Catamarca: Ediciones INTA, 28 p.

FAO. (2020). El estado mundial de la agricultura y la alimentación 2020. https://doi.org/10.4060/cb1447es.

Gravital Agency. (s. f.). Sistemas de Cultivo Hidropónico. Puerto Rico Farm Credit. Recuperado 4 de abril de 2021, de https://prfarmcredit.com/sistemas-de-cultivos-hidroponicos/

Hernández-Barajas J., S. Mondragón J., Muñiz O., (2010), Síntesis a nivel de laboratorio de poliacrilatos de sodio de bajo peso molecular para la industria cerámica: comparación de diferentes alternativas de síntesis, *Revista Iberoamericana de Polímeros*, ISSN-e 0121-6651, Vol. 11, Nº. 6: 392-406.

Horacio, M. V., Juan, P. P., Moisés, R. M. & Enrique, V. G. (2010). Fertirrigación de chile serrano con riego por goteo en el sur de Tamaulipas. ISBN: 978-607-425-465-5.

Los hidrogeles de poliacrilato en la agricultura. (7 de mayo 2018). Portal Fruticola. Recuperado 4 de abril de 2021, de website: https://www.portalfruticola.com/noticias/2018/05/07/los-hidrogeles-de-poliacrilato-en-la-agricultura/

Robinson, J. (2014, 24 abril). Chiles perfectos en 4 etapas. Hortalizas. https://www.hortalizas.com/cultivos/chiles-pimientos/chiles-perfectos-en-4-etapas/#:%7E:text=Los%20chiles%20se%20desarrollan%20bien,cuando%20el%20suelo%20requiere%20magnesio.

Zohuriaan-Mehr M. J., Omidian H., Douroudiani S., Kabiri K., (2010), Advances in nonhygienic applications of superabsorbent hydrogel materials. *Journal of Material Science 45*:5711-5735. https://doi.org/10.1002/pc.21046.

AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Long-term response planting method on wheat under conservation agriculture

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ABSTRACT

Objective: To compare different planting systems: narrow beds, wide beds, and flat soil, on the growth and yield of wheat grown under conservation system.

Design/methodology/approach: Wheat crop was grown during five seasons. Treatments were established on a complete block design with three replicates: wide beds (100 cm), narrow beds (80 cm) and planting on flat soil. Response variables were dry weight of 50 stems, weight of 1000 grains, number of spikes (m^2), harvest index and yield. The relationship between yield and chill hours, and degree-days were identified.

Results: Flat soil reached the highest dry weight of 50 stems, whereas narrow beds had the maximum number of spikes per m^2. Yield was equal between flat soil and narrow beds. No differences were found in the harvest index between the evaluated treatments. When comparing results between years, dry weight of 50 stems increased and the harvest index decreased, negatively affecting the yield. A negative association was found between chill hours and yield.

Study limitations/implications: The use of wide beds reduces wheat crop yield, and the use are not recommended.

Findings/conclusions: Yield was the same with narrow beds and flat soil planting. The yield reduction was mainly associated with of reduction in chill hours occurring in each season.

Keywords: climate; environment; grain yield; plant density; topological arrangement.

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INTRODUCTION

The Mexicali valley is in the state of Baja California in Northwest Mexico. It has an approximate area of 105,000 irrigated hectares, of which 98,000 hectares are crops of wheat (*Triticum aestivum* L.) cultivated during the months of November through May (SIAP, 2014). Traditionally, most growers employ the flat planting system for wheat under conventional tilling practices. The system includes the subsoiling, disking and mould board plough. Besides, straw is usually burned after harvest, which substantially affects microbial activity in soils (Wuest *et al.*, 2005).

Image by Kira Hoffmann at Pixabay

Wheat grain yield attained by the use of this technology has reached an average of 6.8 Mg ha^{-1} (Salinas-Zavala, Salvador, Lluch-Cota & Fogel, 2006). However, it can be enhanced by increasing the number of irrigations (Kakar & Iqbal, 2015) and employing different management practices such as bed planting system. Additionally, profitability of this crop can be increased by employing conservation tillage system (minimum tillage, crop rotation, straw incorporation) (Govaerts *et al*., 2009).

In conservation agriculture system, the soil is ploughed as in conventional system during the first year. After that time, soil is not disturbed; crop rotation and all crop remains are incorporated over the surface. The planting method consists of raised beds (80-100 cm) with two or three rows of plants on the top of the bed (Limon-Ortega, Sayre, Drijber, & Francis, 2002).

With conservation agriculture, growers can achieve fuel costs savings up to 30% (Lal, 2004) increase nitrogen concentration, organic matter content in soil (Zuber *et al*., 2015) and keep stable yield (Kassam *et al*., 2009). Fahonga, Xuqinga & Sayre (2004) mention that bed planting system provides advantages in water saving (30%) when compared to flat soil planting. They also indicate that soil crusting problems are reduced because the seed bed surface improves physical structure. Besides, N use efficiency is increased up to 30%, microclimate favors crop stand, reduces lodging and presence of plant diseases. Weed management and fertilization timing practices are substantially improved (Govaerts, Sayre, & Deckers, 2005).

The use of bed planting in wheat crops has been recommended for more than a decade in the Yaqui valley (state of Sonora) as well as in high valleys of central Mexico (Govaerts *et al*., 2005). Nevertheless, the use of this technology in Mexicali valley is under research due to different cropping systems (type of soil, irrigation water quality and environment) which are contrasting to the places above mentioned. In that sense, a study was conducted during five years in order to compare different bed planting systems (flat soil, wide beds (100 cm) and narrow beds (80 cm) on the growth and yield of wheat under conservation system.

MATERIALS AND METHODS

The research was carried out at the experimental platform for conservation agriculture located at the Agricultural Science Institute at University of Baja California (32° 24’ 12.34” N, 115° 11’ 47.37” W). The weather of this region is arid with periods of rain in winter (BW [h’] hs [x´] [e’]; (INIFAP, 2010), summer temperatures up to 50 °C and winter temperatures of −7 °C, the mean annual temperature is 22.3 °C and the mean annual precipitation is 58 mm.

The soil from the experimental site is classified as clayed (vertisol), bulk density of 1.16 g cm3, electrical conductivity (EC) of 4.44 dS m^{-1}, pH 7.83, 33 ppm of phosphorus content, 395 ppm of potassium, 5236 ppm of calcium, 1255 ppm of magnesium and 672 ppm of sodium respectively. Irrigation water flowed from Colorado River, the pH was of 8.18, EC of 1.28 dS m^{-1} and 122.0, 61.4, 154.8, 177.1, 243.5, 421.8 mg L^{-1} of calcium, magnesium, sodium, bicarbonates, chlorides and sulfates approximately.

The experiment was established in a big area of land under conservation agriculture system. The tillage techniques were those recommended by CIMMYT (2009). The first year of the study (2011-2012), land was ploughed using conventional system (subsoiling, disking and leveling). Subsequently, all treatments were established on a complete block design with three replicates. Plots had a length of 140 m and 12 m wide. Treatments were: A) wide beds (furrows at 100 cm), wheat was planted in twin line (two rows 27 cm apart) (Figure 1) and seed density of 80 kg ha^{-1}; B) narrow beds (furrows at 80 cm) (two rows 27 cm apart) and the same seeding rate; C) flat soil with a seed density of 160 kg ha^{-1}.

Planting and harvest dates were as follow: A) December 6th, 2011, and June 15th 2012 first growing season; B) December 15th 2012 and June 15th 2013 second growing season, C) November 15th 2013 and June 7th 2014 third growing season, D) November 22nd 2014 and June 4th 2015 fourth growing season, E) November 25 2015 and June 15th 2016 the fifth growing season. The variety planted was Rio Colorado during all growing seasons.

Planting on wide and narrow beds was realized with a multifunctional seed drill (manufactured by Industrias Vázquez S.A de C.V); flat soil planting system was realized with a special seed drill (Dobladense 290-17®). Fertilization rates were of 276 kg of nitrogen using urea [$CO(NH_2)_2$] and 78 kg of phosphorus using monoammonium phosphate (MAP-11-52-00). Total P and 25% of N were pre-plant applied, 33% of N applied in the first post-plant irrigation, 25% in the second post-plant irrigation while the last 17% in the third post-plant irrigation. Pest and weed management were realized following the guideline provided by Hernández, Guzmán & Valenzuela (2010).

Response variables were dry weight of 50 stems (season 2013-14; 2014-15 and 2015-16), number of spikes (m^2) (season 2014-15 and 2015-16), weight of 1000 grains (season 2013-14, 2014-15 and 2015-16), harvest index (season 2011-12, 2013-14 and 2015-16) and yield (from 2011 through 2016). It was also identified the ratio of annual yield relative to chill hours and degree-days from planting to February (year). The reason for considering February is because heading usually occurs during this time (Verhulst, Kienle, Sayre, Deckers, & Raes, 2014).

To determine $DW_{50\ stems}$, plant material was taken at harvest, placed on a forced air-dry oven (65 °C) until constant dry weight. Harvest index (HI) was obtained by the ratio of dry weight of 50 stems relative to grain weight. Number of spikes were also counted (m^2). Yield was estimated by harvesting plants on 2 m^2 segment. Climatic conditions were monitored

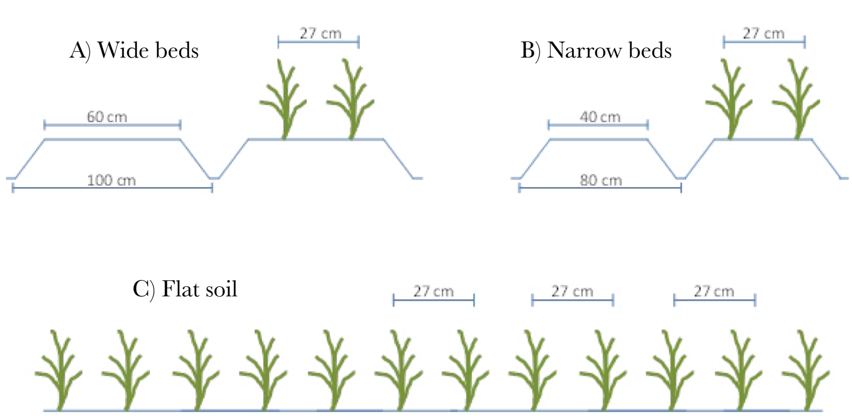

Figure 1. Different planting systems on wheat production.

with a meteorological station located at 840 m from the experimental site during all years of the study. The daily chill hours and degree-days were obtained from a meteorological network station (SIMABC, http://www.simarbc.gob.mx/).

All data obtained was analyzed separately due to different variables measured. Analysis of variance was conducted between treatments, treating years as the fixed factor, ANOVA between years considering treatments as the fixed factor, mean differences between treatments or years, were separated using Tukey's least significance difference (LSD) at $P \leq 0.05$. Additionally, regression models were fitted between accumulated chill hours and degree-days with respect to grain yield.

RESULTS AND DISCUSSION

Table 1 shows the maximum and minimum temperatures during the time of the experiment. It also shows the average monthly maximum and minimum temperatures from 1961 through 2003. It is shown that temperatures obtained during the study were very similar to those recorded from 1962 to 2003. Maximum temperatures registered from January through may increase from 4.9 a 8.7 °C as compared to those registered in the period of 1961 to 2003.

Table 2 shows the monthly Degree-days (DD) and chill hours (CH) accumulation during the study. As the time passed during the period of the experiment, the months from February through March exhibited a reduction in the CH accumulation and increased the DD accumulation.

According to analysis of variance, there were significant differences in variables of dry $weight_{50\ stems}$, spikes (m^2) and yield of crop by the effect of planting methods (Table 3). The greatest weight of 50 stems was registered on flat soil planting. The greatest number of spikes was recorded on narrow bed planting. Lastly, the lowest yield was attained on wide bed planting. However, there were no significant differences on weight of 1000 grains and HI.

Significant differences were found in variables of $DW_{50\ stems}$, $DW_{1000\ grains}$, HI and yield of crop during the years of the study (Table 4). The highest value of $DW_{50\ stems}$ was attained during the season 2015-2016. The yield and HI decreased as the time passed. In addition, $DW_{1000\ grains}$ decreased on the season 2014-15 as compared to seasons 2013-14 and 2015-16. Furthermore, the number of spikes (m^2) was not affected during the time of the experiment.

Table 4 shows the analysis of variance and mean comparison for the variables of $DW_{50\ stems}$, spikes m^2, $DW_{1000\ grains}$, HI and yield in five years of study. It was observed that as time passed, $DW_{50\ stems}$ significantly increased ($P>0.01$) to double the weight attained during the season 2013-14 as compared to season 2015-16. However, there were no significant differences on the number of spikes (m^2) during the years of study ($P>0.05$). The highest value of $DW_{1000\ grains}$ was attained on the seasons 2013-14 and 2015-16 as compared to season 2014-15. HI increased on seasons 2011-12 and 2013-14, but it decreased on season 2015-16. The same tendency was observed when analyzing yield between years. Yield potential significantly decreased as time passed.

Table 1. Maximun and minimum temperatures registered on wheat crop under conservation agriculture. Mexicali, Baja California, México.

Month	Year cero (2011-12)		Year two (2012-13)		Year two (2013-14)		Year three (2014-15)		Year four (2015-16)		Average 2011-16		1961-2003†	
	Mínimum	Máximum	Mínimum	Máximum	Mínimum	Máximum	Mínimum	Máximum	Mínimum	Máximum	Mínimum	Máximum	Mínimum	Máximum
November	7.4	24.7	11.8	31.8	9.4	25.3	9.4	27.0	-‡	-	9.5	26.9	9.3	26.0
December	2.6	19.2	4.5	27.5	5.1	22.0	7.1	20.8	-	-	4.8	22.1	6.4	21.4
January	4.6	23.7	3.3	18.8	6.5	24.3	6.4	22.6	5.1	20.8	5.1	26.3	6.4	21.4
February	6.2	24.0	3.7	21.3	8.1	25.7	9.4	27.5	8.3	26.3	7.1	29.7	7.7	23.9
March	7.1	26.6	9.9	28.8	11.3	30.5	11.7	29.2	9.1	27.8	9.8	33.8	9.7	26.4
April	11.5	30.7	11.4	31.0	11.5	31.2	11.7	30.4	12.2	29.4	11.6	36.5	12.0	29.8
May	14.8	36.2	16.1	36.6	15.5	35.2	13.3	31.9	13.7	32.9	14.6	41.3	15.5	33.9
June	19.0	39.5	19.7	39.6	19.6	39.6	19.6	39.9	21.4	39.0	19.8	47.2	19.1	38.5

†: Average from 1961-2001. Ruiz-Corral *et al.*, 2006.
‡: Not determined.

Table 2. Degree-days (DD) and chill hours (CH) accumulation registered on wheat crop under conservation agriculture. Mexicali, Baja California, México†.

Month/year	2011-12		2012-13		2013-14		2014-15		2015-16	
	CH	DD	CH	DD	CH	DD	CH	DD	CH	DD
November	139	254	0	113	70	292	76	337	267	0
Dicember	375	105	0	0	306	154	211	180	235	59
January	269	206	0	0	195	242	188	211	266	150
February	203	199	195	110	110	269	67	322	107	303
March	139	309	61	392	23	384	36	441	40	365
April	25	446	12	455	12	456	8	442	9	443
May	0	626	0	631	0	602	7	529	0	549
June	0	731	0	747	0	716	0	743	0	767
TOTAL	1150	2876	268	2448	716	3115	593	3205	924	2636

†: SIMARBC. 2016. http://www.simarbc.gob.mx/

Table 3. Effect of planting method on dry weight of 50 stems, spikes (m^2), weight of 1000 grains, HI and yield of wheat crop (2011-12 through 2015-16).

Planting method	$DW_{50\ stems}$ (g)	Spikes m^2	$DW_{1000\ grains}$ (g)	HI	Yield (t ha^{-1})
Flat soil	193.54 a	270 b	77.61	0.393	6.45 a
Narrow beds	158.83 b	370 a	73.73	0.385	6.40 a
Wide beds	176.21 b	255 b	76.66	0.398	5.23 b
Significance	**	*	NS	NS	*

NS: non significant; *, **. Significant at P<0.05, P<0.01, respectively.

Table 4. Mean comparison of yield components on wheat in five growing seasons (2011 to 2016).

Year	$DW_{50\ stems}$ (g)	spikes m^2	$DW_{1000\ grains}$ (g)	HI	Yield (t ha^{-1})
2011-12	-	-	-	0.433 a	6.87 a
2012-13	-	-	-	-	6.98 a
2013-14	117.07 c	-	83.22 a	0.414 a	6.03 a
2014-15	160.93 b	298	63.12 b	-	5.33 ab
2015-16	250.58 a	309	81.66 a	0.327 b	4.94 b
Significance	**	NS	*	*	*

NS: non significant; *,
; *. Significant at P<0.05, P<0.01, y P<0.001 respectively.

The regression model showed that yield increased as the number of chill hours units also increased (R^2=0.4355, P<0.007; Figure 2). The regression equation indicated that yield increased 1.35 t ha^{-1} for every 100 for every 100 chill hours accumulated to February. A different behavior was observed with respect to degree-days accumulation and yield (Figure 3). The regression equation showed that yield decreased 0.94 t ha^{-1} for every 100 degree-days accumulated to February (R^2=0.4487; P<0.006).

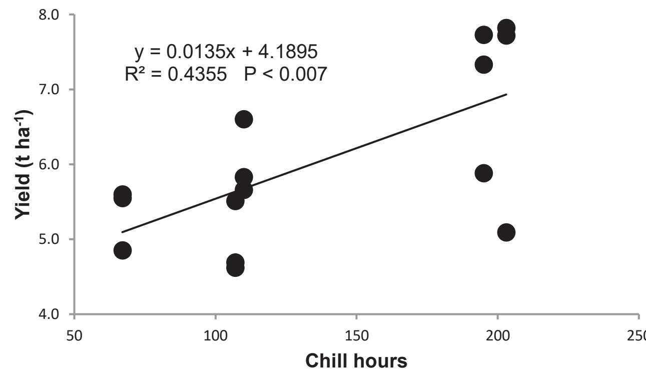

Figure 2. Relationship between chill hours accumulation and yield of wheat in five years.

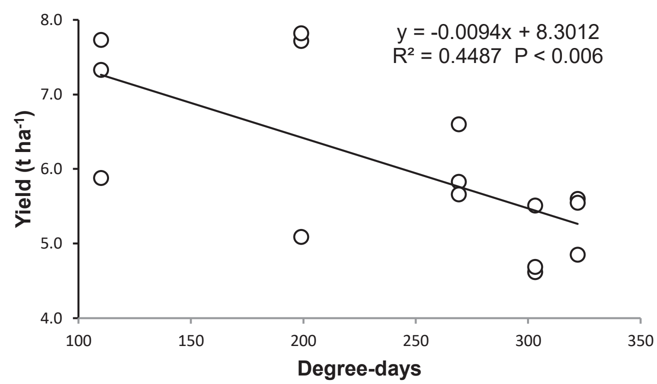

Figure 3. Relationship between degree-days accumulation and yield of wheat in five years.

It is estimated that conservation agriculture system worldwide is growing at a rate of 5.3 million of hectares per year (Kassam, Friedrich, Shaxson & Pretty, 2009). Recently, number of studies show the benefits of this production system. This practice involves the evaluation of climatic and edaphic conditions for each region and the crop response to them (Verhulst, Kienle, Sayre, Deckers, Limon-Ortega, Tijerina-Chavez & Govaerts, 2011). It is observed that yield of wheat under raised bed planting system has not exceed the yield obtained under flat planting system in regions with arid climate and soil saline conditions (Saifuzzaman *et al.*, 2011). However, when there is an increase in yield, it has been associated to improvement in soil conditions (incorporation of crop remains) (Limón-Ortega *et al.*, 2011).

This study evaluated three planting systems on the growth and yield of wheat under environmental conditions of Mexicali Baja California. The flat planting and the narrow bed planting system provided the same yield; while the yield under wide bed planting system was reduced. It is possible that decrease in yield on this system was due to spacing between them. Fischer *et al.* (2019) have also documented this response. Nevertheless, they stated that yield could fluctuate between years because of low temperatures at the time of planting and crop emergence. They also mentioned that wheat late plantings (past January) lead to better yields.

It was identified a significant relationship between yield drop and increase of temperatures on February (P<0.006). According to Verhulst *et al.* (2011), it is just the period for completing the stage of heading and beginning of stem elongation. This process could have modified the pattern of dry matter partitioning to stems, leading to a decrease in harvest index (Banerjee and Krishnan, 2015). The hypothesis is contrasting because of the increase in chill hours during the same month (February). Nuttall *et al.* (2018) indicated that heat waves in period before flowering affected the yield. On the other hand, Calderini *et al.* (1999) exhibited that lowering yields are associated to temperature drop between stages of growth.

CONCLUSIONS

Stem dry weight, number of spikes and yield of crop were affected by the planting system. The lowest yield was attained on the wide bed planting system. Meanwhile, grain weight and harvest index were not affected by the planting method. Furthermore, the weight of stems was increased but the harvest index was reduced by the effect of year. There was also an association between the yield and cold units accumulated during the month of February. Overall, the narrow bed planting system can be a potential management practice in maintaining yield potential of wheat.

REFERENCES

Banerjee, V. & Krishnan P. (2015). Effect of high temperature stress on biomass partitioning in wheat (*Triticum aestivum* L.) at different growth stages. *Journal of Agricultural Physics, 15*(2) 122-126. Doi: 10.13140/RG.2.2.26670.00320

Calderini, D. F., Abeledo, L. G., Savin, R. & Slafer, G. A. (1999). Final grain weight in wheat as affected by short periods of high temperature during pre- and post anthesis under field conditions. *Aust. J. Plant Physiol. 26*: 453-458. Doi: 10.1071/PP99015

Fahong, W., Xuqing, W. & Sayre, K. (2004). Comparison of conventional, flood irrigated, flat planting with furrow irrigated, raised bed planting for winter wheat in China. *Field Crops Research 87*(1): 35-42. Doi: 10.1016/j.fcr.2003.09.003

Fischer, R. A., Moreno Ramos, O. H., Ortíz Monasterio, I. & Sayre, K. D. (2019). Yield response to plant density, row spacing and raising beds in low latitude spring wheat whit ample soil resources: An update. *Fields Crops Research 232*: 95-105. Doi: 10.1016/j.fcr.2018.12.011

Govaerts, B., Sayre, K. D., & Deckers, J. (2005). Stable high yields with zero tillage and permanent bed planting? *Field Crops Research, 94*(1), 33–42. Doi: 10.1016/j.fcr.2004.11.003

Govaerts, B., Sayre, K. D., Goudeseune, B., De Corte, P., Lichter, K., Dendooven, L., & Deckers, J. (2009). Conservation agriculture as a sustainable option for the central Mexican highlands. *Soil and Tillage Research, 103*(2), 222–230. Doi: 10.1016/j.still.2008.05.018

Hernández Vázquez, B., Guzmán Ruiz, S de C. y Valenzuela Palafox, J. A. (2010). *Guía para producir trigo en los valles de Mexicali, B. C. y San Luis Rio Colorado, Son*. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias Centro de Investigación Regional del Noroeste Campo Experimental Valle de Mexicali Mexicali, B. C., Noviembre de 2010. Folleto para Productores, 28 pp. http://www.oeidrus-bc.gob.mx/sispro/trigobc/Produccion/Paquetes/Trigo2010.pdf

Kakar, K. M., & Iqbal, A. (2015). Effect of irrigation levels and bed-system of planting on seed fill duration, seed growth rate, yield and yield components of spring wheat (*Triticum aestivum*) under semiarid condition. *Pure Appl. Biol., 4*(4), 511–521. Doi: 10.19045/bspab.2015.44009

Kassam, A., Friedrich, T., Shaxson, F., & Pretty, J. (2009). The spread of Conservation Agriculture: justification, sustainability and uptake. *International Journal of Agricultural Sustainability, 7*(4), 292–320. Doi: 10.3763/ijas.2009.0477

Lal, R. (2004). Carbon emission from farm operations. *Environment International, 30*(7), 981–990. Doi: 10.1016/j.envint.2004.03.005

Limon-Ortega, A., Sayre, K. D., Drijber, R. A. & Francis, C. A. (2002). Soil attributes in a furrow-irrigated, bed planting system in northwest Mexico. *Soil & Tillage Research 1635*: 1–10. Doi: 10.1016/S0167-1987(01)00230-6

Nuttall, J. G., Barlow, K. M., Delahunty, A. J., Christy, B. P. & O´Leary, G. J. (2018). Acute high temperature response in wheat. *Agron. J. 110*:1296-1308. Doi: 10.2134/agronj2017.07.0392

Ruiz-Corral, J. A., Díaz-Padilla, G., Guzmán-Ruiz, S. D., Medina-García, G., & Silva-Serna. M. M. (2006). *Estadísticas Climatológicas Básicas del Estado de Baja California (Período 1961-2003)*. Instituto Nacional de Investigaciones, Forestales, Agrícolas y Pecuarias. http://www.simarbc.gob.mx/descargas/estadclimatologica-inifap.pdf

Saifuzzaman, M., Rawson, H. M., Amin, M., Farhad, M., Helal Uddin, M., Sydur Rahman, M., Farhad Hossain M. & Enamul Haque, M. (2011). Beds or flat-planting for southern wheat crops. *Report number: ACIAR Technical Report 78*. https://www.researchgate.net/publication/264574527_Beds_or_flat-planting_for_southern_wheat_crops

Salinas-Zavala, C. A, Salvador E, Lluch-Cota, & Fogel, I. (2006). Historic development of winter-wheat yields in five irrigation districts in the sonora desert, Mexico. *Interciencia, 31*(4), 254-261. http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0378-18442006000400004&lng=es&nrm=iso

SIAP (2014) Servicio de Información Agroalimentaria y Pesquera. *Avance de siembras y cosechas. Resumen nacional por cultivo*. http://infosiap.siap.gob.mx:8080/agricola_siap_gobmx/AvanceNacionalSinProgramado

Verhulst, N., Sayre, K.D., Vargas, M., Crossa, J., Deckers, J., Raes, D. & Govaerts, B. (2011). Wheat yield and tillage-straw management system x year interaction explained by climatic co-variables for an irrigated bed planting systems in northwestern Mexico. *Fields Crops Research 124*:347-356. Doi: 10.1016.j.fcr.2011.07.002

Verhulst, N., Kienle, F., Sayre, K. D., Deckers, J., & Raes, D. (2014). Soil quality as affected by tillage-residue management in a wheat-maize irrigated bed planting system. *Plant Soil 340*, 453-466. Doi: 10.1007/s11104-010-0618-5

Verhulst, N., François, I. & Govaerts, B. (2015) Agricultura de conservación, ¿mejora la calidad del suelo a fin de obtener sistemas de producción sustentables?. *Centro Internacional de Mejoramiento de Maíz y Trigo. Modernización Sustentable de la Agricultura Tradicional*. https://repository.cimmyt.org/bitstream/handle/10883/4408/56985.pdf?sequence=4&isAllowed=y

Wuest, S. B., Caesar-Tonthat, T. C., Wright, S. F., & Williams, J. D. (2005). Organic matter addition, N, and residue burning effects on infiltration, biological, and physical properties of an intensively tilled silt-loam soil. *Soil and Tillage Research, 84*(2), 154–167. Doi: 10.1016/j.still.2004.11.008

Zuber, S. M., Behnke, G. D., Nafziger, E. D., & Villamil, M. B. (2015). Crop rotation and tillage effects on soil physical and chemical properties in Illinois. *Agronomy Journal, 107*(3), 971–978. Doi: 10.2134/agronj14.0465

AP AGRO PRODUCTIVIDAD

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Yield and forage quality in maize (*Zea mays* L.) inbred lines

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ABSTRACT

Objective: To analyze the genetic diversity (agronomic attributes, forage yield, and fiber content) of 100 maize inbred lines and to identify genotypes with potential for the breeding of plants with the said traits.

Design/methodology/approach: One-hundred maize inbred lines were evaluated in two environments, in a 10×10 complete block experimental design, with two repetitions. Days to male flowering, days to female flowering, ear height, plant height, stem diameter, green forage yield, neutral detergent fiber, and acid detergent fiber data were recorded.

Results: Significant differences were observed in the environmental sources of variation and genotypes for all the traits evaluated. The first four components account for 81% of the total variation observed and the first two account for 66% of the variation. The variables which have the highest absolute value and which strongly influence the dispersion of the genotypes, as well as the formation of groups, were: plant height, forage yield, and acid detergent fiber (component one) and days to female flowering and days to male flowering (component two). Consequently, the genotypes were dispersed in four groups.

Study limitations/implications: It was not possible to conduct genetic diversity studies using molecular markers.

Findings/conclusions: The grouping pattern of inbred lines shows the genetic diversity present; this can be an important tool in genetic improvement programs aimed at obtaining hybrids with specific characteristics.

Keywords: Forage yield, plant breeding, forage quality, principal component analysis.

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INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereals in the world, since it supplies nutritional elements for human diets and animal feed, and it provides the industry with a basic raw material. It is used as fresh forage, silage or stubble for animal consumption, mainly during the dry season (Luna *et al.*, 2013).

The maize plant, transformed into silage, has been used with great success by the dairy and beef industries. In addition, after the grain is harvested, the dry leaves and

Image by carolalves at Pixabay

the upper part (including the tassel) are also used to provide relatively good forage for ruminants (Hassan *et al.*, 2018); this activity is carried out by many small farmers in various agricultural regions of Mexico.

In 2019, 553,095 ha were sown with maize for forage purposes in Mexico, achieving a total production of 15,101,860 t and an average yield of 31.1 t ha^{-1}. Based on the area in which maize for green forage is cultivated, the most important states are: Jalisco, Zacatecas, Aguascalientes, Durango, Chihuahua, State of Mexico, and Coahuila with 211,712 ha, 101,231 ha, 53,327 ha, 51,164 ha, 37,868 ha, 22,471 ha, and 22,382 ha, respectively (SIAP, 2019).

In genetic improvement programs, the genetic knowledge of the base populations facilitates the selection of superior lines. To choose the improvement scheme, the type of inheritance or specific gene action must be determined in order to establish quantitative traits of agronomic interest (Peyman *et al.*, 2009). Studying genetic diversity is a useful tool to select appropriate genotypes for hybridization, because divergent parents can produce highly heterotic effects (Falconer, 1960), with a significant impact on the genetic improvement of the crop (Hallauer *et al.*, 1988). Maize breeders have constantly emphasized the significant contribution of diverse parental genotypes for heterotic hybrids (Xia *et al.*, 2005).

The objective of this work was to analyze the genetic diversity (agronomic attributes, forage yield, and fiber content) of 100 maize inbred lines and to identify genotypes with potential for the genetic improvement of the said traits.

MATERIALS AND METHODS

Genetic material

One-hundred maize lines with different levels of inbreeding and diverse origins were evaluated. The seed was obtained from the genetic improvement programs for the tropics and subtropics of the germplasm bank of the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMyT).

Experimental sites and growing cycles

The field experiment was established at the Dr. Ernest W. Sprague experimental station, Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMyT) in Agua Fría, Puebla, Mexico (20° 27’ N, 97° 38’ W, 812 masl) and at the Rancho Santa Margarita, Puerto Vallarta, Jalisco (20° 44’ N, 105° 10’ W, 731 masl). In both locations, cultural management practices common to the region were carried out, keeping the crop free from pests and diseases; sowing and harvesting were done manually.

Design and experimental unit

The experiments were established based on an experimental design with 10×10 complete blocks, with two repetitions, which included the 100 accessions. The experimental unit consisted of two 5-m long furrows, with 0.80 m between furrows and 0.15 m between plants, resulting in a planting density of 83,000 plants per hectare.

Traits measured

Regarding flowering, the days from the sowing to the female (FF) and male (FM) flowering were recorded: the first when 50% of the plants of the experimental unit had exposed stigmas and the second when 50% of the plants presented pollen emission.

The following characteristics were measured in five randomly selected plants from the useful plot: ear height (AMZ) was measured from the soil surface to the insertion of the first ear; plant height (AP) was measured from the union of the root and the stem to the base of the male inflorescence; diameter of the stem base (DT) was measured using a vernier caliper and the results were reported in centimeters.

To determine green forage yield (RFV), when the plants reached physiological maturity, ten plants were harvested from each experimental unit and the total weight per genotype was recorded. The forage was harvested from two central furrows of each useful plot, discarding the plants that grew one meter from both ends of the furrow, to avoid edge effect and to obtain fully competitive plants. A 30-kg Eura Brand M2/50 digital scale was used.

To determine neutral detergent fiber (FDN) and acid detergent fiber (FDA), the internodes were collected: second to fifth above the ground from five plant stems for each inbred line. With these samples, the determinations were made based on the principle of van Soest (1991), using an ANKOM 220 fiber analyzer.

Statistical analysis

A combined analysis of variance was performed across localities. An analysis of the principal components was applied based on the correlation matrix; the dispersion of the accessions in the plane determined by the first two biplot components was plotted; and a cluster analysis —in which the Euclidean distance coefficient and the unweighted pair group method with arithmetic (UPGMA) were used— was carried out. The Statistical Analysis System (SAS) version 9.3 for Windows and the Infostat statistical packages were used. All effects were considered as random effects.

RESULTS AND DISCUSSION

Analysis of variance

Significant differences were observed for the environmental variation sources and the genotypes for all the characteristics evaluated; meanwhile, in the genotypes × environments interaction, significant differences were only observed for the plant height, ear height, stem diameter, and forage yield variables (Table 1). The significant differences observed between environments can be attributed to the effects of temperature and relative humidity, which were different in the evaluation environments. As a consequence of the random fluctuation of the environment, the agronomic evaluation of maize germplasm must be carried out in different environments, allowing a more accurate estimation of the value of the genetic components and the separation of the environmental genetic effect (Gutiérrez *et al.*, 2004; Alejos *et al.*, 2006).

The mean values for the plant and ear height (AP and AMZ) variables were 173.81 and 87.61 cm, respectively. Alfaro (2009) reported similar results for these variables describing the morphology of maize cultivars from southern Venezuela. Bejarano *et al.* (2000)

Table 1. Mean squares of the analysis of variance for yield traits and forage quality of 100 inbred maize lines evaluated in Agua Fría, Puebla and Puerto Vallarta, Jalisco, Mexico.

Variable	**Variation source**				**Mean**	**SV (%)**
	Localities (Loc)	**Genotype (Gen)**	**Interaction Gen × Loc**	**Error**		
DF	1	99	99	399		
Days to female flowering	4134.3**	80.1**	13.4^{NS}	13.60	78.7	4.7
Days male flowering	3795.1**	56.5**	13.1^{NS}	11.88	79.2	4.3
Plant height (cm)	2654.5**	2654.5**	249.8**	43.95	173.8	3.8
Ear height (cm)	25973.8**	1208.9**	144.1**	19.15	87.6	4.9
Stem diameter (mm)	148.1**	87.1**	5.1**	1.07	20.5	5.1
Forage yield (t ha^{-1})	10031.6**	702.1**	117.1**	0.30	40.2	10.3
Acid detergent fiber (%)	1337.1**	146.9**	0.1^{NS}	0.95	46.6	12.1
Neutral detergent fiber (%)	3911.7**	162.6**	1.3^{NS}	1.04	69.9	11.5

SV: Variation source, DF: Degree of freedom.

evaluated the AP and AMZ variables in simple maize crosses from lines with three different inbreeding levels and reported significant differences. In general, the lines evaluated in this study are short, as a consequence of the inbreeding depression that occurs when the lines are subjected to a successive self-fertilization in order to achieve homogeneity (Poehlman and Sleper, 1995).

The genotypes with the highest forage yield potential exceeded the national average of 31.1 t ha^{-1} (SIAP, 2019). The lines with the highest values were: CLWN701, CLWN345, CML472, CML216, CLWQ232, and CML528, with 74.8, 72.2, 67.7, 65.8, 63.8, and 62.5 t ha^{-1}, respectively. These genotypes could be used in subsequent genetic improvement-oriented evaluations and for the generation of maize hybrids for forage production. These results are similar to those recorded by Peña *et al.* (2008) and Castillo *et al.* (2009), with 70-95 t ha^{-1} yields.

The average acid detergent fiber (FDA) value was 46.62%. However, the CLRCY017, CLWQH2N75, CSL1612, CSL1620, CLYN548, CLYN482, CSL1732, and CLYN631 lines are the genotypes with the lowest FDA concentration, with 34.8, 36.9, 37.7, 38.612, 39.4, 39.8, 40.1, and 40.2%, respectively. In this regard, De la Cruz *et al.* (2007) report 25-35% FDA values in maize lines from two locations. The acid detergent fiber value (FDA) is used to estimate the energy value of maize silage. Forage digestibility is mainly linked to FDA, because it consists of cellulose, lignin, and proteins (Castillo *et al.*, 2009): as FDA increases, plant digestibility decreases. Forage crop quality depends on <28% FDA values. However, this percentage depends on factors such as days to harvest after sowing, sowing density, and fertilization, among others (Gallegos *et al.*, 2012).

The average neutral detergent fiber (FDN) value was 69.91% (with 59.01-87.48% values). The CLYN482, CSL1612, CSL1612, CLRCY017, CLYN531, CML427, CLWQH2N75, CLYN540, and CLYN548 genotypes had the lowest FDN concentration, with 59.01, 59.12, 60.52, 60.87, 61.65, 61.98, 62.27, 62.58, and 63.746%, respectively. In contrast, De la Cruz *et al.* (2007) reported 41-61% determinations of FDN in a group of maize inbred lines. Núñez *et al.* (2001) evaluated the FDN concentration of eleven maize hybrids of temperate origin and seven of tropical origin and obtained values of 59.80 to 44.70%. This study identified inbred lines with a lower FDN content than those reported by Gallegos-Ponce *et al.* (2012), Amodu *et al.* (2014), and Fortis *et al.* (2009) for diverse maize genotypes evaluated under contrasting environmental conditions.

FDN content in forages is negatively correlated with intake and digestibility (Oramas and Vivas, 2007); therefore, obtaining forages with high energy value requires the use of maize hybrids with less than 50.0% of this type of fiber (Gallegos *et al.*, 2012).

Main component analysis

The result of the main components analysis indicated that the four components account for 81% of the total variation observed and the first two account for 66% of the variation. Ferraz *et al.* (2013) mention that a high proportion —explained by a low number of components— allows a better interpretation of the variation and the studies about the traits related to maize yield and shows that the models that involve biplot graphs are useful to determine the intensity and the nature of maize populations under certain environmental conditions (Martínez *et al.*, 2016). The classification of the main effects obtained through a biplot graphic representation can be easily interpreted, allowing a better observation of the relationship of the genotypes and the variables evaluated (Akinwale *et al.*, 2014).

In this study, the variables which have the highest absolute values —in the coefficients of the eigenvectors of the first two components— and which strongly influence the dispersion of the genotypes, as well as the formation of groups, were: plant height, forage yield, and acid detergent fiber (component one) and days to female flowering and days to male flowering (component two) (Table 2).

The dispersion of the accessions according to the first two main components enables the differentiation of four groups (Figure 1). The first group consists of the CLWN701,

Table 2. Eigenvalues and eigenvectors associated with the first four main components of the analysis of eight agronomic and forage quality variables in 100 maize inbred lines.

Variable	Eigenvector			
	CP 1	CP 2	CP 3	CP 4
Acid detergent fiber (FDA)	0.41	−0.07	0.54	−0.08
Neutral detergent fiber (FDN)	0.39	−0.01	0.55	−0.27
Plant height (AP)	0.46	−0.02	−0.33	−0.21
Ear height (AMZ)	0.40	0.01	−0.42	−0.43
Stem diameter (DT)	0.35	−0.13	0.06	0.76
Forage yield (RF)	0.42	−0.02	−0.34	0.32
Days to female flowering (DFF)	0.03	0.70	0.05	0.07
Days male flowering (DFM)	0.10	0.69	0.01	0.07
Eigenvalues	3.37	1.95	1.18	0.81
Proporción de la varianza explicada (%)	42	24	15	10

CP1, CP2, CP3, and CP4: Main components 1, 2, 3, and 4, respectively.

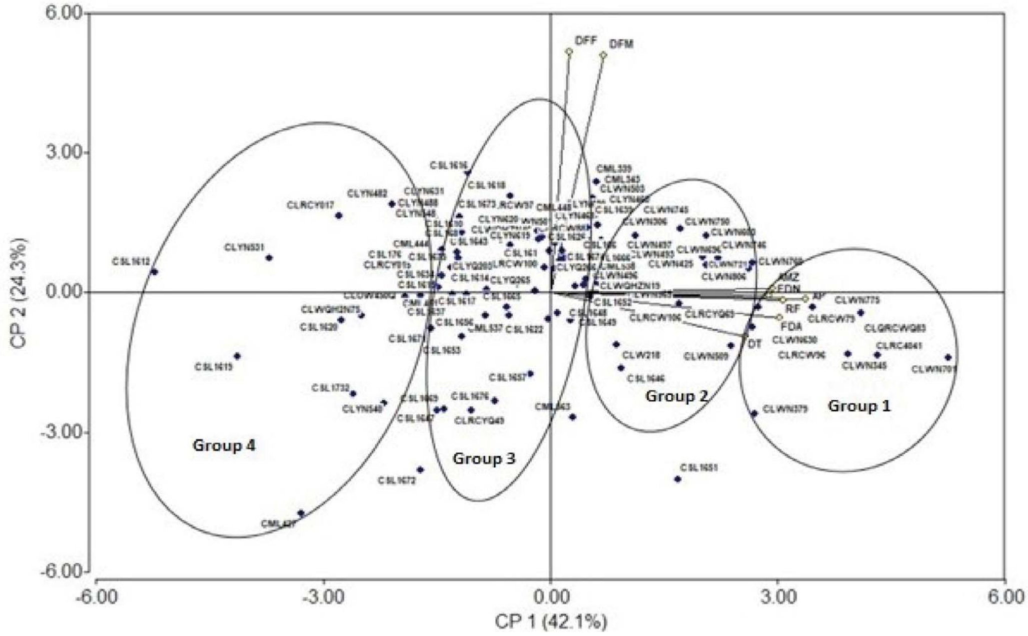

Figure 1. Dispersion of 100 maize inbred lines based on the first two main components.

CLWN630, CLWN775, CLWN345, CLWN379, CLRCW96, CLRC4041, and CLRCW79 genotypes and is mainly associated with the plant height, ear height and forage yield variables. Group two consists of the CLWN746, CLWN721, CLWN745, CLWN750, CLWN760, CSL166, CSL1652, CSL1648, and CSL1551 genotypes which are, associated with days to male flowering and days to female flowering variables. Group three shows the greatest association in the neutral detergent fiber and acid detergent fiber variables. Group four includes the CLYN631, CLYN482, CLYN531, CLYN548, CLYN540, CLYN488, CSL1612, CSL1620, CSL1619, and CSL1732 genotypes, which are mainly associated with stem diameter and forage yield.

Estimating genetic diversity and relationships between germplasm accessions facilitates the selection of parents with diverse genetic backgrounds, which is an essential component of the breeding program (Chinyere *et al*., 2020). The considerable morphological variation found in this study was mainly due to genetic factors, although environmental factors were also involved (Table 2, Figure 1). Several works about the genetic diversity of maize inbred lines have also reported variability in different morphological traits (Enoki *et al*., 2002; Azad *et al*., 2012; Boakyewaa *et al*., 2019).

CONCLUSIONS

The grouping pattern of inbred lines in this study shows the presence of genetic diversity. This diversity is an important tool in genetic improvement programs aimed to obtain maize hybrids with specific characteristics. The variability we detected can potentially contribute to the efficient use of inbred lines in the exploitation of heterosis and the formation of genetically diverse populations in maize breeding programs.

REFERENCES

Akinwale, R.O., Fakorede, M.A.B., Badu-Apraku, B., Oluwaranti, A. (2014). Assessing the usefulness of GGE biplot as a statistical tool for plant breeders and agronomists. *Cereal Research Communications 42*(3), 534–546. doi:10.1556/crc.42.2014.3.16

Alfaro, S.V. (2009) Formation, evaluation and description of single cross yellow maize (*Zea mays* L.) INIA 21. *Revista UDO Agrícola 9*, 499–508.

Alejos, G., Monasterio, P., Rea, R. (2006). Análisis de la interacción genotipo-ambiente para el rendimiento de maíz en la región maicera del estado Yaracuy, Venezuela. *Agronomía Tropical 56*(3), 369–384.

Amodu, J.T., Akpensuen, T.T., Dung, D.D., Tanko, R.J., Musa, A., Abubakar, S.A., Hassan, M.R., Jegede, J.O., Sani, I. (2014). Evaluation of maize accessions for nutrients composition, forage and silage yields. *Journal Agricultural Science 6*(4), 178–187.

Azad, M.A.K., Biswas, B. K., Alam, N., Alam, S.S. (2012). Genetic diversity in maize (*Zea mays* L.) inbred lines. *The Agriculturists 10*(1), 64–70.

Bejarano A, Segovia V, Marín C (2000). Evaluation of simple maize crossings from lines with three different levels of inbreeding. *Agronomía Tropical 50*, 461–476.

Boakyewaa, A.G., Badu-Apraku, B., Akromah, R., Garcia-Oliveira, A.L., Awuku, F.J., Gedil, M. (2019). Genetic diversity and population structure of early-maturing tropical maize inbred lines using SNP markers. *PLoS ONE 14*(4), e0214810. https://doi.org/10.1371/journal.pone.0214810

Castillo-Jimenez, M., Rojas- Bourrillón, A., WingChing-Jones, R. (2009). Nutritional value of silage made with a mixture of corn and mung bean (*Vigna radiata*). *Agronomía Costarricense 33*, 133–146.

Chinyere A., Badu-Apraku, B., Gracen, V., Tongoona, P., Gedil, M., Unachukwu, N., Kwame-Offei, S., Kwadjo-Dzidzienyo, D., Hearne, S., Garcia-Oliveira, A.L. (2020). Genetic diversity and Inter-trait relationships among maize inbreds containing genes from *Zea diploperennis* and hybrid performance under contrasting environments. *Agronomy 10*, 1478. doi:10.3390/agronomy10101478

De la Cruz-Lázaro, E., Rodríguez-Herrera, S.A., Palomo-Gil, A., López-Benítez, A., Robledo-Torres, V., Gómez-Vázquez, A., Osorio-Osorio, R. (2007). Combining ability of protein high quality maize inbred lines for forage characteristics. *Universidad y Ciencia 23* (1), 57–68.

Enoki, H., Sato, H., Koinuma, K. (2002). SSR analysis of genetic diversity among maize inbred lines adapted to cold regions of Japan. *Theoretical and Applied Genetics 104*, 1270–1277.

Falconer, D.S. (1960). Introduction to quantitative genetics. Oliver & Boid, London. 304 p.

Ferraz, T.Y., Permuy, A.N., Acosta, R.R. (2013). Evaluación de accesiones de maíz (*Zea mays* L.) en condiciones de sequía en dos zonas edafoclimáticas del municipio Gibara, provincial Holguin. Evaluación morfoagronómica y estudios de la interacción genotipo × ambiente. *Cultivos Tropicales 34*, 24–30.

Fortis-Hernández, M., Leos-Rodríguez, J.A., Preciado-Rangel, P., Orona-Castillo, I., García-Salazar, J.A., García-Hernández, J.L., Orozco-Vidal, J.A. (2009). Application of organic fertilizers in the production of forage corn with drip irrigation. *Terra Latinoamericana 27*(4), 329–336

Gallegos-Ponce, A., Martínez-Ríos, A., Fernando-Sánchez, M., Figueroa-Viramontes, R., Berumen-Padilla, S., Venegas-Soto, J., Quevedo-Guillen, J. de D., Escobedo-López, D. (2012). Calidad nutricional de maíz forrajero (*Zea mays* L.) bajo condiciones limitadas de agua de riego. *Agrofaz 12*(1), 59–66.

Gutiérrez-del Rio, E., Espinoza-Banda, A., Palomo-Gil, A., Lozano-Garcia, J.J. y Antuna-Grijalva, O. (2004). Aptitud combinatoria de híbridos de maíz para la Comarca Lagunera. *Revista Fitotecnia Mexicana 27*(1), 7–11.

Hallauer, A.R., Filho, J.B. (1988). Quantitative genetics in maize breeding, 2nd. edn. Iowa State University Press.

Hassan, A.A., Abdikadir, M., Hasan, M., Kalam-Azad, A., Hasanuzzaman, M. (2018). Genetic variability and diversity studies in maize (*Zea mays* L.) inbred lines. *Journal of Agriculture and Veterinary Science 11*(11), 69–73.

Luna-Ortega, J.G., Garcia-Hernández, J.L., Preciado-Rangel, P., Fortis-Hernández, M., Espinoza-Banda, A., Gallegos-Robles, M.A., Chavarría-Galicia, J.A. (2013). Evaluation of hybrids from simple crosses using maize elite landraces with forage outstanding characteristics for a Mexican arid land. *Tropical and Subtropical Agroecosystems 16*(1), 119–126.

Martínez, S.J., Espinosa, P.N., Villegas, A.Y. (2016). Interacción genotipo ambiente en poblaciones de maíz nativo de Chiapas. *Revista Mexicana de Agroecosistemas 3*, 38–48.

Núñez, H.G., Faz, R.C., Tovar, G.M., Zavala, G.A. (2001). Corn hybrids for forage production with high digestibility in northern Mexico. *Técnica Pecuaria en México 39*, 77–88.

Oramas-Wenholz, C., Vivas-Quila, N.J. (2007). Evaluation of two hybrids and one variety of corn (*Zea mays*) in intensive crop and association with bean (*Phaseolus vulgaris*), for silage. *Revista de la Facultad de Ciencias Agropecuarias 5*(1): 28–35.

Poehlman, J.M., Sleper, D.A. (1995). Breding field crops. 4th ed. Iowa State University Press/Ames. 494 p

Peña, R.A., González-Castañeda, F., Núñez-Hernandez, G., Preciado-Ortiz, R., Terrón-Ibarra, A., Luna-Flores, M. (2008). H-376, corn hybrid for forage and grain production in el bajío and the north central part of México. *Revista Fitotecnia Mexicana 31*, 85–87.

Peyman, S., Hamid, D., Ali, M., Mohammad, M. (2009). Genetic and genotype × enviroment interaction effects for appearance quality of rice. *Agricultural Science China 8*, 891–901.

SIAP (Servicio de Información Agroalimentaria y Pesquera) (2019). Cierre de la producción agrícola por cultivo. http:// infosiap.siap.gob.mx:8080/agricola_siap_gobmx/ResumenProducto.do

van Soest, P.J., Robertson, J.B., Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science 74*, 3583–3597.

Xia, X. C., Reif, J. C., Melchinger, A. E., Frisch, M., Hoisington, D. A., Beck, D., Pixley, K., Warburton, M. L. (2005). Genetic diversity among CIMMYT maize inbred lines investigated with SSR markers. *Crop Science 45*, 2573–2582.

AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Native palms as an economically important non-timber forest product among rural communities in the Yucatán peninsula, Mexico

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ABSTRACT

Objective. To analyze the valuation, use, and preservation of native palms as a non-timber forest product (NTFP) of high economic importance for rural communities in the Yucatán Peninsula, Mexico.

Design/methodology/approach: The available literature on palms and their use in the Yucatán Peninsula (YP) was examined using the snowball method. Subsequently, different online flora databases were consulted in order to examine the taxonomic identities of palm species present in the YP. The reported uses were classified.

Results: The YP has 20 native species that belong to 13 genera and three subfamilies. All of them are economically exploited as NTFPs, especially in construction (85%) and honey production (70%), followed by food and medicinal use (35% each), craftwork (30%), ornamental use (25%), and fodder (10%). These data confirm that native palms are an important livelihood means for the inhabitants of Mayan communities in the region.

Study limitations/implications: The research faced a limited database of encyclopedias, anthologies, directories, books, or articles that interpret works or research about this topic.

Findings/Conclusions: The local and regional use of palms represents an additional income for the people who use this natural resource on different productive scales, both in rural communities and city centers. An alternative to ensure the sustainability of palms would be to establish governmental programs for their reproduction.

Key words: Arecaceae, Uses, Construction, Campeche, Quintana Roo, Yucatán.

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INTRODUCTION

The species that belong to the Arecaceae or Palmae family are commonly known as palms. They make up over 2,400 species of cosmopolitan distribution, comprised in 183 genera and five subfamilies (Dransfield *et al.*, 2008). Palms are classified as part of the monocotyledons and are characterized by morphological traits such as generally non-ramified woody stems and generally composite large leaves that form a crest at the tip

(Alvarado-Segura *et al.*, 2012). Palms are distributed in the tropics and subtropics (0-3,200 masl), mainly in tropical rain forests (Dransfield *et al.*, 2008; Galeano and Bernal, 2010). The diversity of their life forms and their abundance in the canopy or the understory have made them ecologically relevant elements for the function of some ecosystems. For this reason, they have been used in the tropics as a model for the study of the diversity of species, their causes, and maintenance (Quero and Flores, 2004; Dransfield *et al.*, 2008; Alvarado-Segura, 2014). Human beings have used palms as a source of natural resources for thousands of years; different cultures have used them as a non-timber forest product (PFNM), as well as for food, oils, fibers, medicines, construction materials, and other uses (Pérez-García and Rebollar-Domínguez, 2008). Currently, their ornamental value contributes to the economy of several countries and to the lifestyle of their inhabitants (Quero and Flores, 2004). In Mexico, the exploitation of the flora as a social practice is mainly carried out in rural areas (Challenger, 1998); in this regard, the country registers around 100 species of native palms, comprised in 22 genera and three subfamilies (Quero and Flores, 2004). In the Yucatán Peninsula (YP), rural communities exploit a wide variety of vegetable species, including palms. They are economically important, because all their species are widely used in the population's livelihood (de la Torre *et al.*, 2009). This study consists of a thorough bibliographic review of sources about the potential of palms as a non-timber forest product, in order to encourage their knowledge, preservation, and the appreciation of the goods and services they provide to both ecosystems and human beings in the YP, Mexico.

MATERIALS AND METHODS

First of all, the available literature on palms and their use in the YP (which comprises the states of Campeche, Quintana Roo, and Yucatán) was reviewed using the snowball method (Bernard, 2006). Subsequently, several online flora databases were consulted in order to examine the taxonomic identities of the palm species found in the YP. Some of these databases included: Tropicos® (www.w3tropicos.org); Flora: Península de Yucatán—CICY (https://www.cicy.mx/sitios/flora%20digital/); The Plant List (www.theplantlist.org); and the International Plant Names Index (www.ipni.org). Based on the information that was gathered, the uses of palms were classified as follows: 1) food; 2) fodder (used to feed livestock); 3) craftworks (used to make tools and decorative objects); 4) construction (used to build traditional housing, fences, etcetera); 5) medicinal uses (used to treat human health issues); 6) ornamental use (used to decorate public areas or private houses); 7) honey production (used as food in production activities for apiculture and meliponiculture). Based on the gathered information, descriptive analyses about the native palms of the YP were carried out and later documented with field photographs.

RESULTS AND DISCUSSION

Twenty native palm species grow in the YP (Table 1). They comprise 13 genera and three subfamilies: *Acoelorrhaphe*, *Coccothrinax*, *Cryosophila*, *Thrinax*, and *Sabal* (Coryphoideae); *Acrocomia*, *Attalea*, *Bactris*, *Chamaedorea*, *Desmoncus*, *Gaussia*, and *Roystonea* (Arecoideae); and *Pseudophoenix* (Ceroxyloideae) (Quero and Flores, 2004; Carnevali *et al.*, 2010). These

Table 1. Distribution and uses of native palms in the Yucatán Peninsula (YP), Mexico. Uses: AL=Food, AR=Craftwork, CO=Construction, FO=Fodder, MD=Medicinal, ME=Honey production, OR=Ornamental. Conservation status (CS) (based on Pérez-Sarabia *et al.*, 2020): EN=Endangered, LC=Least concern. NT=Near-threatened.

Taxa	Common name	Distribution in the YP	Uses	CS*
Acoelorraphe wrightii (Griseb. & H. Wendl.) H. Wendl. ex Becc.	Guano prieto, Tasiste	CAM, QROO, YUC	CO, ME	LC
Acrocomia aculeata (Jacq.) Lodd. ex Mart.	Cocoyol, tuk', coyol	CAM, QROO, YUC	AL, AR, CO, MD, ME	LC
Attalea butyracea (Mutis ex L. f.) Wess. Boer	Corozo, coyol real, palma real	CAM	AL, CO, ME	LC
Attalea guacayule (Liebm. ex Mart.) Zona	Corozo	CAM	AL, CO	LC
Bactris major Jacq.	Caña brava, coyol, Jawacte'	CAM, YUC	AL, AR, FO	LC
Bactris mexicana Mart.	Coyolillo, Jahuactillo, Jawuacte' de montaña, Jawacte'	CAM, YUC	OR	LC
Chamaedorea oblongata Mart.	Xiat	CAM, YUC	CO, OR	LC
Chamaedorea seifrizii Burret	Xiat	CAM, QROO, YUC	MD, OR	LC
Coccothrinax readii H. J. Quero R.	Náaj k'aax, nak'as	QROO, YUC	CO, MD	LC
Cryosophila stauracantha (Heynh.) R. Evans	Escobo, palo de escoba, huano k'uum, k'uum	CAM, YUC	CO, ME	LC
Desmoncus chinantlensis Liebm. ex Mart.	Bayal, janan	CAM, YUC	AR, CO, ME	LC
Gaussia maya (O. F. Cook) H. J. Quero R.	Gausia	CAM, YUC	CO, ME	NT
Pseudophoenix sargentii H. Wendl. ex Sarg.	Palma bucanero, palma de guinea, kuka', ya'ax jalalche'	QROO, YUC	ME, OR	LC
Roystonea dunlapiana P. H. Allen	Desconocido	CAM, YUC	CO, ME	LC
Roystonea regia (Kunth) O. F. Cook	Palma real	CAM, QROO, YUC	CO, ME, OR	LC
Sabal gretherae H. J. Quero R.	Huano, xa'an	QROO, YUC	CO, ME	EN
Sabal mauritiiformis (H. Karst.) Griseb. & H. Wendl.	Botan, huano, xa'an	CAM, YUC	AR, CO, MD, ME	LC
Sabal mexicana Mart.	Guano, guano bon, bon xa'an, xa'an	CAM, YUC	AL, AR, CO, FO, MD, ME	LC
Sabal yapa C. Wright. ex Becc.	Guano, guano macho, julok' xa'an, xa'na	CAM, QROO, YUC	AL, AR, CO, MD, ME	LC
Thrinax radiata Lodd. ex Schult. & Schult. f.	Chi'it	CAM, QROO, YUC	AL, CO, MD, ME	LC

palms, found in the YP, are important or predominant floral elements in medium and high semi-evergreen tropical forests, subdeciduous tropical forests, and coastal sand dune scrubs (Miranda, 1958; Orellana, 1992; Sánchez and Islebe, 2002).

Current and potential uses

All native YP palms are exploited economically as non-timber forest product (NTFPs). They are predominantly used in construction (85%) and honey production (70%), followed by food and medicinal uses (35% each), craftwork (30%), ornamental use (25%), and fodder (10%) (Table 2). This confirms that palms are an important means of livelihood for the Mayan communities in the region. Two of the most widely used species are *huano* (*Sabal mexicana*) and *S. yapa*, which have been used since the times of the ancient Mayas (Martínez-Ballesté *et al.*, 2008): these types of palms have been used for approximately more than 3,000 years to build roofs for houses, and also as food for humans and fodder (Pulido and Caballero, 2011). The widespread use of these two species led to the creation of the NOM-006-SEMARNAT-1997 (SEMARNAT, 1997), which establishes the procedures, criteria, and specifications for the exploitation, transport, and storage of palm leaves in natural environments in the Mexican territory. This regulation serves as a control measure for the exploitation of this NTFP, which is highly important for the livelihood of the inhabitants of the YP rural communities. According to interviews with key inhabitants of several YP towns (Tenabo, Hopelchén, Champotón, Calkiní in Campeche; Felipe Carrillo Puerto in Quintana Roo; Mérida and Maxcanú in Yucatán), the cost per cut leaf unit of the *huano* (*Sabal*) species fluctuates between one and five pesos, except for Iturbide, El Platanar, and other towns of the Hopelchén municipality, Campeche, where the *huano* leaf can be bought for 0.50 pesos. Palm leaves are sold by unit (leaf) or by roll. Thus, in Yucatán, 1 roll (50 palm leaves) costs 180 pesos; in the Tenabo municipality of Campeche, 1 roll (50-60 palm leaves) costs 80-100 pesos; meanwhile, in the Holpechén municipality, palm is only sold by leaf and costs between 0.50-1 and 2-3 pesos, in Iturbide and Ukum, respectively. The exploitation of *huano* leaves in Campeche is an economic activity that represents a source of income for workers, whose daily wage (called *jornal*) can reach 200 pesos. Depending on its dimensions, building a roof for a traditional Mayan construction may require an investment of up to 6,000 pesos —only for the acquisition of the leaves (Figure 1).

Table 2. Use of native palms in the Yucatán Peninsula, Mexico.

Use	Number of species	Percentage of use
Construction	17	85 %
Honey production	14	70 %
Food	7	35 %
Medicinal	7	35 %
Craftwork	6	30 %
Ornamental	5	25 %
Fodder	2	10%

Figure 1. Uses of *Sabal mexicana* in the Yucatan Peninsula. A. *Sabal mexicana*. B. Huano Leaf. C. House construction. D-E. Houses with a huano roof. F. Palapa for tourism. G-H. Palapas to protect mayas stelae in archaeological zones.

At present, only some palm species have been spreading widely in the region, as a result of their ornamental potential. One of them is *Roystonea regia*, which is grown in most cities and piers, and whose wild populations are rare and little known. Governmental programs' nurseries should propose palms as a NTFP that can generate additional income, and have them certified in order to guarantee that their commercialization is fair and does not damage wild populations. Likewise, the *Sabal yapa* and *S. mexicana* species should be grown in deforested areas for their exploitation. In addition, the establishment of nurseries that use seeds to spread palm species with an ornamental potential —such as *Gaussia maya*, *Chamaedora seifrizii*, and *C. oblongata*— is feasible. This would also help their natural populations to remain unaltered.

Distribution in the Yucatán Peninsula

Some palm species have a limited distribution in the YP. *Sabal gretherae*, for instance, grows in a pasture sector of the northern area of Quintana Roo; *Gaussia maya* and *Chamaedorea oblongata* only grow in the southeastern end of the peninsula (Campeche and Quintana Roo); and *Pseudophoenix sargentii* generally forms small populations, with a discontinuous pattern along the coastal area of Yucatán and Quintana Roo (Quero and Flores, 2004). *G. maya* is endemic to the Yucatán Peninsula Biotic Province, while *Coccothrinax readii* and *S. gretherae* are endemic to the Mexican section of the Peninsula (Quero and Flores, 2004). In contrast, the remaining species are widely distributed in the region, as in the case of *Acrocomia aculeata* and *Sabal yapa*, which are apparently favored by disturbance conditions (Orellana 1992; Quero and Flores, 2004) (Figure 2).

Conservation status

According to the Official Mexican Standard (DOF, 2010), nine native palms of the YP are listed in a risk category: *Coccothrinax readii*, *Cryosophila stauracantha*, *Gaussia maya*, *Pseudophoenix sargentii*, and *Thrinax radiate* appear as endangered, while *Bactris major*, *Roystonea dunlapiana*, *R. regia*, and *Sabal gretherae* appear under special protection. However, according to Pérez-Sarabia *et al*. (2020), 18 out of the 20 species are listed under the category of least concern, one as near-threatened (*G. maya*), and the other one as endangered (*S. gretherae*). The main threat for these species is the loss of habitat as a consequence of the land use conversion for anthropogenic activities.

CONCLUSION

The different economic uses and the lack of planning for the adequate management of palms have led to several palm species being listed in preservation risk categories. The wild palm populations have been impacted by the exploitation of stems, leaves, and complete individuals linked to an excessive extraction rate. In addition, deforestation, extensive cattle farming, intensive agriculture, and tourism-related urbanization have reduced palm habitats to fragments of vegetation. The local and regional use of palms represents an additional income for people who use this natural resource on different productive scales, both in rural communities and city centers. An alternative to ensure the sustainability of palms would be to establish governmental programs to reproduce them on a large scale, through seeding in forest nurseries that would enable their sustainable use and the preservation of wild populations.

Figure 2. Arecaceae ecosystems in the Yucatan peninsula, Mexico. A. Deforested areas for crops where they keep *Sabal mexicana* in Hopelchén, Campeche. B. Cultivation of *Roystonea regia* in Xamantún, Campeche. C. *Acoelorraphe wrightii* in its natural habitat, Río Hondo, Quintana Roo. D. *Sabal yapa* populations conserved in the archaeological zone of Xtampú, Yucatán. E. *Attalea guacayule* populations conserved in the archaeological zone of Kohunlich, Quintana Roo.

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la Península de Yucatán: estrategia para integrar y promover el conocimiento etnobotánico con fines de investigación, formación de recursos humanos, conservación, uso y manejo sustentable".

REFERENCES

Alvarado-Segura A.A., Calvo-Irabién L.M., Duno de Stefano R. & Balslev H. (2012). Palm richness, abundance and diversity along an environmental gradient on the Yucatan Peninsula. *Nordic Journal of Botany 30*: 613-622.

Alvarado-Segura A.A. (2014). Patrones de diversidad en comunidades de palmas (Arecaceae) en selvas medianas de la Península de Yucatán: determinantes ambientales y espaciales. Tesis doctoral. Centro de Investigación Científica de Yucatán, A.C., Mérida, Yucatán, México.

Bernard H.R. (2006). Research methods in anthropology: qualitative and quantitative approaches, 4th edn. Library of Congress, Washington, DC.

Carnevali F.C.G., Tapia-Muñoz J.L., Duno de Stefano R. & Ramírez-Morillo I. (editores generales). (2010). Flora Ilustrada de la Península de Yucatán: Listado Florístico. Centro de Investigación Científica de Yucatán A.C., Mérida Yucatán, México.

Challenger A. (1998). Utilización y conservación de los ecosistemas terrestres de México, pasado, presente y futuro de México. CONABIO, Instituto Biológico. UNAM: Agrupación Sierra Madre S. C. P., México.

de la Torre L., Calvo-Irabién L.M., Salazar C., Balslev H. & Borchsenius F. (2009). Contrasting palm species and use diversity in the Yucatan Peninsula and the Ecuadorian Amazon. *Biodiversity and Conservation 18*, 2837-2853.

DOF (Diario Oficial de la Federación) 2010. Norma Oficial Mexicana NOM-059-Semarnat, México, D.F.

Dransfield J., Uhl N.W., Asmussen C.B., Baker W.J., Harley M.M. & Lewis C.E. (2008). Genera Palmarum: The Evolution and Classification of Palms. London, UK: Kew Publishing-Royal Botanic Gardens.

Galeano G. & Bernal R. (2010). Palmas de Colombia. Guía de Campo. Bogotá, Colombia: Universidad Nacional de Colombia.

Martínez-Ballesté A., Martorell C. & Caballero J. (2008). The effect of Maya traditional harvesting on the leaf production, and demographic parameters of Sabal palm in the Yucatán Peninsula, Mexico. *Forest Ecology and Management 256*, 1320-1324.

Miranda F. (1958). Estudios acerca de la vegetación [de la Península de Yucatán]. En: Beltrán E. Ed. Los Recursos Naturales del Sureste y su Aprovechamiento, Tomo II. pp. 215-271. México, D.F.: Instituto Mexicano de Recursos Naturales Renovables, A.C.

Orellana R. (1992). Síndromes morfológicos y funcionales de las palmas de la Península de Yucatán. *Bulletin de l'Institut Francais d' Etudes Andines 21*, 651-667.

Orellana R., Herrera P., Rebollar S., Escalante J., López G., Escalante S. & Gus L. (1999). Studies on the potential uses of some native palms of the Yucatan Peninsula (Mexico) as substitutes of Rattan. *Acta Horticulturae 486*, 291-295.

Pérez-Sarabia, J.E. R. Duno de Stefano, G. Carnevali Fernández-Concha, I. Ramírez Morillo, N. Méndez-Jiménez, P. Zamora-Crescencio, C. Gutiérrez-Báez, & W. Cetzal-Ix. (2017). El conocimiento florístico de la Península de Yucatán, México. *Polibotánica 44*, 39-49.

Pérez-García M. & Rebollar-Domínguez S. (2008). Formas de aprovechamiento de algunas palmas de la Península de Yucatán. *Contactos 69*, 53-60.

Pulido Silva M.T. & Caballero Nieto J. (2011). Uso de la palma de huano. En: Ozo, C., Armijo Canto, N. Y Calmé, S. Editoras. Riqueza Biológica de Quintana Roo. Un análisis para su conservación. Tomo I. pp. 157-160. México, D. F.: El Colegio de la Frontera Sur (ECOSUR), Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), Gobierno del Estado de Quintana Roo y Programa de Pequeñas Donaciones (PPD).

Quero H. & Flores J.S. (2004). Arecaceae: Taxonomía, Florística y Etnobotánica. Etnoflora Yucatanense: 23. México: Universidad Autónoma de Yucatán, Yucatán.

Sánchez S.O. & Islebe G.A. (2002). Tropical forest communities in southeastern Mexico. *Plant Ecology 158*, 183-200.

SEMARNAT. (1997). Norma Oficial Mexicana NOM-006-SEMARNAT-1997, Procedimientos, criterios y especificaciones para realizar el aprovechamiento, transporte y almacenamiento de hojas de palma. Diario Oficial de la Federación 28 de mayo 1997.

SEMARNAT. (2012). Plan de manejo tipo para palma chit (*Thrinax radiata*), manejo extensivo para la conservación y aprovechamiento sustentable. Ciudad de México: Secretaría de Medio Ambiente, Recursos Naturales y Pesca.

AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Evaluation of different antioxidants during *in vitro* establishment of allspice (*Pimenta dioica* L. Merrill): a recalcitrant species

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ABSTRACT

Objective: To evaluate the effect of different antioxidant agents during *in vitro* establishment of allspice (*Pimenta dioica* L. Merrill).

Design/methodology/approach: The effect of different antioxidant agents (Methylene blue, L-cysteine, and silver nanoparticles [AgNPs]) added to Murashige and Skoog culture medium at different concentrations were studied during axenic establishment of *P. dioica*. A completely randomized experimental design was used. All trials were performed in triplicate. The percentage of survival, oxidation, contamination was determined, the phenols content, antioxidant capacity and lipid peroxidation.

Results: The highest survival occurred with the addition of L-cysteine. The lowest percentage oxidation were observed in explants treated with L-cysteine. Treatments with 100 and 200 mg L^{-1} AgNPs had the lowest contamination values. L-cysteine and 50 and 100 mg L^{-1} AgNPs resulted in an increase in the content of soluble phenols.

The highest contents of cell wall-linked phenols were obtained in treatments with 200 mg L^{-1} methylene blue, L-cysteine, and 200 mg L^{-1} AgNPs. In this study, all treatments had a reaction of scavenging/reduction mechanisms free radicals. The highest content of malondialdehydes was observed in the control treatment and 200 mg L^{-1} methylene blue. The highest content of malondialdehydes was observed in the control treatment and 200 mg L^{-1} methylene blue.

Limitations on study/implications: The highest percentage of oxidation was observed in the control treatments, 100 and 200 mg L^{-1} methylene blue, causing cell death.

Findings/conclusions: The addition of L-cysteine to the culture medium is alternative to reduce oxidation during *in vitro* introduction of *P. dioica*.

Keywords: methylene blue, antioxidant capacity, L-cysteine, silver nanoparticles, lipid perioxidation, polyphenols.

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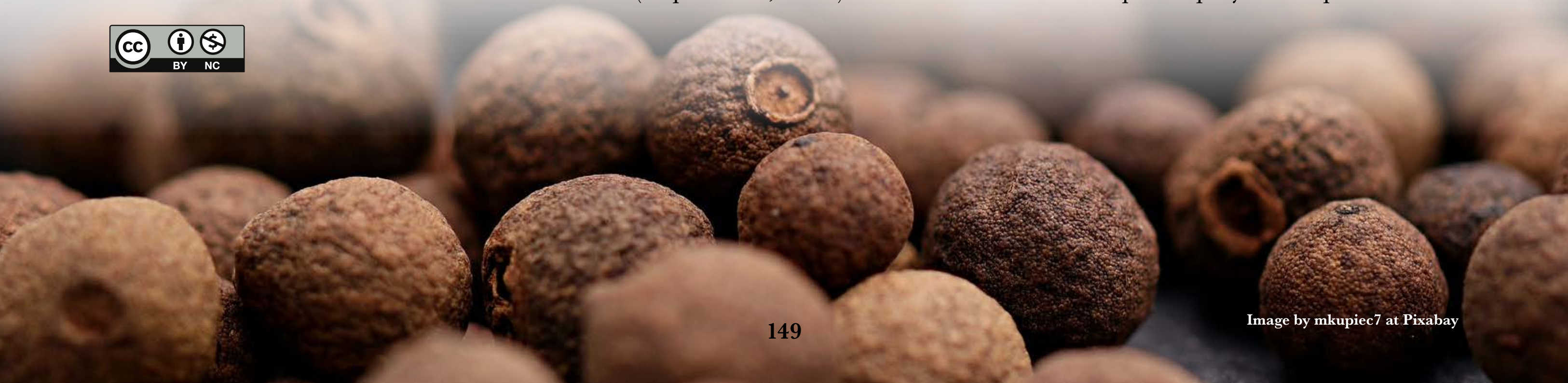
INTRODUCTION

The "pimienta gorda" or allspice (*Pimenta dioica* L. Merrill), is native to Mexico and Central America (López *et al.*, 2021). The cultivation of this species plays an important role



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in the agro-food and pharmaceutical industry because its fruits are used as a spice and in therapies due to their high level of eugenol (Marques *et al*., 2019). Allspice is propagated through seeds and cuttings. The propagation by seeds presents high genetic variability, while the propagation method by cuttings does not guarantee phytosanitary quality for planting. The Plant Tissue Culture is an alternative for *in vitro* production of pest and disease free clonal seedlings (Cardoso *et al*., 2018). However, the *in vitro* establishment stage is the main constraint on initiating commercial micropropagation of plants, due to the *in vitro* manipulation causes tissue damage producing the oxidation of phenols, causing the darkening of the tissue affecting its growth (Martínez Rivero *et al*., 2020). This oxidation and other stress conditions *in vitro* trigger the overproduction and accumulation of Reactive Oxygen Species (ROS) in plant cells (Zhou *et al*., 2021), the generation of ROS promote changes in phenolic content, antioxidant capacity and lipid peroxidation (Wei *et al*., 2019).

During *in vitro* establishment, in some species, the use of antioxidants is necessary to prevent oxidation of the explant (Taghizadeh *et al*., 2020). Methylene blue, L-cysteine and AgNPs are an alternative of reducing oxidation of phenolic compounds and maintaining a survival rates of the explants in species recalcitrant to *in vitro* morphogenesis. The aim of this study was to evaluate different antioxidant agents to reduce oxidation, as well as the phenolic content, antioxidant capacity and lipid peroxidation of *P. dioica* explants during *in vitro* establishment.

MATERIALS AND METHODS

Plant material and *in vitro* establishment

Nodal cuttings 2 cm high of female plants of *P. dioica* ecotype Totonacapan from the Totonacapan region, Veracruz, Mexico was taken as explants. The explants were washed with running water and Tween® 20 soap (Sigma-Aldrich®, St. Louis, MO, USA) in continuous circulation for 30 min. Subsequently, they were rinsed with drinking water. The explants were transferred to a laminar flow hood where they were immersed in 70% (v/v) ethanol for 1 minute and rinsed three times with sterile distilled water; they were then immersed in sodium hypochlorite (NaClO, 6% active ingredient Clorox®; Monterrey, NL, Mexico) at 0.6 and 0.9% (v/v) for 15 and 10 min, respectively, adding 2 drops of Tween® 20 per 100 mL of solution. The explants were rinsed three times with sterile distilled water and were cultured in different treatments with different antioxidant agents.

Treatments with antioxidant agents

The antioxidant response of methylene blue (Merck; Darmstadt, Germany), L-cysteine (Sigma-Aldrich®) and Silver nanoparticles (Argovit™) at concentrations of 0, 50, 100 and 200 mg L^{-1} was evaluate to equalize conditions. The antioxidant agents were added to MS (Murashige and Skoog, 1962) culture medium, supplemented with 3% sucrose (w/v) (Fermont®; Monterrey, NL, Mexico) and 2.5 g L^{-1} phytagel (Sigma-Aldrich®) as gelling agent. The pH was adjusted to 5.8 and sterilized in an autoclave at 120 °C and 115 kPa for 20 min. Finally, 10 explants per treatment were seeded individually in test tubes (15×2 cm) containing 10 mL of culture medium. After 30 days of culture, the percentage of survival, oxidation, contamination, phenol content, antioxidant capacity and lipid peroxidation were evaluated. Explants were incubated at 25±1 °C with an irradiance of 45 μmol m^{-2} s^{-1} provided by 60 W fluorescent lamps (Osram®, Munich, Germany) with a photoperiod of 16 h light.

Determination of phenols

Soluble phenols. Phenolic content was determined according to Payet *et al.* (2006). First, 18 mg dry weight of plant tissue was used in the different treatments with antioxidant agents. Extraction was performed with methanol: water (50:50) and 180 μL of the supernatant were taken by adding 100 μL of 10% Folin-Ciocalteua reagent (E. Merck), it was homogenized in a vortex (Corning® LSE™; Tewksbury, MA, U.S.A.), 30 μL of 20% calcium carbonate (Sigma-Aldrich®) were added and then it was incubated for 2 h at 26 °C. Finally, the absorbance was measured at 765 nm using distilled water as a blank. Phenolic content was calculated from a gallic acid calibration curve (0-10000 μg/mL) and expressed as milligrams of gallic acid equivalents (GAE) per g of dry weight (g DW) of *P. dioica* nodal explants.

Cell wall-linked phenols. The determination was carried out according to Payet *et al.* (2006). First, 250 μL of 1M NaOH (Fermont®) were added to the pellet obtained from the soluble phenols determination, homogenized in a vortex (Corning® LSE ™), and then incubated in a water bath at 70 °C for 16 h. Subsequently, 250 μL of 2 M HCl (Fermont®) were added and 100 μL of extract, 900 μL of distilled water and 100 μL of 10% Folin-Ciocalteu (Merck) reagent were taken; after 5 min, 600 μL of 1 M NaOH (Fermont®) were added, saturated with calcium carbonate (Sigma-Aldrich®) and incubated at 26 °C for 1 h. Absorbance and calibration conditions were the same as those used in the determination of soluble phenol.

Antioxidant capacity

The determination of DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Sigma-Aldrich®, St. Louis MO) was performed by the methodology proposed by Huang *et al.* (2002). An aliquot of 3900 μL of DPPH and 100 μL of methanolic extract obtained in the soluble phenols determination was carried out. A calibration curve with Trolox (Sigma-Aldrich®, St. Louis, Missouri Ohio) was used. The mixture was incubated at 30 °C for 30 min and the absorbance was measured at 515 nm. Data were expressed as Trolox equivalents (TE) per g of dry weight (g DW) of *P. dioica* nodal explants.

Determination of lipid peroxidation

The methodology by Heath and Packer (1968) was used. First, 50 mg of fresh plant material were used by adding 1 mL of 0.1% trichloroacetic acid (TCA) (Sigma-Aldrich®) and homogenizing it in a vortex (Corning® LSE™), after which it was centrifuged at 10,000 xg for 15 min. Next, 500 μL of the supernatant was taken and 1 mL of 20% TCA plus 1 mL of 0.5% thiobarbituric acid (TBA) (Sigma-Aldrich®). The supernatant was incubated in a water bath at 95 °C for 30 min followed by an ice bath. MDA content was calculated by the absorbance difference at 532 and 600 nm.

Experimental design and statistical analysis

A completely randomized experimental design was used. All trials were performed in triplicate. Data were processed with the Statistical Package for the Social Sciences (SPSS) version 22 software for Windows and statistical analysis was carried out using an analysis of variance (ANOVA) and a comparison of means using Tukey's test ($p \leq 0.05$). Values expressed in percentages were transformed by the arcsine function before performing the analysis.

RESULTS AND DISCUSSION

Treatments with antioxidant agents

The evaluation of different antioxidant agents showed significant differences for the variables survival, oxidation and contamination *in vitro* in *P. dioica* nodal explants (Table 1).

The highest survival was observed with the addition of L-cysteine, with percentages greater than 40%, while the lowest survival occurred in the control treatment, 100 and 200 mg L^{-1} methylene blue, with 0, 3.3 and 0% survival, respectively. The highest percentage of oxidation was observed in the control treatment, 100 and 200 mg L^{-1} methylene blue with 96.67% oxidation, while the lowest percentages of oxidation were observed in explants treated with L-cysteine, with 30% oxidation. Regarding the percentage of contamination, treatments with 100 and 200 mg L^{-1} AgNPs had the lowest contamination values, with only 20.00%, while the rest of the treatments had contamination values between 30.00 and 46.67%.

In this study, the use of antioxidant agents had an effect on the survival, oxidation, and contamination *in vitro* of *P. dioica* nodal explants. Adding methylene blue to the culture medium showed a negative effect on survival in the explants; the decrease in survival was probably caused by the high percentages of oxidation observed. Regarding the percentage of contamination, no effect was observed because methylene blue does not

Table 1. Effect of three antioxidant agents on the survival, oxidation and contamination *in vitro* of *Pimienta dioica* (L.) Merrill nodal explants.

Treatment	Concentration (mg L^{-1})	Survival (%)	Oxidation (%)	Contamination (%)
Control	0	0.00 ± 0.00^{c}	96.67 ± 3.33^{a}	46.67 ± 3.33^{a}
Methylene blue	50	13.33 ± 3.33^{b}	66.67 ± 3.33^{b}	43.33 ± 3.33^{a}
	100	3.33 ± 3.33^{c}	96.67 ± 3.33^{a}	46.67 ± 3.33^{a}
	200	0.00 ± 0.00^{c}	96.67 ± 3.33^{a}	43.33 ± 3.33^{a}
L-cysteine	50	43.33 ± 3.33^{a}	30.00 ± 0.00^{c}	43.33 ± 3.33^{a}
	100	43.33 ± 3.33^{a}	30.00 ± 5.77^{c}	46.67 ± 3.33^{a}
	200	40.00 ± 5.77^{a}	30.00 ± 5.77^{c}	43.33 ± 3.33^{a}
AgNPs$^{\alpha}$	50	13.33 ± 3.33^{b}	66.67 ± 3.33^{b}	30.00 ± 0.00^{ab}
	100	33.33 ± 3.33^{ab}	83.33 ± 3.33^{ab}	20.00 ± 5.77^{b}
	200	33.33 ± 3.33^{ab}	83.33 ± 3.33^{ab}	20.00 ± 5.77^{b}

Values represent the mean±SE (standard error). Means with different letters in a column are statistically different (Tukey's test; $p \leq 0.05$), at 30 days of *in vitro* culture. AgNPs: Silver nanoparticles.

present any microbicidal action. Waranusantigui *et al.* (2003) state that methylene blue reduces light penetration and thus prevents oxidation; while Bruchey and Gonzalez-Lima (2008) point out that, at low doses, it decreases superoxide radicals produced in oxidative phosphorylation.

The L-cysteine, it increased survival and decreased the percentage of oxidation, without having an effect on contamination. The reduction of oxidation caused an increase in the survival rate. L-cysteine is an amino acid that has been used to reduce the oxidation of phenolic compounds, as reported in sandalwood (*Santalum album* L.) (Akhtar and Shahzad, 2019). AgNPs did not show a drastic effect on survival rate or oxidation; however, they caused a reduction in contamination rates. The use of AgNPs to reduce *in vitro* contamination has been reported by Spinoso-Castillo *et al.* (2017) in vanilla (*Vanilla planifolia* Jacks. ex Andrews). In fungi, AgNPs break the cell membrane of hyphae altering the mechanisms of infection (Bocate *et al.*, 2019). Lee *et al.* (2019) report that AgNPs that penetrate the cell increase Ag^+ cations, which could affect the electrical potential of the membrane, denaturing proteins, leading to cell cycle arrest. Because *P. dioca* is a woody species, it has a high susceptibility to oxidation during *in vitro* establishment.

Content of soluble phenols and cell wall-linked phenols

P. dioica buds cultured in medium with methylene blue showed no differences in soluble phenol content; however, treatments with L-cysteine and 50 and 100 mg L^{-1} AgNPs had a significant increase in soluble phenol content, with values above 3.843 mg GAE g^{-1} DW, while the rest of the treatments showed the lowest values (Figure 1A). Regarding cell wall-linked phenolic compounds, the highest contents were obtained in the treatments with 200 mg L^{-1} methylene blue, L-cysteine, and 200 mg L^{-1} AgNPs, with concentrations higher than 7.185 mg GAE g^{-1} DW; while the control and the rest of the treatments did not show significant statistical differences (Figure 1B). The production of phenolic compounds is an indicator of defense against the mechanical damage involved in the healing process. The addition of methylene blue showed no effect on the content of soluble phenols; however, changes were observed in the content of cell wall-linked phenols at the highest concentration evaluated, probably because some dyes have a toxic effect on plant tissues, due to the formation of chelates that produce toxicity (Castellar *et al.*, 2018). On the other hand, when L-cysteine was added, an effect on the content of phenolic compounds was observed for due to its ability to remove quinones (Cruz-Gutiérrez *et al.*, 2020). The use of L-cysteine as an *in vitro* antioxidant agent has been reported by Ricco *et al.* (2018) in mistletoe (*Ligaria cuneifolia*). Regarding the use of AgNPs, they did not decrease the production of soluble phenols, probably due to the reaction of Ag^+ with the thiol, carboxylate, phosphate, hydroxyl, amine, imidazole and indol groups of some enzymes, producing their inactivation and cell death (Ashraf *et al.*, 2013). However, the addition of AgNPs increased the content of cell wall-linked phenols at the highest evaluated concentration, due to possible toxic damage caused by an excess in the Ag^+ ion in the culture medium. Toxicity at high concentrations of AgNPs has been reported by Hussain *et al.* (2018) in tangerine (*Citrus reticulata*).

Antioxidant capacity

The evaluation of antioxidant capacity showed that all antioxidant treatments had a reaction of DPPH free radical capture, with values greater than 990 TE g^{-1} DW, differing significantly from the control treatment (Figure 1C). Antioxidant capacity is a defense mechanism against oxidative stress. All treatments with antioxidant agents showed antioxidant capacity. Callaway *et al.* (2004) report that methylene blue at low concentrations can function as a free radical scavenger. However, at high concentrations it can lead to oxidative stress at the cellular level. L-cysteine acts as an intracellular precursor of glutathione biosynthesis, whose function is to remove excess ROS molecules (Koramutla *et al.*, 2021). Haase *et al.* (2012) point out that exposure to AgNPs induces the synthesis of antioxidant enzymes, such as catalase and superoxide dismutase. Pace *et al.* (2015) report that the addition of L-cysteine in lettuce (*Lactuca sativa*) plants increases antioxidant capacity. Regarding the effect of AgNPs, Chung *et al.* (2018) report their antioxidant capacity in cucumber (*Cucumis anguria*) roots.

Lipid peroxidation

Lipid peroxidation showed significant differences in malondialdehyde (MDA) content among treatments with antioxidant agents. The highest MDA content was observed in the

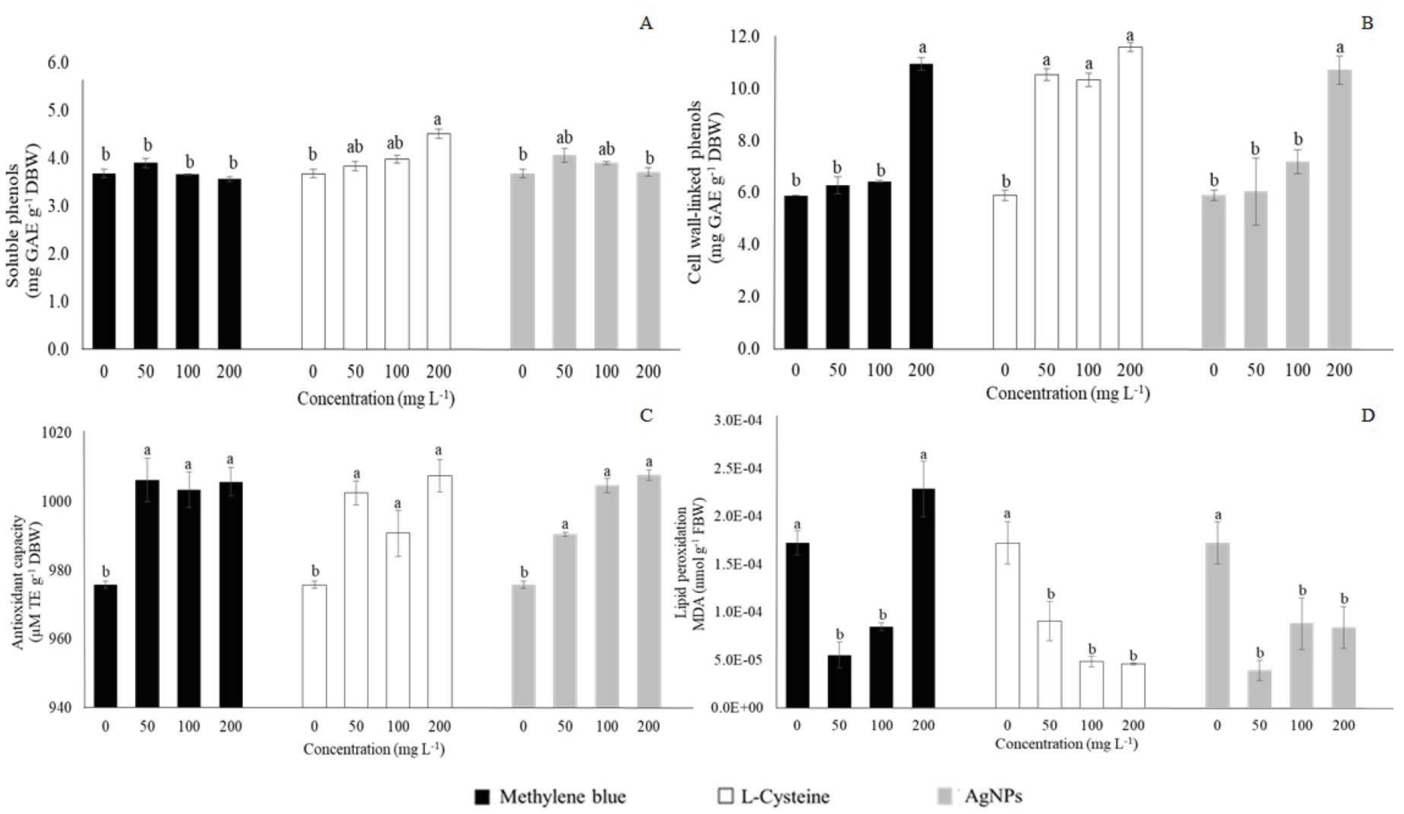

Figure 1. Effect of three antioxidant agents on A) soluble phenols, B) cell wall- linked phenols, C) antioxidant capacity and D) lipid peroxidation in *P. dioica* after 30 days of *in vitro* culture. Different letters denote statistically significant differences according to Tukey's test ($p \leq 0.05$). DBW: Dry biomass weight. FBW: Fresh biomass weight.

control treatment and 200 mg L^{-1} methylene blue, with values higher than 1.72×10^{-4} nmol MDA/g fresh weight, while the lowest content was obtained in the treatments with the addition of L-cysteine and AgNPs, with values lower than 9.07×10^{-5} nmol MDA g^{-1} fresh weight (Figure 1D). An increase in the oxidative degradation of lipids is the result of the capture of electrons that make up the fatty acids present in the cell membrane. The increase in MDA content in the control treatment was due to oxidative stress caused by the lack of antioxidants as reducing agents to prevent and protect oxidative damage, in this regard, Mostofa *et al.* (2015) point out that malondialdehydes produced because of some type of stress can act as molecules that inhibit development. The addition of methylene blue at low concentrations resulted in a decrease in MDA production, because it interacts directly with the mitochondrial electron transport chain (Yang *et al.*, 2020) and having a redox reaction avoiding the oxidative stress of tissues; however, at the highest concentration evaluated it produced greater stress in the *P. dioica* explants, resulting in higher MDA production, the effect on lipid peroxidation using methylene blue has not yet been reported; however, this fact was probably due to toxic damage caused by the high concentration of methylene blue. The addition of L-cysteine resulted in a decrease in MDA content. Ali *et al.* (2016) report decreased MDA production in lychee (*Litchi chinensis*) fruits with the addition of L-cysteine. Regarding the addition of AgNPs, like L-cysteine, they reduced lipid peroxidation. In our study, the decrease in MDA content was probably due to the low effect of silver ions (Nuñez-Anita *et al.*, 2014). Spinoso-Castillo *et al.* (2017) found that 50 mg L^{-1} of AgNPs in *V. planifolia* has an effect on the reduction of MDA.

CONCLUSION

The present study, L-cysteine is an alternative to reduce oxidation of *P. dioica* and could be evaluated for other species with *in vitro* recalcitrance caused by oxidation during establishment.

ACKNOWLEDGMENTS

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REFERENCES

Akhtar, R., & Shahzad, A. (2019). Morphology and ontogeny of directly differentiating shoot buds and somatic embryos in *Santalum album* L. *Journal of Forestry Research, 30* (4), 1179-1189. DOI: 10.1007/s11676-018-0679-5

Ali, S., Khan, A. S., & Malik, A. U. (2016). Postharvest L-cysteine application delayed pericarp browning, suppressed lipid peroxidation and maintained antioxidative activities of litchi fruit. *Postharvest Biology and Technology, 121*, 135-142. DOI: 10.1016/j.postharvbio.2016.07.015

Ashraf, S., Abbasi, A. Z., Pfeiffer, C., Hussain, S. Z., Khalid, Z. M., Gil, P. R., & Hussain, I. (2013). Protein-mediated synthesis, pH-induced reversible agglomeration, toxicity and cellular interaction of silver nanoparticles. *Colloids and Surfaces B: Biointerfaces, 102*, 511-518. DOI: 10.1016/j.colsurfb.2012.09.032

Bocate, K. P., Reis, G. F., de Souza, P. C., Junior, A. G. O., Durán, N., Nakazato, G., & Panagio, L. A. (2019). Antifungal activity of silver nanoparticles and simvastatin against toxigenic species of *Aspergillus*. *International Journal of Food Microbiology, 291*, 79-86. DOI: 10.1016/j.ijfoodmicro.2018.11.012

Bruchey, A. K., & Gonzalez-Lima, F. (2008). Behavioral, physiological and biochemical hormetic responses to the autoxidizable dye methylene blue. *American Journal of Pharmacology and Toxicology, 3*(1), 72-79.

Callaway, N. L., Riha, P. D., Bruchey, A. K., Munshi, Z., & Gonzalez-Lima, F. (2004). Methylene blue improves brain oxidative metabolism and memory retention in rats. *Pharmacology Biochemistry and Behavior, 77*(1), 175-181. DOI: 10.1016/j.pbb.2003.10.007

Cardoso, J. C., Gerald, L. T. S., & da Silva, J. A. T. (2018). Micropropagation in the twenty-first century. *Plant Cell Culture Protocols*, 17-46. DOI: 10.1007/978-1-4939-8594-4-2

Castellar, G., Angulo, E., Zambrano, A., & Charris, D. (2018). Equilibrio de adsorción del colorante azul de metileno sobre carbón activado. *Revista UDCA Actualidad & Divulgación Científica, 16* (1), 263-271. DOI: 10.31910/rudca.v16.n1.2013.882

Chung, I. M., Rajakumar, G., & Thiruvengadam, M. (2018). Effect of silver nanoparticles on phenolic compounds production and biological activities in hairy root cultures of *Cucumis anguria*. *Acta Biologica Hungarica, 69* (1), 97-109. DOI: 10.1556/018.68.2018.1.8

Cruz-Gutiérrez, E. J., Hernández-Fuentes, L. M., & Gómez-Reyes, L. A. (2020). Uso de L-cisteina para el control de oxidación *in vitro* de *Annona muricata* L. *Revista Multidisciplinaria de Avances de Investigación, 6* (3), 1-6

Haase, A., Rott, S., Mantion, A., Graf, P., Plendl, J., Thünemann, A. F., & Reiser, G. (2012). Effects of silver nanoparticles on primary mixed neural cell cultures: uptake, oxidative stress and acute calcium responses. *Toxicological Sciences, 126*(2), 457-468. DOI: 10.1093/toxsci/kfs003

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics, 125* (1), 189-198. DOI: 10.1016/0003-9861(68)90654-1

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Prior, R. L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of Agricultural and Food Chemistry, 50*(16), 4437-4444. DOI: 10.1021/jf0201529

Hussain, M., Raja, N. I., Naz, F., Iqbal, M., & Aslam, S. (2018). Green synthesis and characterisation of silver nanoparticles and their effects on antimicrobial efficacy and biochemical profiling in *Citrus reticulata*. *IET Nanobiotechnology, 12* (4), 514-519

Koramutla, M. K., Negi, M., & Ayele, B. T. (2021). Roles of glutathione in mediating abscisic acid signaling and its regulation of seed dormancy and drought tolerance. *Genes, 12*(10), 1620. DOI: 10.3390/genes12101620

Lee, B., Lee, M. J., Yun, S. J., Kim, K., Choi, I. H., & Park, S. (2019). Silver nanoparticles induce reactive oxygen species-mediated cell cycle delay and synergistic cytotoxicity with 3-bromopyruvate in *Candida albicans*, but not in *Saccharomyces cerevisiae*. *International Journal of Nanomedicine, 14*, 4801. DOI: 10.2147/IJN.S205736

López, I. M., Torres, J. M., & Hernández, D. M. (2021). Diversity of Volatile Organic Compounds in leaves of *Pimenta dioica* L. Merrill at different developmental stages from fruiting and no-fruiting trees. *Journal of the Mexican Chemical Society, 65* (3). DOI: 10.29356/jmcs.v65i3.1498

Marques, C. S., Carvalho, S. G., Bertoli, L. D., Villanova, J. C. O., Pinheiro, P. F., Dos Santos, D. C. M., & Bernardes, P. C. (2019). β-Cyclodextrin inclusion complexes with essential oils: Obtention, characterization, antimicrobial activity and potential application for food preservative sachets. *Food Research International, 119*, 499-509. DOI: 10.1016/j.foodres.2019.01.016

Martínez Rivero, A., Ramírez-Mosqueda, M. A., Mosqueda, F. O., Rivas, P. M., & Bello-Bello, J. J. (2020). Influence of Vitrofural® on sugarcane micropropagation using temporary immersion system. *Plant Cell, Tissue, and Organ Culture, 141*(2), 447-453. DOI: 10.1007/s11240-020-01800-x

Mostofa, M. G., Hossain, M. A., & Fujita, M. (2015). Trehalose pretreatment induces salt tolerance in rice (*Oryza sativa* L.) seedlings: oxidative damage and co-induction of antioxidant defense and glyoxalase systems. *Protoplasma, 252* (2), 461-475. DOI: 10.1007/s00709-014-0691-3

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum, 15* (3), 473–497. DOI: 10.1111/j.1399-3054.1962.tb08052.x

Nuñez-Anita, R. E., Acosta-Torres, L. S., Vilar-Pineda, J., Martínez-Espinosa, J. C., de la Fuente-Hernández, J., & Castaño, V. M. (2014). Toxicology of antimicrobial nanoparticles for prosthetic devices. *International Journal of Nanomedicine, 9*, 3999. DOI: 10.2147/IJN.S63064

Pace, B., Capotorto, I., Ventura, M., & Cefola, M. (2015). Evaluation of L-cysteine as anti-browning agent in fresh-cut lettuce processing. *Journal of Food Processing and Preservation, 39*(6), 985-993. DOI: 10.1111/jfpp.12312

Payet, B., Shum Cheong Sing, A., & Smadja, J. (2006). Comparison of the concentrations of phenolic constituents in cane sugar manufacturing products with their antioxidant activities. *Journal of Agricultural and Food Chemistry, 54*(19), 7270-7276. DOI: 10.1021/jf060808o

Ricco, M. V., Bari, M. L., Ricco, R. A., Wagner, M. L., & Álvarez, M. A. (2018). Análisis de polifenoles e iniciación de cultivos *in vitro* de *Ligaria cuneifolia* (R. et P.) Tiegh (Loranthaceae) de ejemplares provenientes de la localidad La Población, provincia de Córdoba, Argentina. *Dominguezia, 34* (1), 53-64

Spinoso-Castillo, J. L., Chavez-Santoscoy, R. A., Bogdanchikova, N., Pérez-Sato, J. A., Morales-Ramos, V., & Bello-Bello, J. J. (2017). Antimicrobial and hormetic effects of silver nanoparticles on *in vitro* regeneration of vanilla (*Vanilla planifolia* Jacks. ex Andrews) using a temporary immersion system. *Plant Cell, Tissue and Organ Culture, 129* (2), 195-207. DOI: 10.1007/s11240-017-1169-8

Taghizadeh, M., & Dastjerdi, M. G. (2020). Inhibition of browning problem during the callogenesis of *Spartium junceum* L. *Ornamental Horticulture, 27*, 68-77. DOI: 10.1590/2447-536X.v27i1.2230

Waranusantigui, P., Pokethitiyook, P., Kruatrachue, M., & Upatham, E. S. (2003). Kinetics of basic dye (methylene blue) biosorption by giant duckweed (*Spirodela polyrrhiza*). *Environmental Pollution, 125* (3), 385-392. DOI: 10.1016/S0269-7491(03)00107-6

Wei, Z., Shi, X., Wei, F., Fan, Z., Mei, L., Tian, B., ... & Shi, G. (2019). The cotton endocycle-involved protein SPO11-3 functions in salt stress via integrating leaf stomatal response, ROS scavenging and root growth. *Physiologia Plantarum, 167* (1), 127-141. DOI: 10.1111/ppl.12875

Yang, L., Youngblood, H., Wu, C., & Zhang, Q. (2020). Mitochondria as a target for neuroprotection: role of methylene blue and photobiomodulation. *Translational Neurodegeneration, 9*, 1-22. DOI: 10.1186/s40035-020-00197-z

Zhou, X., Joshi, S., Patil, S., Khare, T., & Kumar, V. (2021). Reactive oxygen, nitrogen, carbonyl and sulfur species and their roles in plant abiotic stress responses and tolerance. *Journal of Plant Growth Regulation*, 1-24. DOI: 10.1007/s00344-020-10294-y

AP AGRO PRODUCTIVIDAD

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Diversity of VAM in soils used to cultivate sugarcane (*Saccharum* spp.) in Tabasco, Mexico

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ABSTRACT

Objective: A study was carried out in the Ingenio Santa Rosalía sugar mill supply area with the aim of identifying the soil subunits cultivated with sugar cane and to quantify the degree of mycorrhization of *Saccharum officinarum*.

Design/Methodology/Approach: Rhizosphere samples were collected at a depth of 0-30 cm and root segments with diameters of ≤1 mm and 1-2.0 mm were studied. The intersect method and the fungal structures method were comparatively analyzed to determine the mycotrophic state of the roots. The study also quantified the number of spores in the soils and classified the morpho-species of vesicular-arbuscular mycorrhiza (VAM).

Results: The results show that seven soil subunits exist in the area, but the intersect method did not detect differences in colonization by root diameter or by root colonization ($\bar{X}$=68.5%). For its part, the fungal structures method showed differences in colonization between soil subunits ($\bar{X}$=69.5%), being higher in thin roots, and statistical differences wer found for vesicles and spores. At the sites, an average of 696 spores was quantified per 100 g of soil, which indicates a high presence of vesicular-arbuscular mycorrhizal fungi in the study area. Finally, six species of mycorrhizae were identified, of which four are present in various soil subunits: *Glomus* aff. *deserticola*, *Glomus etunicatum*, *Glomus viscosum*, and *Paraglomus occultum*.

Study Limitations/Implications: This was an exploratory study that indicates the potential of VAM on sugarcane.

Findings/Conclusions: The presence of hyphae, vesicles, arbuscules, and spores indicates that there is an active mycotrophic process between VAM and sugarcane cultivation in the soils of the study area.

Index words: arbuscular mycorrhizae, endophytes, sugar mill, vesicles.

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INTRODUCTION

The Ingenio Santa Rosalía is the second most economically important sugarcane mill in Tabasco, Mexico, both for the cultivated area and for its production of grinding stalks. During the 2017/2018 season, 823,264 tons of sugarcane were harvested in an area of 12,268 ha. The average field yield for this cycle was 67 t ha^{-1} with a factory sugar yield of 7,298 kg ha^{-1} (UNC, 2018). The yields of grinding stalks and sugar are low and are due in part to the poor management of fertilization, since the specific fertilizer recommendations for each type of soil in the supply area have not been followed (Salgado *et al.*, 2005). In recent decades, the term biofertilizers has been introduced in the scientific literature, and refers to consortia of bacteria and mycorrhizal fungi that can provide N, P, and water for crop nutrition, as well as growth-promoting substances (gibberellins and indoleacetic acid), sulfur oxidation, increased root permeability, and siderophore production (Requena *et al.*, 2007; Azevedo, 2008). The subject has awakened interest due to the possibility of lowering fertilization costs, as well as reducing the impacts of excessive fertilizer use, especially nitrogen.

The active dynamics of nutrient exchange, reproduction, and survival in adverse conditions have endowed mycorrhizal fungi with highly specialized structures such as hyphae, vesicles, arbuscules, and spores, all of which could be indicators of the mycotrophic state of the plants with which they are associated (Barea *et al.*, 2005). For its part, the root system of sugarcane is made up of segments with different thicknesses and degrees of lignification that could cause mycorrhizal structures to be differentially present (Sánchez-Reyna *et al.*, 2020). The intersect method is the most commonly used to determine the degree of mycorrhization (Giovannetti and Mose, 1980), while the fungal structures method (McGonigle *et al.*, 1990) that takes into account several structures of the fungus present in the root (hyphae, vesicles, arbuscules, and spores) is less common. Therefore, the objective of this study was to determine the mycotrophic status of VAM in soils cultivated with sugarcane within the supply area of the Ingenio Santa Rosalía sugar mill in Tabasco, Mexico.

MATERIALS AND METHODS

Description of the study area. The Santa Rosalía (ISR) sugar mill is located in the Municipality of Cárdenas, Tabasco, at an altitude of 12 m above sea level. The region is part of the Gulf Coastal Plain, with average annual temperature of 26 °C, average rainfall of 1870 mm per year, and average annual relative humidity of 83% (Salgado *et al.*, 2005). The ISR supply area covers a surface of more than 12,000 ha of sugarcane plantations (UNC, 2018).

Collection of soil and roots. Seven soil subunits growing sugarcane were identified, corresponding to the Fluvisol, Vertisol, and Gleysol types, with neutral or moderately acidic pH (Salgado *et al.*, 2005). Four rhizosphere samples were collected from each soil subunit, which were considered repetitions. Each sample was taken at 20 cm from the stump and at a depth of 0-30 cm. Between each sampling, tools were fired to avoid cross contamination (Salgado *et al.*, 2013). The samples were stored in labeled bags and transported (±4 °C) to the Biotechnology laboratory of the Instituto Tecnológico de la Zona Olmeca.

Clarifying and staining of roots. Thirty (30) root segments were taken (thin and thick), which were washed with running water to remove clinging soil; excess water was removed by placing them on absorbent paper. Afterwards, 2 cm-long portions of the root were cut and then immersed in the FAA solution for clarifying and staining. They were then rinsed to eliminate excess FAA solution and KOH at 10% was added, covering the roots completely; they were immersed in this solution for 24 hours or more, until the roots were clarified. Subsequently, excess KOH was decanted and the roots were washed with running water and soaked for 5 to 10 min in HCl at 10%. Subsequently, the HCl was decanted and the roots were washed with running water. Finally, trypan blue 0.05% was added for 24 h; after this time, the dye was decanted and the roots were placed in Petri dishes with lactoglycerol to remove excess dye (Phillips and Hayman, 1970).

Study variables

Root thickness. The root segments of each thickness, including thin roots (diameter $\leq$1mm) and thick roots (diameter 1 mm to $\leq$2.0 mm), were observed independently to determine the degree of mycorrhization with each of the methods used.

Intersect method (Giovannetti and Mosse, 1980). The root segments were evenly distributed in 8.5 cm diameter, glass square Petri dishes with grids (0.5×0.5 in). Observations were made with a Leica brand 35 X stereo microscope using the following equation:

$$C=(TRCH+TRCV / TRH+TRV)\times 100$$

where: C=Colonization (%); $TRCH$=Total colonized horizontal roots; $TRCV$=Total colonized vertical roots; TRH=Total horizontal roots; TRV=Total vertical roots.

Fungal Structures method (McGonigle *et al.*, 1990). The roots were placed in permanent preparations on microscope slides, with 12 horizontal roots and 100 observations under the microscope for each preparation (100%). Afterward, 2 or 3 drops of PVLG were added, they were left to dry for 5 min, and then covered with a coverslip; these preparations were allowed to dry for a week. Subsequently, the preparations were read by fields of view with the 10x and 40x objectives in a Leica brand microscope, until 100 observations were made. Colonization by hyphae, vesicles, arbuscules, or spores was calculated using the following equation:

$$C=(TCC / TCO)\times 100$$

where: C=Colonization (%); TCC=Total colonized fields; TCO=Total observed fields.

Spore extraction. The successive wet sieving and decantation technique was used in combination with the sugar flotation technique (Salgado *et al.*, 2014). Observations and counts were done under a Leica brand 35 X stereo microscope.

Morphological classification of species. Once the spores were extracted from the soil samples, they were grouped and quantified according to their morphological characteristics by size, color, support hypha, and spore shape. The taxonomic classification of the morpho-species was made based on the INVAM (2018) keys.

Statistical analysis. Analyses of variance (ANOVA) were performed in a 7×2 factorial design (7 soil subunits and 2 root thicknesses) with four repetitions using the STATGRAPHICS plus 5.1 software; Tukey's means comparison test (α=0.05) was also used.

RESULTS AND DISCUSSION

Fungal Structures method. Figure 1 shows the fungal structures observed in the cane roots. The hyphae and spores branch out inside the cortical cells and between the cells of the epidermis, confirming that they do not invade the root pith and that the mycorrhization process occurs naturally in this crop, as reported by Jiménez *et al.* (2010) for the soils cultivated with sugarcane from the Ingenio Presidente Benito Juárez (IPBJ) sugar mill in Tabasco.

The ANOVA performed by fungal structures showed significant differences in the main effect of root thickness on hyphae, vesicles, and arbuscules (Figure 2), with the highest percentages observed in thin roots. This indicates that the symbiotic association is very dynamic and that it is taking place differentially in the plant roots, which could be influenced by their functions. That is, in addition to different functions, the diameter of the roots could implicate different ages and lignification, among other factors that could affect colonization. The mean colonization with this method was 69.5%, similar to the intersect method, which allows to infer that there is an interaction between VAM and sugarcane cultivation (Soria *et al.*, 2001), although it is unclear if this relationship is positive or negative since VAMs can demand up to 15% of the carbohydrates produced by the host. The structures with the greatest presence were hyphae and vesicles, whose functions are transport and storage, respectively, without dismissing the important role they play in the spread of mycorrhizal fungi. The presence of arbuscules was low and since they are the structures through which mycorrhizal fungi deposit nutrients in the cell cytoplasm (Smith and Read, 2008), this could indicate that the nutritional state is not being benefitted. The presence of spores within the root system could be indicative of the mycorrhiza species present, since not all have the ability to form these structures within the roots (Souza, 2006).

Significant differences were found in the main effect of the soil subunits on all fungal structures (Table 1). A greater hyphae colonization was found in the moderately acidic

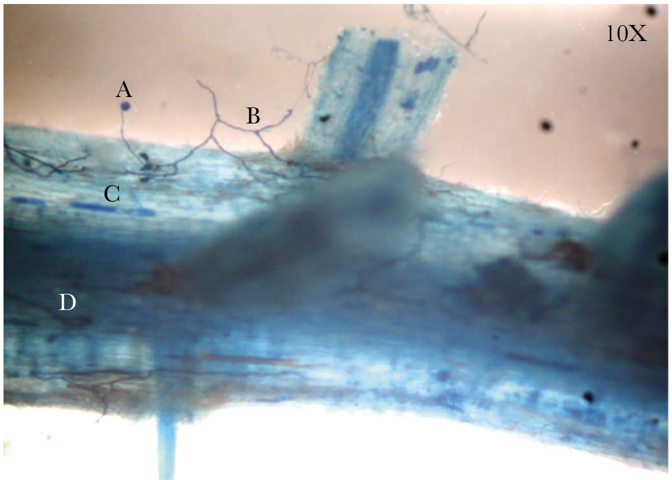


Figure 1. Fungal structures of VAM observed in sugarcane roots cultivated in the supply area of Ingenio Santa Rosalía: A) extra-root spore, B) extracellular hyphae, C) vesicles and D) intracellular hyphae.

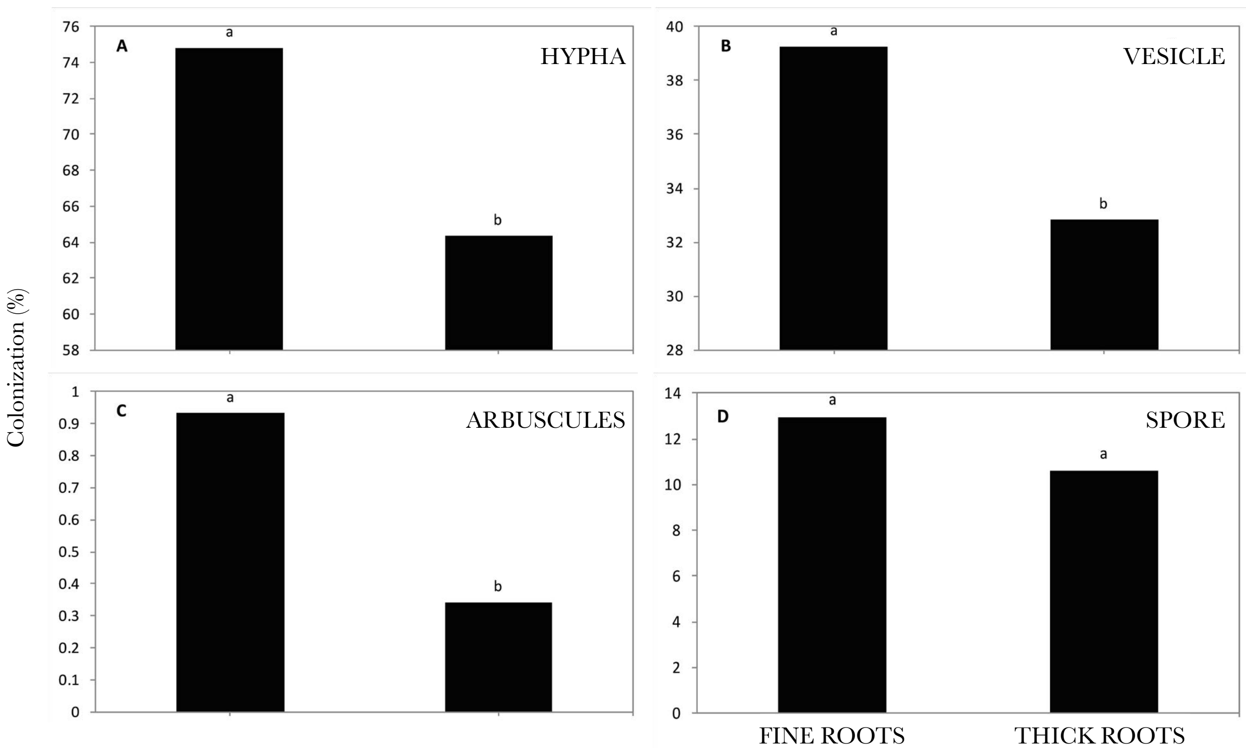


Figure 2. Mycorrhizal colonization (%) by fungal structures (McGonigle *et al*., 1990), in fine roots (diameter ≤1mm) and thick roots (diameter 1 to 2.0 mm) of sugarcane grown at Santa Rosalia sugar mill. Values with different literals indicate significant statistical difference (Tukey $p \leq 0.05$).

Eutric Fluvisol (90.0%), while the moderately acidic Eutric Vertisol was the one that showed the lowest percentage (54.2%). Most of the soil subunits are grouped within the range of 62% to 79%. These structures are the most abundant since they manage to disperse widely within the root tissue, from which other more specialized structures are formed.

The colonization by vesicles was higher in the eutric-gleyic-neutral Fluvisol subunits (50%), in the moderately acidic eutric-gleyic Fluvisol (52% a), and in the neutral pelic Vertisol (45%), and lower in the moderately acidic Eutric Vertisol plot (11%). The colonization by vesicles could indicate that the nutritional status of the sugarcane crop is adequate for the VAMs, which are storing nutritional reserves.

In relation to the other structures, colonization by arbuscules was minimal (<1.9%) and colonization by spores was greater in the moderately acidic Eutric Fluvisol (37%), followed by the moderately acidic eutric-gleyic Fluvisol (25.3%), while the rest of the subunits showed lower percentages.

Intersect method. The ANOVA showed that there is no difference between the main effect of both root diameters; VAMs colonize both types of roots without showing statistically significant differences in colonization. It was observed that the thicker root segments are difficult to clarify and generally stain excessively; however, the cortical tissue of these segments was dissected, confirming the presence of fungal structures.

No significant differences were found in the main effect by soil subunit (Table 1), although arithmetic variations were observed. The highest mycorrhizal colonization is

Table 1. Mycorrhizal colonization (%) and colonization index by subunit of soil cultivated with sugar cane, in the supply area of Santa Rosalia sugar mill, Tabasco.

Subunit of soil		Colonization (%)[Z]	Colonization index (%)[Y]			
			hyphae	vesicles	arbuscules	spores
1	Fluvisol eutric neutral	72.19 a	76.19 a	44.75 a	0.750 a	9.00 b
2	Fluvisol eutric moderately acidic	64.91 a	63.38 a	21.25 bc	0.125 a	10.13 b
3	Fluvisol eutric-gleyic moderately acidic	61.55 a	69.46 a	43.13 a	0.875 a	23.71 a
4	Gleysol eutric neutral	63.51 a	75.13 a	31.63 ab	1.250 a	8.63 b
5	Gleysol eutric moderately acidic	78.20 a	75.88 a	33.00 ab	0.625 a	3.25 b
6	Vertisol eutric moderately acidic	62.35 a	54.25 a	11.00 c	0.625 a	3.38 b
7	Vertisol pelic neutral	78.47 a	67.88 a	40.31 a	0.125 a	7.50 b

Values with different literals indicate statistical significant difference (Tukey ($p \leq 0.05$). [Z]Giovannetti y Mosse, (1980); [Y]McGonigle *et al.*, (1990).

seen in the neutral pelic Vertisol (78.5%), and the lowest in the moderately acidic eutric-gleyic Fluvisol (61.5%); this subunit of soil presents serious waterlogging problems in which the cane roots die from excess moisture. No trend was observed between the subunits and the soil pH, with a mean root colonization of 68.5%, considered to be acceptable according to various authors (Cracogna *et al.*, 2002; Salgado *et al.*, 2014; Tahuico, 2005), who report colonization at 70%, 88.9%, and 69.8%, respectively. The results indicate that mycorrhization is occurring in the ISR soils naturally in cultivated sugar cane.

Colonization index (%) Y
Vesicles Arbushes Spores

Spores in the soil

Figure 3 shows the number of spores in 100 g of soil. Tukey's test establishes three statistical groups. The highest number of spores was observed in the moderately acidic

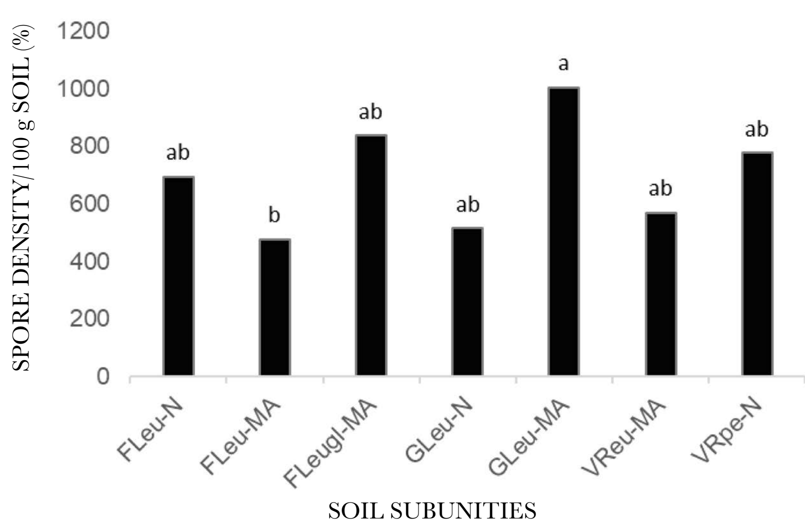


Figure 3. Density of spores per 100 g of soil in seven soil subunits cultivated with sugar cane at Santa Rosalia sugar mill supply area. Bars with different literals indicate statistically significant difference (Tukey $p \leq 0.05$).

eutric Gleysol subunit (1005); and the lowest in the moderately acidic eutric Fluvisol subunit (477). The rest of the subunits presented values between 514 and 837 spores per 100 g of soil. The mean was 696 spores per 100 g of soil in the ISR, lower than that observed in the IPBJ (943 spores per 100 g of soil), which is explained by the greater variability in edaphoclimatic conditions as observed by Reis *et al.* (1999). In three localities cultivating sugarcane in Pernambuco and Rio de Janeiro, Brazil, a variation from 18 to 2070 spores per 100 g of soil was reported. The most abundant varieties CB 14-16 and SP 70-1284 presented 1630 and 1080 spores in 100 g of soil. The number of spores found in the ISR indicates a high presence of VAMs, compared to the 4 spores per 100 g of soil from the Ingenio Tres Valles sugar mill in Honduras (Thauico, 2005); or compared to saline soils cultivated with ancho pepper, sunflower, and corn, where 210, 400, and 280 spores are reported per 100 g of soil (Tapia-Goné *et al.*, 2008).

Classification of VAM species. Six species of VAM were identified (Table 2). However, only four of them are present in more than four soil subunits: *Glomus* aff. *deserticola*, *Glomus etunicatum*, *Glomus viscosum*, and *Paraglomus occultum*. These results coincide with the predominance of the *Glomus* genus in sugarcane soils (Reis *et al.*, 1999). However, the number of species in the soils of the ISR is lower when compared to the 16 species of VAM reported in the soils of the IPBJ (Salgado *et al.*, 2014).

CONCLUSIONS

The analysis of mycorrhizal colonization by the intersects method did not show differences between the type of roots (thin or thick) but did detect significant differences in root colonization according to the soil subunits. The structures method detected significant differences by root type and showed significance in the colonization of hyphae, vesicles, and arbuscules, but not in the colonization by spores. In addition, statistically significant differences were observed in the colonization of roots according to the soil subunits. The highest number of spores was observed in the moderately acidic eutric Gleysol subunit (1005), and the lowest number in the moderately acidic eutric Fluvisol subunit (477). The rest of the subunits presented values between 514 and 837 spores per 100 g of soil. The average of 696 spores per 100 g of soil found in the ISR soils studied indicates a high presence of VAM. Six VAM species were found, of which four are present in more than four soil subunits: *Glomus* aff. *deserticola*, *Glomus etunicatum*, *Glomus viscosum*, and *Paraglomus occultum*. The presence of hyphae, vesicles, arbuscules, and spores indicates that there is an interactive process between VAM and sugarcane roots. Further research is recommended to determine if this relationship is symbiotic or parasitic given the low yields of grinding stalk obtained in the ISR.

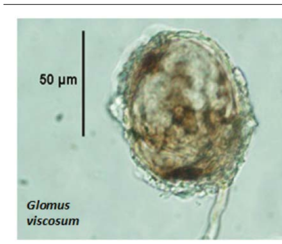
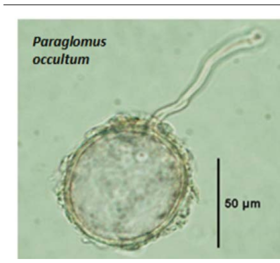
REFERENCES

Azevedo, B.L.C. 2008. Comunidades de fungos micorrícicos no solo e raízes da cana-de-açúcar. Tese de Doutor. Escola Superior de Agricultura Luiz de Quiroz, USP. Piracicaba, Brasil. 111 p.

Barea, J.M., Pozo, M.J., Azcón, R., and Azcón-Aguilar, C. 2005. Microbial cooperation in the rhizosphere. *Journal of Experimental Botany, 56*(417):1761-1778.

UNC (Unión Nacional de Cañeros A.C. CNPR). 2018. Boletín Técnico de la Unión Nacional de Cañeros (35): 1- 7. Disponible en línea: www.caneros.org.mx. Fecha de consulta 29/11/2018.

Table 2. Description and characteristics of morpho-species of hmva found in seven subunits of soil in the supply area of Santa Rosalia sugar mill, Mexico.

Photography	Description of spores	Presence in Soil Subunits
	*Acaulospora morrowiae* Spain & Schenck (HMA_ Blaszkowski. Spores pale yellow (3A3) to yellow (3A6), globose to sub-globose; (70-) 80 (-120) μm in diameter, sometimes ovoid, 70-80 x 100-120 μm.	*4*, *7*
	*Acaulospora scrobiculata* Trappe (HMA_Blaszkowski). Yellowish white (3A2) to pale yellow (3A3) spores, globose to sub-globose; (90-) diameter 120 μm (-135).	*7*
	*Glomus* aff. *deserticola* Trappe, Bloss & Menge HMA Blaszkowski). Spores pale yellow (3A3) to orange (6A6), globose to sub-globose; (70-) 89 (-115) μm diameter, sometimes pear-shaped ovoid, 70-80 x 85 to 100 μm, with a subtend hypha.	*1*, *3*, *6*, *7*
	*Glomus etunicatum* Becker & Gerdemann HMA_ Blaszkowski). Spores pale yellow (3A3) to yellow (3A6), globose to subglobose; (75-) 95 (-135) μm in diameter, occasionally ovoid, 110-160 x 140-180 microns, with a subtend hypha.	*1*, *3*, *5*, *7*
	*Glomus viscosum* Nicol. Subhyaline spores (0-0-5-0) to pale yellow (0-5-20-0); spore surface appears duller due to surface organic matter or debris; globose to subglobose, rarely irregular. Many spores form in loose branching aggregates of a common hypha, 50-120 μm (82 m, n=160).	*4*, *5*, *6*, *7*
	*Paraglomus occultum* (Walker) Morton & Redecker (INVAM). Subhyaline spores (0-0-0-0) to cream (0-0-5-0), globose to subglobose, somewhat irregular, 60-140 μm, with a mean equal to 85 μm (n=120).	*1*, *2*, *5*, *6*, *7*

Cracogna, M.F., Fogar, M.N., Iglesias, M. y Rosso, J. 2002. Estudio exploratorio de la infección por micorrizas en caña de azúcar. www1.unne.edu.ar/cyt/2002/05-Agrarias/A-050.pdf, Fecha de consulta 02/03/2012.

Giovannetti, M. and Mosse, B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist 84* (3): 489-500.

INVAM (International culture collection of vesicular arbuscular mycorrhizal fungi). 2018. http://fungi.invam.wvu.edu/the-fungi/classification.html. Fecha de consulta 30/11/2018.

Salgado, G.S., Castelán E.M., Jiménez, J.R., Gómez, L.J.F., Osorio, M.M. 2014. Diversidad de hongos micorrícicos arbusculares en suelos cultivados con caña de azúcar en la región de la Chontalpa, Tabasco. *Revista Mexicana de Micologia. 40*: 2-16.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., and Swan, J.A. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist 115* (3): 495-501.

Paula, M.A., Reis M.V., and Dobereiner, J. 1991. Interactions of *Glomus clarum* with *Acetobacter diazotrophicus* in infection of sweet potato (*Ipomoea batatas*), sugarcane (*Saccharum* spp.), and sweet sorghum (*Sorghum vulgate*). *Biol. Fertil. Soils 11*:111-115.

Philips, J.M. and Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid asessment of infection. *Transactions of the British Mycological Society, 55*: 158-160.

Reis, M.V., Puala, A.M., and Dobereiner, J. 1999. Ocorrência de micorrizas arbusculares e da bacteria diazotrofica *Acetobacter Diazotrophicus* em cana-de-açúcar. *Pesq. agropec. bras. Brasília, 34* (10):1933-1941.

Requena, N., Serrano, E., Ocón, A., and Breuninger, M. 2007. Plant signals and fungal perceptions during arbuscular mycorrhizal establishment. *Phytochemistry, 68*: 33-40.

Salgado, G.S., Palma, L.D.J., Lagunes, E.L.C., Ortiz, G.C.F., y Ascencio, R.J.M. 2005. Bases para generar un programa sustentable de fertilización en un ingenio de Tabasco, México. *Interciencia 30*(7): 395-403.

Salgado, G.S., Palma, L.D., Castelán-Estrada M., Lagunes, E.L. y Ortiz, L.H. 2013. Manual para el muestreo de suelos, plantas y aguas e interpretación de análisis para la producción sostenible de alimentos. Colegio de Postgraduados. México. 108 p.

Sánchez-Reyna, L., Salgado-García, S., Córdova-Sánchez, S., Hernández-Cuevas, L., Gómez-Leyva, J.F., Palma-López, D.J., Castañeda-Ceja, R., García de la Cruz, R. 2020. Capacidad infectiva de propágulos de Hongos Micorrícicos Arbusculares en el cultivo de caña de azúcar. *Agroproductividad 13*(1): 59-66.

Smith, S.E., and Read, D. 2008. Mycorrhizal Symbiosis (Third Edition). Academic Press, New York. 219 p.

Soria, A.E.M., Reyes, E.C., Occeguera, A.Z., y Pereira, M.C. 2001. Micorrización de plántulas micropropagadas de caña de azúcar (*Saccharum officinarum*). *Agricultura Técnica (Chile), 61*(4):436-443.

Souza, L.S. 2006. Análise do proteoma de raízes de cana-de-acucar e da expresao de uma perosidase apoplástica responsiva a micorriza arbuscular. Tese de Doutor em Agronomia. Microbiologia Agricola. ESALQ-USP. Brasil. 102 p.

Tahuico, R.J.S. 2005. Respuesta de caña de azúcar a la inoculación con micorrizas vesículo-arbusculares en el Ingenio Tres Valles, Honduras. Tesis de licenciatura. Zamorano, Honduras. 70 p.

Tapia-Goné, J., Ferrera-Cerrato, R., Varela-Fregoso, L., Rodríguez, O.J.C., Lara, M.J., Soria, C.J.C., Cuellar, T.H., Tiscareno, I.M.A. y Cisneros, A. R. 2008. Caracterización e identificación morfológica de hongos formadores de micorriza arbuscular, en cinco suelos salinos del estado de San Luis Potosí, México. *Revista Mexicana de Micologia. 26*: 3-7.

Weier, K.C., McEwan, C.W., Vallis, I., Catchoole, V.R., and Myers, R.J. 1996. Potential for biological denitrification of fertilizer nitrogen in sugarcane soils. *Aust. J. Agric. Res. 47*: 67

AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Optimal fertilization dose in castor bean (*Ricinus communis* L.) using partial budgets analysis

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ABSTRACT

Objective: To determine the economically adequate fertilization dose for a castor bean (*Ricinus communis* L.) crop that improves the producer's benefits using the partial budgeting technique.

Design/methodology/approach: The experiment was established at a site located 500 m from the Centro de Chiapas experimental field, with an altitude of 800 m. Two production factors were studied: nitrogen (40, 60, and 80) and phosphorus (20, 40, and 60) with three levels each. The treatments had nine combinations in a randomized complete block experimental design and four replications. Phenological and morphological variables, seed yield, and variable costs economic components were evaluated as a response.

Results: With the average yields the obtained net benefits were determined; an adjustment of the benefits was made, and its dominance was determined. Four experiments were dominated, and five showed favorable profits for the producer. The 80-60-00 formulation had a return rate (RR) of 23%.

Conclusions: The 40-20-00, 40-40-00, 40-60-00, 80-40-00 and 80-60-00 formulations were dominant. The 80-60-00 formulation reported the highest RR, an increase in variable cost of US$9.64, like other doses of lower costs and benefits. For this dose, the net benefits increase was US$ 222.61.

Keywords: *Ricinus communis*, variable costs, net benefit, return rate.

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INTRODUCTION

The optimization of resources is key to increasing net profits within the agricultural production process. Within this production process, fertilizers cover between 20 and 37% of total production costs (Salinas *et al.*, 2017); for this reason, its correct use is essential to maintain a balance in the benefit-cost relationship, in addition to contributing to the reduction of the emission of pollutants into the environment. Due to the increase in the food demand caused by population growth, many farmers put more pressure on the land, without concerning an adequate return of nutrients; others fertilize in excess and abuse their use. From agricultural fields fertilization, some fertilizing elements have a detrimental impact on stratospheric chemistry, thus worsening regional and global climate

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change (IPCC, 2013). Therefore, the development and implementation of methodologies and techniques to reduce emissions from agricultural land are an urgent need.

In intensive agriculture, even when the used fertilizers volumes are calculated from a soil diagnosis and the yield to be obtained, an economic approach must be added to precisely know how that fertilizer dose maximizes the efficiency of all the financial resources. Thus, an economically optimal dose is defined as the amount of fertilizer that maximizes the crop's profitability (Nelson *et al.*, 1985; Pagani *et al.*, 2008). In this sense, Salvagiotti *et al.* (2011) comment that a way to calculate the economically optimal dose that maximizes resources is through the adjustment of response curves between fertilizer doses and yield. In this way, it uses the first derivative of the function to calculate the economic response of each level of applied fertilizer. According to Cerrato and Blackmer (1990), the optimal economic dose can also be estimated using the quadratic and exponential models, the Linear-Plateau model, and the Quadratic-Plauteau model. The economically optimal dose of fertilizer should be established with practical methods for farmers; so using the aforementioned methods would not provide results with the desired characteristics.

Other authors suggest using the partial budgeting technique. Partial budgeting is a tool used to evaluate the costs and benefits associated with a specific change in a farm; it is a planning and decision-making framework used to compare the costs and benefits of the alternatives faced by an agricultural company (Soha, 2014). Soha (2014) used partial budgets to estimate the effect on the net benefit of changing from one nitrogen fertilization level to another; also evaluated the costs and benefits associated with a specific change. Shaaban and Kisetu (2014) used partial budgets to evaluate the response of the Irish potato to NPK-based fertilization applications and its economic rate of return when grown in a Ultisol. For their part, Agumas *et al.* (2014) evaluated the response of irrigated onions to different nitrogen and phosphorus doses in northwestern Ethiopia.

At the same time, the world is faced with the need to incorporate new raw materials options for biofuels production. Currently, there is a downward trend in hydrocarbon production, mainly due to increased exploration and extraction costs, reservoir depletion, regulation of environmental impact, and effects on the climate. Faced with this situation, renewable energies, besides being friendlier to health and the climate than fossil fuels, facilitate access to electricity with a lower cost advantage (Kühne *et al.*, 2019). In this sense, castor bean (*Ricinus communis*) is an attractive option for biodiesel production. Some of its genotypes have a great capacity to produce excellent quality oil as an input for biodiesel. The main advantages of castor oil are that it preserves its viscosity at high temperatures and resists low temperatures without freezing, it comes from a renewable source, it is non-toxic, non-flammable, easy to transport, biodegradable, sustainable, and sulfur and aromatic compounds free (Raya-Pérez *et al.*, 2016). Another advantage is its drought tolerant and adapted to a great diversity of climates (Barrios-Gómez *et al.*, 2018). Due to the great adaptation potential of castor bean to the Mexican climates and the state of Chiapas, and to the degree is adopted as an industrial crop, its technological package could be complemented with an economically optimal dose of fertilization; This would undoubtedly support low-income producers' economy, as well as help them to conserve the soils of their plots.

The objective of this research was to determine the economically optimal dose of fertilization for castor bean cultivation using the partial budget technique. The information in this research will be useful for farmers and companies dedicated to the cultivation of this species in the state of Chiapas. Above all, it will help to calculate the fertilizer's volumes, and to reduce the possibility of capital flight and pollution of natural resources.

MATERIALS AND METHODS

Experiment location

The castor bean experiment was established at Ocozocoautla de Espinosa, Chiapas, Mexico (16° 45" N and 93° 22" W, at 800 m). The soil is sandy-clayey in texture, light brown when dry, and with a 5% slope. The climate is Aw type, warm humid with rains in summer, and subject to midsummer drought effects.

Treatments description

Two production factors were studied: nitrogen and phosphorus; each with three levels, for nitrogen 40, 60, and 80; while for phosphorus 20, 40, and 60. The treatments were a combinatorial arrangement with nine combinations total (Table 1). The experimental design was randomized complete blocks with four repetitions.

Partial budgets

Partial budget is a methodology that only takes into account the costs associated with the decision to use a treatment or not (Reyes, 2001). This technique is based on the principle that small changes in the production system could generate one or more of the following effects (Duque-Orrego, 2005):

1) Originate additional costs; 2) Eliminate or reduce some returns; 3) Originate additional returns; 4) Eliminate or reduce some costs In this research, the partial budgeting technique was applied following the recommendations of the International Maize and Wheat Improvement Center (CIMMYT, 1988).

Table 1. Evaluated fertilization treatments, nitrogen (N) and phosphorus (P).

T	N	P
	(kg ha^{-1})	
1	40	20
2	60	20
3	80	20
4	40	40
5	60	40
6	80	40
7	40	60
8	60	60
9	80	60

RESULTS AND DISCUSSION

Variable costs

Table 2 shows the variable costs per treatment, the costs generated by the urea application plus that of DAP fertilizer are considered; to these market costs, the cost of transporting the product are added, then adding both to establish the field cost considered in the partial costs analysis as the one disbursed by the producer, according to the variable cost. The highest value purchase product was urea. The doses varied for each treatment. The treatments were formed with a dose of urea and another dose of DAP, so the sum of both was the total variable cost for the nine treatments.

The first 40-20-00 (N-P-K) treatment generated the lowest costs. In general, the varying costs ranged from US$ 69.65 to US$ 79.1, with two urea and one of DAP applications. The highest costs are found in the 80-40-00 and 80-60-00 treatments with urea applications of 139 and 123 kg and DBH of 86 and 130 kg respectively; therefore, the total of US$ 99.1 and US$ 108.75 was paid.

Economic profit and adjusted profit

Calculating the economic benefits was based on the yield obtained per treatment for the purchase price of castor seed, which was US$ 0.46 per kilogram sold in the year of the analysis; the yields obtained are presented in Table 3 for each treatment according to the applied fertilization dose.

According to the obtained data, treatment 9, with doses of DAP and urea 80 and 60 respectively yielded 2.1 t ha^{-1}, the highest benefit, with a total of US$ 945, although it was also the one that disbursed the highest costs; while treatment 3, the one with the lowest yield, with 124 kg, and therefore had the lowest net economic obtained benefit, in addition to the generated costs were a total of US $ 89.65, which means that it was not the treatment with the lowest profit and production, it was the one with the lowest gender cost.

After calculating the net benefit and according to the process, an adjustment is made to the net benefit, this can vary from 10 to 25 % according to the recommendation (CIMMYT,

Table 2. Variable costs by applied treatment.

n°	Description (kg ha^{-1})	Quantity		Unit cost		Number of applications		Total variable costs US$
		urea (kg)	DAP (kg)	urea (US $/kg)	DAP (US$/kg)	urea (kg)	DAP (kg)	
1	40-20-00	69.65	43.47	0.46	0.4	2	1	49.42
2	60-20-00	113.43	43.7	0.46	0.4	2	1	69.65
3	80-20-00	156.91	43.7	0.46	0.4	2	1	89.65
4	40-40-00	52.93	86.95	0.46	0.4	2	1	59.12
5	60-40-00	96.41	86.95	0.46	0.4	2	1	79.12
6	80-40-00	139.89	86.95	0.46	0.4	2	1	99.12
7	40-60-00	36.08	130.43	0.46	0.4	2	1	68.76
8	60-60-00	79.56	130.43	0.46	0.4	2	1	88.76
9	80-60-00	123.05	130.43	0.46	0.4	2	1	108.77

DAP: Diammonium phosphate.

Table 3. Economic benefit for established treatment.

Number	Description (kg ha^{-1})	Yield (t ha^{-1})	Economic benefit US$
1	40-20-00	0.704	316.80
2	60-20-00	0.904	406.80
3	80-20-00	0.124	55.80
4	40-40-00	0.841	378.45
5	60-40-00	0.984	442.80
6	80-40-00	1.518	683.10
7	40-60-00	1.03	463.50
8	60-60-00	1.2	540.00
9	80-60-00	2.1	945.00

1988). The adjustment made was 15%, which was calculated on the initial net profit and subsequently subtracted from this income, resulting in the adjusted net profit (Table 4).

Dominance analysis

By definition, the first treatment is not dominated (Reyes, 2001); later, when going from treatment 1 to 4, it is observed if there is an increase in benefits because this treatment was considered not dominated, from treatment 4 to treatment 7 there is an increase, which is why it is considered not dominated. It was found that from treatment 7 to treatment 2 the net benefit is lower and therefore considered dominated (Table 5).

By having a dominated treatment, treatment 7 was used as not dominated and it was determined that treatment 5 is dominated, therefore, treatment 7 will continue to be the basis for determining the following treatments. Treatment 8 is not mastered, while Treatment 3 is mastered. Treatments 6 and 9 are not dominated.

According to Table 4, fertilization with the 80-20-00 combination had variable costs of US$ 89.65 and a net benefit of US$ 51.91, which represents a high cost and a low net benefit, unlike the other different fertilization doses.

Table 4. Adjusted economic benefit.

Number	Description (kg ha^{-1})	Economic benefit US$	Adjustment to 15% US$	Adjusted economic benefit US$
1	40-20-00	316.80	47.52	269.28
2	60-20-00	406.80	61.02	345.78
3	80-20-00	55.80	8.37	47.43
4	40-40-00	378.45	56.77	321.68
5	60-40-00	442.80	66.42	376.38
6	80-40-00	683.10	102.47	580.64
7	40-60-00	463.50	69.53	393.98
8	60-60-00	540.00	81.00	459.00
9	80-60-00	945.00	141.75	803.25

Table 5. Dominance analysis.

Number	Description (kg ha^{-1})	Costs that vary (CV) US$	Net profit (NP) US$	Observation of treatment change	Conclusion of the observation
1	40-20-00	49.427	269.28		not dominated
4	40-40-00	59.128	321.6825	De 1 a 4	not dominated
7	40-60-00	68.769	393.975	De 4 a 7	not dominated
2	60-20-00	69.658	345.78	De 7 a 2	dominated
5	60-40-00	79.1285	376.38	De 2 a 5	dominated
8	60-60-00	88.7695	459	De 5 a 8	not dominated
3	80-20-00	89.6585	47.43	De 8 a 3	dominated
6	80-40-00	99.1295	580.635	De 3 a 6	not dominated
9	80-60-00	108.775	803.25	De 6 a 9	not dominated

Rate of return

The rate of return is established through the increases in the net economic costs and benefits, for its calculation, only the non-dominated treatments are considered, which for this case would be those described in the following Table 6.

Partial budgets can be used when there is a minimum change in agricultural activity and generally these changes are carried out in a short term, seeking to evaluate the change in both income and expenses (Santilla-Simbaña, 2020); such is the case of the analysis that was carried out to the fertilization changes in castor bean trials to determine the optimal dose is technically and economically most viable to obtain the lowest cost and the greatest benefit.

In this analysis, the variable costs are determined based on the use of fertilizers according to the dose or treatment, determining their field costs. These vary with the production and are intended to be less than the economic benefits to be obtained, since it could only be economic to continue production in the short term, as long as the income is greater than the costs of production variable (Cooperative Extension, 2021).

CONCLUSIONS

The treatment with the highest costs was the 80-40-00 dose (US$ 99.12) and the 80-60-00 (US$ 188.77), from these treatments the obtained economic benefits were US$

Table 6. Rate of return of the different treatments.

Number	Description (kg ha^{-1})	Costs That vary (CV) US$	Net profit (NP) US$	Increase in NP US$	Increase in CV US$	TRM (%)
1	40-20-00	49.427	269.28			
4	40-40-00	59.128	321.6825	52.40	9.70	5.40
7	40-60-00	68.769	393.975	72.29	9.64	7.50
8	60-60-00	88.7695	459	65.03	20.00	3.25
6	80-40-00	99.1295	580.65	121.64	30.36	4.01
9	80-60-00	108.775	803.25	222.62	9.65	23.08

683.1 and US$ 945, with yields of 1.5 and 2.1 ton per hectare respectively. According to the dominance analysis, treatments 40-20-00, 40-40-00, 40-60-00, 80-40-00, and 80-60-00 presented no dominance, so they could be the optimal doses for fertilization in the production of castor bean. In the rate of return analysis, it is determined that the 80-60-00 dose is the economic optimum because the cost increase was only US$ 9.64 equivalent to the 40-40-00 and 40-60-00 treatments. the treatment with the highest cost variation was 80-40-00 with a total of US$ 30.36. While the benefits increase is presented in the 80-60-00 treatment with US$ 222.61. According to the partial budget methodology, the optimal economic treatment is the 80-60-00 since their costs increase are low and the net benefit is greater than that of the rest of the recommended doses.

REFERENCES

Agumas B., Abewa A. and Abebe D. (2014). Response of Irrigated Onion (*Allium cepa* L.) to Nitrogen and Phosphorus Fertilizers at Ribb and Koga Irrigation Schemes in Amhara Region, North western Ethiopia, *International Research Journal of Agricultural Science and Soil Science.* 4(5). 95-100.

Barrios- Gómez, E. J., Canul- Ku, J., Hernández- Arenas, M. G., Solís- Bonilla, J. L., (2018). Evaluación de dos ciclos de higuerilla en Morelos, México: siembra y rebrote. *Revista Mexicana de Ciencias Agrícolas.* Volumen 9 (8): 1663-1673. Doi: 10.29312/remexca.v9i8.679

Cerrato M. E., Blackmer A. M. (1990). Comparison of models for describing corn yield response to nitrogen fertilizer. *Agronomy Journal*. 82(1). 138-143.

CIMMYT. (1998). La formulación de recomendaciones a partir de datos agronómicos: Un manual metodológico de evaluación económica. Edición completamente revisada. México D. F., México: CIMMYT.

Cooperative Extension. 2021. Presupuestos para tomar decisiones agrícolas. PennState Extensión. Alternativas Agrícolas. P. 12.

Duque-Orrego, H., Posada-Suárez, H.E., Alvarado-Alvarado, G. (2005). Análisis económico de la adopción de las variedades castillo regionales resistentes a la roya. *Cenicafé.* 56(3). 197-215.

Kühne, K., Sánchez, L., Roth, J., Tornel, C., Gerasimchuk I. (2019). Más allá de los combustibles fósiles: Transición fiscal en México. The International Institute for Sustainable Development, Manitoba, Canada. P. 49.

Nelson L. A., Voss R. D., Pesek J. (1985). Agronomic and statistical evaluation of fertilizer response. *In*: OP Engelstad (ed.) Fertilizer technology and use. Pp 53-90. ASA. Madison – Wisconsin – USA.

Pagani A., Echeverria H. E., Sainz Rozas H. y PA Barbieri. (2008). Dosis óptima económica de nitrógeno en maíz bajo siembra directa en el Sudeste bonaerense. *Ciencia del Suelo*. 26(2). 179-188.

Reyes-Hernández. M. (2001). Análisis económico de experimentos agrícolas con presupuestos parciales: Re-enseñando el uso de este enfoque. Centro de Información Agro socioeconómica. *Boletín informativo* 1-200, p-33.

Salvagiotti F., Castellarin J. M., Ferrugati F. J., Pedro H. M. (2011). Dosis optima económica de nitrógeno en maíz según potencial de producción y disponibilidad de nitrógeno en la región Pampeana norte. *Ciencia del suelo.* 29 (2).199-212.

Santilla-Simbaña. K. M. (2020). Desarrollo de manual para presupuestos parciales para el proceso de toma de decisiones agrícolas. Escuela Agrícola Panamericana. Carrera de administración de agronegocios. Zamorano, Honduras.

Shaaban H., Kisetu E. (2014). Response of Irish potato to NPK fertilizer application and its economic return when grown on an Ultisol of Morogoro, Tanzania. *Journal of Agricultural and Crop Research*. 2 (9). 188-196.

Soha M. E.-D. 2014. The partial budget analysis for sorghum farm in Sinai Peninsula, Egypt. *Annals of Agricultural Sciences.* 59(1). 77-81. Doi:10.1016/j.aoas.2014.06.011

AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Productive and technological characteristics of goat farmers in Comondú, Baja California Sur

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ABSTRACT

To identify productive characteristics through the technological indicators adopted by goats farmers at the Comondú municipality, state of Baja California Sur, Mexico. A survey consisting of semi-structured questions was designed to obtain multi-criteria information on the variables of technological, economic, social, and multilevel indicators. The production units (PUs) were classified by the cluster analysis of means statistical method in the SAS software, allowing to identify four groups of producers of 10.33, 69, 10.33, and 10.33 % of them. The social indicator demonstrated differences between UPs regard their education level, non-inclusion of women in the activity, total annual income, and whether or not they are members of an association. The technological indicator revealed differences in their distance from the UPs to the community, road conditions, area of the UP, available area for planting, water sources, herd size, availability to facilities, machinery, and equipment. The economic indicator showed differences between UPs in their agricultural complementary activities, economic dependence, milk production, cheese production, animal wastes, goat production, and their respective sales values. The conclusion is that this information is useful for particularly attending to the needs and will allow defining the precise kind of intervention in the management practices or the required management.

Keywords: goats, technology transfer, adoption.

Objective: Identify the productive characteristics through technological indicators implemented by goat farmers at the Comondú Municipality, Baja California Sur, Mexico.
Design/methodology/approach: A survey consisting of semi-structured questions was designed to obtain multi-criteria information on variables of technological, economic, social, and multilevel indicators. To determine the sample size, the formula suggested by Snedecor and Cochran (1967 and cited by Rojas, 1979); Cadena, (2004); Uzcanga *et al.* (2021) was used. It states that the elements must be selected by a random draw

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with replacement in the case of the producers representing each FPU, who participate in the PRODETER program, and according to the numerical characteristics of the universe of producers.
Results: The production units (PU) were classified using the cluster analysis of means statistical method with the SAS software, which allowed to identify four groups of producers with 10.33, 69, 10.33, and 10.33% of them. The social indicator revealed differences between PUs in the degree of schooling, the non-inclusion of women in the activity, total annual income, and whether or not they are members of an association. The technological indicator found differences in the distance of the PU from the community, road conditions, area of the PU, area available for planting, water sources, herd size, availability of facilities, machinery, and equipment. The economic indicator indicated differences between the PUs in the complementary agricultural activities, economic dependence, production of milk, production of cheese, cull animals, production of goats, and their respective sales values.
Findings/conclusions: Technology indicators, herd size, and distance from the PUs to the municipal seat and community, allowed grouping and understanding the producers in a better way based on their social characteristics, technological and economic indicators. Such information will allow to precisely define the required management practices or management interventions.

INTRODUCTION

The public policy issue in the general provisions and operating guidelines of the rural development program of the Secretaria de Agricultura y Desarrollo (Secretary of Agriculture and Rural Development, Mexico) for the 2019 fiscal year (DOF-28/02/2019) and modified on 01/11/2019 indicate that the aids of the Rural Development Program are destined to small producers, located in high and very high marginated areas, applying social inclusion and gender equity criteria. Family Production Units (FPU) are a group of associated producers with a common goal, with no legal formality, or constituted as associative figures according to the national legal order. For this purpose, three parties join forces: extensionism, research, and productive sector to create synergy and for innovation management.

The Secretaria de Agricultura y Desarrollo Rural (Secretary of Agriculture and Rural Development) defined territorial development as an action where two segments converge: infrastructure investment and knowledge investment. so that farmers, producers, and ranchers (for this document, using any of the three concepts will be considered as synonymous among each other, and differentiates ranchers given the activity they develop)

Figure 1. Elements that make up the development strategy of PRODETER. Source SADER, 2019.

are considered subjects and not as beneficiaries and recipients of government programs that contribute to their benefit and that of their families.

In this regard, INIFAP is directly involved with the research segment, committing to three fundamental actions, based on the available technologies, providing technical support to the FPUs grouped in functional organizations to achieve or manage innovation, based on a territorial diagnosis through the characterization of the FPU, for which detected problems prioritization was carried out and a differential work model was used in the assessed territories.

The Mexican Secretaría de Agricultura (Secretary of Agriculture) identified four opportunity areas to operationalize the above graphically framed:

I. Capacity Building, Extension, and Rural Advisory Services.
II. Production Chains Economic Integration.
III. Strengthening of Family Production Units.
IV. Research and Technology Transfer. In this area, for which the Instituto Nacional de Investigaciones, Forestales, Agrícolas y Pecuarias (National Institute of Forestry, Agricultural and Livestock Research, INIFAP) is responsible, three deliverables were agreed upon:

1. Technical-productive diagnosis of the Family Production Units, which consisted of the following sections or activities:

a. Technological characterization of the Family Production Units.
b. Identification of production problems.
c. Definition and estimation of productive indicators, baseline diagnosis to contrast the annual advances of the technological intervention.

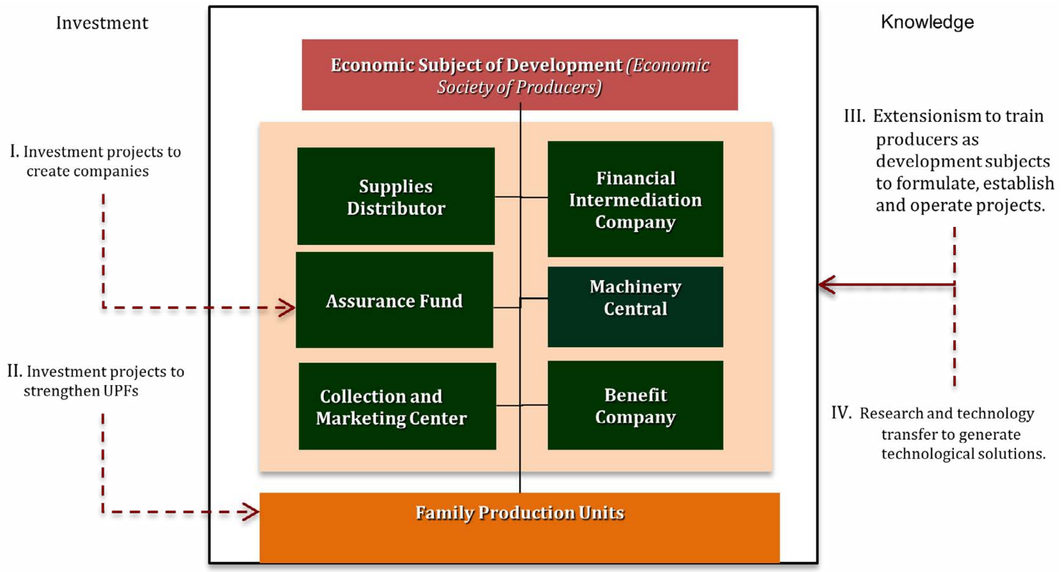

Figure 2. Areas of opportunity for development the PRODETER. Source SADER, 2019.

d. Proposal of a technological model based on the available technologies by INIFAP or other higher education/research institutions.

2. Technology Transfer Proposal

a. Demonstration modules (on the producers' land) and demonstration events.
b. Execution of a program for the development of technical capacities for extensionists and producers.
c. Sessions to exchange experience and knowledge among producers.

3. Technical Support Strategy

The objective was to provide feedback to the producers and extensionists in applying technological components, allowing a direct interrelation between extensionists, producers, and the researcher in the field; a concept widely described by Everett Rogers (1983) in the 1960s. Who in that decade, proposed the Traditional Model of Diffusion and Adoption of Innovations. There, the author proposed that new knowledge generation through scientific research on agricultural aspects was a basic necessity, a task developed in experimental stations or fields in different conditions and circumstances than those of the producers. Once the technology was developed, it was transmitted to technicians or "change agents", so that they bridged between researchers and the producers (Axinn, 1993). Therefore, the process was one-way vertical from research to farmers. This way, the transfer was focused on scientific and technological achievements and not on the farmers. Eponou (1993) defined the model proposed by Rogers as a linear, or vertical model, where the activities are separated, without interrelation or feedback between the integrating elements, and mentioned that there is a clear labor division in each of the described stages. Researchers carry out the research, extensionists deliver the technologies and the farmers use them. Validation or testing is conducted on farmers' land; this process is oriented by the researchers, but strongly involves extensionists; the process seeks to refine the technology and test it for technical, financial, and environmental adaptation of the farmers.

During the early '60s, Rogers stated that the approach was a continuous process, that started by generating innovations, followed by a validation stage, to be later extended among producers through programs led by agricultural development offices. After which, farmers are expected to adopt the innovations (Rogers, 1983), detailed concepts and discuss different situations and contexts (Cadena *et al.*, 2018). Based on this model, technical support was carried out on producers' and ranchers' plots and/or ranches. A program with visits within the assessed territory was designed by the researchers, considering: the phenological stages of the crop, physiological stages of the animals, recommended technological components, and other specific aspects. Although it is not specifically described, the model used in the characterization of the goat farmers is closer to that by Röling in 1988, where there is an interrelation between the components, *i.e.*, research, extension, and producers (Röling, 1988; Röling, 1990; Lionberger1986; Kaimowitz *et al.*, 1990; Manzo, 1994; Cadena 2004).

MATERIALS AND METHODS

A survey consisting of semi-structured questions was designed to obtain multi-criteria information of the variables of technological, economic, social, and multilevel indicators. To determine the sample size, the formula suggested by Snedecor and Cochran (1967, cited by Rojas, 1979), Cadena (2004), Uzcanga *et al.*, 2021) was used. This indicates that the elements should be selected through a random draw with replacement in the case of those producers representing each FPU, participating in the PRODETER program and according to the numerical characteristics of the universe of producers. To accomplish this, the lists of producers provided by each promoter or extensionist were used, the producers were then selected from the universe in each of the strategies following the formula:

$$n = \frac{\frac{Z^2 p_n q}{d^2}}{1 + \frac{Z^2 p_n q}{N\, d^2}}$$

The mathematical equation details as follows based on a hypothetical universe: Where: Z=Confidence Level, D=Precision level, p_n=Proportion of the population from to the group of interest q=(1−pn), N=Population size, N=Sample size.

A questionnaire that was personally applied to 14.5% of the producers (n=29 surveys) was designed considering a simple random sampling and taking as a basis the list of participants in the PRODETER program (N=200); semi-structured interviews were applied to the producers to obtain multi-criteria information on several variables of the technological, economic, social and multilevel indicators.

The information from the semi-structured interviews at the production unit was systematized in a database in Excel software. To classify the production units (PU), the technological development index proposed by Nahed *et al.* (2021) was used. The indicators which comprise it were infrastructure possession, machinery, and equipment (grade), which made up thirteen variables (Table 1), and management practices application (grade) made up of ten variables (Table 2). Tables 1 and 2 show the indicators, their variables, and the evaluated characteristics to obtain the technological development indexes. Each indicator's value is the average of the scores of its component variables.

The PUs were classified using the cluster analysis statistical method for means, for which the herd size and distance from the PUs technological development indexes were used as grouping variables. The multivariate statistical method of cluster analysis, groups homogeneous intra-group data (minimum variance) allows to differentiate heterogeneous inter-group data (maximum variance) and generates a vector of PU membership in the clusters.

Subsequently, the clusters or groups of PUs identified were characterized by the variables of the social, technological, and economic indicators. The social indicator variables were: age of the producers (years), women's inclusion in the activity (%), educational level of the

Table 1. Evaluated variables and characteristics used to elaborate the identification of technological indicators regard the possession of facilities, machinery and equipment (grade). INIFAP, 2021.

Variable	Feature	Scoring criteria
Corral	Own	yes=1 No=0
Milking room	Own	yes=1 No=0
Drinking fountain	Own	yes=1 No=0
Eatable	Own	yes=1 No=0
Wine cellar	Own	yes=1 No=0
Dairy workshop	Own	yes=1 No=0
Plough	Own	yes=1 No=0
Tractor	Own	yes=1 No=0
Drag	Own	yes=1 No=0
Water pump	Own	yes=1 No=0
Scale	Own	yes=1 No=0
Rennet tubs	Own	yes=1 No=0
Pickup truck	Own	yes=1 No=0

Table 2. Evaluated variables and characteristics to elaborate the identification of technological indicators regard management practices (grade). INIFAP, 2021.

Variable	Feature	Sciring criteria
Records management	Aretado	Yes=1 No=0
	Lotifica herd	Yes=1 No=0
	Productive records	Yes=1 No=0
	Qualification	Maximum Summation=3
Zootechnical management	Ensures colostrum consumption	Yes=1 No=0
	Use evaluated players	Yes=1 No=0
	Supplement stallions	Yes=1 No=0
	Supplement females	Yes=1 No=0
	Deworming	Yes=1 No=0
	Perform mastitis tests	Yes=1 No=0
	Adjust animal load	Yes=1 No=0
	Qualification	Maximum Summation=7

producer (none, elementary, secondary, high school or bachelor's degree), family members working on the ranch (number), total annual income including cheese sales, cull animals, capons and goal kids ($), type of owned property (private, ejido, communal or rented) and whether they are part of an association (%); the technological indicator variables were: distance from the PU to the municipality head or to the community (km), percentage of producers with good, regular or bad road conditions, area of the PU (ha), planted available area (ha), availability (%) of water for the livestock (stream, dam, spring, well or jar), percentage of producers by production system (semi - stabled or extensive), herd size (head), breed diversity (number), breeding females (head), sires (head), birth rate (%), abortions (%), availability of the facilities (grade), machinery and equipment availability (grade), record keeping (grade) and husbandry practices application (grade); the economic indicator variables were: complementary activities (agricultural and livestock), PU income from goat farming (<50, between 50-99 and 100%), average milk production (L), cheese production (kg), cheese sales value ($), cull animal sale (heads), cull animal sale value ($), capon sale (heads), capon sale value ($), cabrito sale (heads), goat kids sale value ($), goat kids sale (heads), and sale value per goat kid ($).

Statistical analyses were performed with the SAS statistical software (2014).

RESULTS AND DISCUSSION

Production units' classification. The cluster analysis allowed to identify four groups of producers. Clusters 1, 2, 3, and 4 grouped 10.33, 69, 10.33, and 10.33% of them, respectively.

Social characterization of the production units based on technological indexes. Table 3 presents the variables for the social indicator. The main observed difference was recorded in the educational level, the non-inclusion of women in the activity, the highest total annual income, and being part of an association; these last three among cluster 4 producers, while the cluster 3 producers had a lower educational level. Producers in cluster 2 were more diverse regard their education level and the type of property they owned.

Technological characterization of the production units based on technological indexes. Table 4 shows the variables of the technological indicator. The cluster 1 producers present the shortest distance from the community, larger production unit area, birth rate, facilities availability, planting area, and a greater degree of record keeping. Producers in cluster 2

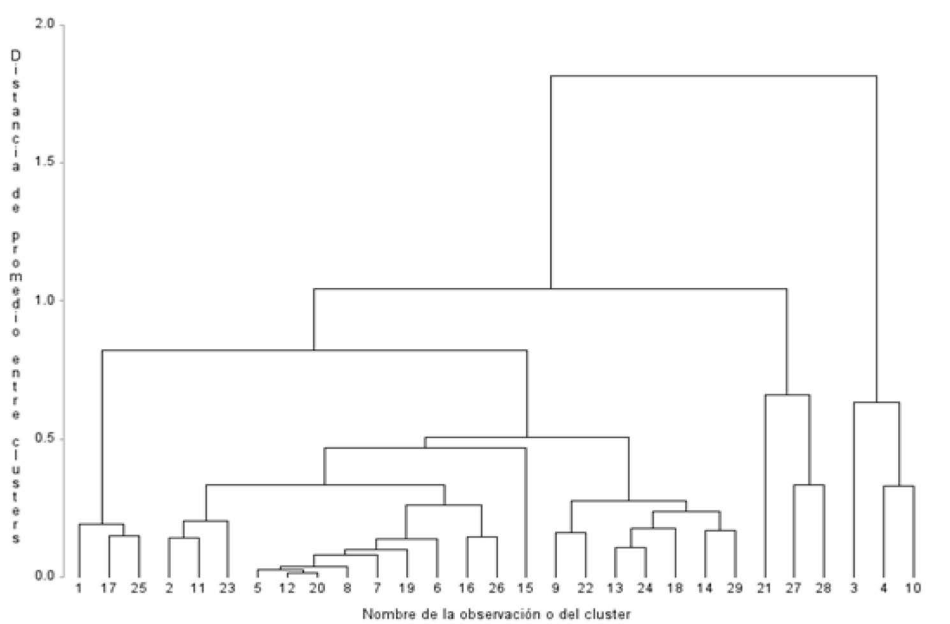

Figure 3. Number of integrated clusters according to the adopted technologies. INIFAP, 2021.

Table 3. Variables of the social indicator. INIFAP, 2021.

Variable	Conglomerate 1	Conglomerate 2	Conglomerate 3	Conglomerate 4
Production units	3	20	3	3
Age (years)	49.6	50	53	46
Schooling (%)				
Primary	0	30	100	67
High school	100	30	0	33
High school	0	20	0	0
Degree	0	10	0	0
No	0	10	0	0
Family members working on the ranch (number)	3.3	2.3	2.3	3
Inclusion of women (%)	33	35	33	0
Total income ($/year)	126 967	52 134.7	31 200	141 033
Type of ownership (% of producers)				
Private	67	30	67	33
Ejidal	33	60	33	67
Communal	0	5	0	0
Rented	0	5	0	0
Be part of an association (%)	67	85	67	100

reported the shortest distance from the municipality, better road conditions, more water source diversity for the livestock, semi-stabled production systems, most diverse breeds, and the lowest number of breeding and milking goats. The third cluster grouped producers with the longest distance to the community, water availability from dams and springs, high abortion rates, lowest facilities availability, and highest equipment and machinery availability. Cluster 4 groups the producers with the greatest distance to the municipality, worst road conditions, smallest PU area, no available planting surface, available water for cattle from streams, extensive production system, largest herd size, smaller diversity of breeds, a higher number of breeding and milking females, more stallions and the lowest abortion percentage.

Zootechnical management practices (degree) are similar among producers in the different clusters.

Economic characterization of the production units based on technological indexes. Table 5 shows the variables of the economic indicator. The producers in cluster 1 have the highest percentage of complementary activities, the highest milk production, cheese, castrated animals, and the higher income from the sale of cheese and capons. Producers in cluster 2 have a higher dependence on the goat activity and a lower animals disposal rate. Cluster 3 groups producers with the lowest milk production, the lowest income from cheese sales, and with no castrated animals or got kids production from sales. Producers in cluster 4 are exclusively dedicated to complementary livestock activities, have the highest number of castrated animals and goat kids, so the highest income is obtained from these activities.

Table 4. Variables of the technological indicator. INIFAP, 2021.

Variable	Conglomerate 1	Conglomerate 2	Conglomerate 3	Conglomerate 4
Production units	3	20	3	3
Distance from production unit (km)				
Municipal seat	168	159	162	192
Community	23	29	199	39
Road conditions (%)				
Well	0	5	0	0
Regular	33	35	33	0
Bad boy	67	60	67	100
Surface of the UP (ha)	1 300	448	1 292	53.5
Area available for sowing (ha)	1.5	0.24	0.66	0
Water sources for livestock (%)				
Brook	100	65	0	100
Dams	0	10	67	0
Spring	0	5	33	0
Well	0	10	0	0
Jars	0	10	0	0
Production system				
Semi - stabled	0	25	0	0
Extensive	100	75	100	100
Herd size (heads)	255	113	145	453
Diversity of breeds (number)	5	7	5	4
Breeding females (heads)	133	56	66	240
Females in milking (heads)	73	32	46	177
Stallions (heads)	3.3	2.5	3.3	6
Birth rate (%)	100	80	67	67
Abortions (%)	20	19	28	10
Availability of facilities (grade)	0.38	0.24	0.11	0.16
Availability of machinery and equipment (grade)	0.19	0.12	0.24	0.14
Records management (grade)	2	1.45	1.67	1.33
Zootechnical management (degree)	3.3	2.95	3.4	3

Nubian and Saanen breeds are the most used in the PUs. Only producers in clusters 1 and 2 use Creole goats. The Boer breed is utilized by producers in clusters 2 and 4

Comondú goat farmers milk an average of four of the six milk production months, calves are nursed for two months, during this time some goat farmers also alternate with minimal milking. In this sense, the estimated milk production for the group of producers in cluster 1 was 164 liters per goat per year or 1.3 liters per goat per day. For this variable, the estimated milk production would be 1.14, 0.26, and 0.43 liters per goat per day for

Table 5. Variables of the economic indicators. INIFAP, 2021.

Variable	Conglomerate 1	Conglomerate 2	Conglomerate 3	Conglomerate 4
Production units	3	20	3	3
Complementary activities				
Agricultural	100	25	33	0
Livestock	100	95	100	100
Admission to the UP for goat farming (%)				
<50	33	10	0	0
50 - 99	0	5	33	33
100	67	85	67	67
Milk production (L)	12 000	4 360	1 433	9 040
Cheese production (kg)	1 520	734.8	400	1 383
Sale value of cheese ($)	77 600	37 399.7	20 000	73 700
Waste animals (heads)	20	6	10	33
Sale value of waste animals ($)	23 200	6 450	11 200	37 333
Capons (heads)	17	5	0	5
Sale value of capons ($)	19 600	5005	0	5000
Production of goats (heads)	13	7	0	50
Sale value of goats ($)	6 667	3 280	0	25 000

producers in clusters 2, 3, and 4, respectively. Cluster 1 producers have the highest birth percentage, facilities availability, land availability for sowing, and a higher discard of unproductive or cull animals (8%), have higher milk production, and consequently the highest cheese production. The reduced distance from the community could also be an advantage to commercialize their products compared to the rest of the PUs. The cluster 2 PUs have similar characteristics to those of cluster 1 PUs, their main difference lies in the number of producers that practice the semi-stabled production system, the number of animals they own, the ranch surface, and that the higher income of the PU depends on the goat's activity and have better road conditions. Both clusters of the producers (1 and 2), in contrast to the other two, employ Creole animals, which in the local climatic and topographic conditions have a longer adaptation period than the rest of the introduced breeds of the herds in the state, this adaptive advantage, with the crossbreeding of other breeds, can also be the result of higher milk production.

Based on the technological and economic indicators, the producers grouped in cluster 3 are the ones with the greatest productive lag. Probably because of their lower educational degree and lower facilities availability, although they have the highest equipment and machinery availability, as well as water from dams and springs and planting area. This is not reflected, probably due to the lack of technical support in the efficient usage of these resources. Another important factor that may affect this situation is the distance they have to travel to their community for education or products commercialization. Therefore, it is assumed that the production of goats and goat kids is for self-consumption.

Table 6. Distribution (%) of the main breeds used by production unit. INIFAP, 2021.

Variable	Conglomerate 1	Conglomerate 2	Conglomerate 3	Conglomerate 4
Production units	3	20	3	3
Alpine	33	20	67	33
Saanen	67	25	100	33
Toggenburg	0	10	67	0
Nubia	100	100	67	100
Boer	0	25	0	33
Murcia	33	15	33	0
Creole	33	40	0	0

Producers in cluster 4, who have the highest annual income, have a low milk yield per goat per day (0.43 L). Therefore, the income reflects the higher number of animals and not of their efficiency. Additionally, using Boer breed animals is efficient for meat production, but inefficient to produce milk. If animals born of these breeds are left as breeding stock, there is a risk that they develop accessory mammary glands, resulting in a greater efficiency loss in the milk production process.

As for the variables of the indexes of technological development in the PUs, in all the assessed groups, low adoption of technologies was observed, ranging from 11 to 38% for the facilities available, from 12 to 24% in the machinery and equipment availability, management of records degree ranging from 1.33 to 2, based on a 3 grading, and in the implementation of zootechnical practices from 2.9 to 3.4 based on a maximum implementation grade out of 7. This last one is the most important in the productive efficiency of the PUs.

Although this overview of the characteristics of the producers regard their technological levels, allow to identify different groups of goat farmers and the destination they give to their primary products, and offers opportunity areas for them to be educated in agribusiness or provide added value to the milk. So that, in addition to the milk production period, they offer feeding schemes that allow to increase slightly more period or use specialized breeds to increase production or sale calves to demanding markets (Cadena *et al.*, 2019).

The grouping considers the information and offers the possibility of prioritizing the research of the PUs according to their limitations, potentialities, and opportunities, improving the decision-making process (Falconi and Burbano, 2004; Munda, 2004) for planning and developing the public policy on the livestock of the region or state.

CONCLUSIONS

The technological indexes, herd size, and distance from the PUs to the municipality and the community allowed to group and understand the assessed producers, based on their social characteristics, technological and economic indicators. This information will make it possible to precisely define the kind of intervention required, in terms of the management practices.

REFERENCES

Axinn, G.H. (1993). Guía de los Distintos Enfoques de la Extensión. Organización de las Naciones Unidas para la Agricultura y la Alimentación. Roma, Italia. p 51

Cadena-Iñiguez, P. (2004). Actores, estrategias y dinámicas de organización en el agro de La Frailesca, Chiapas. Tesis Doctorado en Ciencias. Programa de Estudios del Desarrollo Rural. Colegio de Postgraduados. Montecillo, Estado de México. 154 p.

Cadena-Iñiguez, P., Guevara-Hernández, F., Argüello-Aguilar, R.A., Rendón-Medel, R. (2018). Proceso de comunicación, extensionismo y adopción de tecnologías. *Revista Mexicana de Ciencias Agrícolas*. 9 (4). 851-862.

Cadena-Iñiguez, P., Rodríguez-Hernández, R., Morales-Guerra, M., Berdugo-Rejón, J.G., Salinas-Cruz, E., Camas-Gómez, R., Guevara-Hernández, F., Pinto-Ruíz, R. (2019). Model of Management for Innovation in Small Farmers of Latin America; A Successful Proposal. *International Journal of Agricultural Sciences and Natural Resources*. 2019. 6(1). 1-7. 2375-3773

Eponou, T. (1993). Partners in Agricultural Technology: Linking Research and Technology Transfers to serve farmers. ISNAR. Research Report Nº 1. The Hague: International Service for National Agricultural Research. The Netherlands. 85 p.

Falconi, F., Burbano, R. (2004). Economic tools for environmental management: mono-criteria vs. multicriteria decisions. REVIBEC. 1. 11-20.

Kaimowitz, D., Snyder, M., Engel, P. (1990). A conceptual Framework for studying the links between Agricultural Research and Technology Transfer in developing countries. *In*: Kaimowitz, D. (Ed), making the link: Agricultural Research and Technology Transfer in developing countries. Boulder, Colorado; Westview Press.

Lionberger, H.F. (1986). Toward and Idealized System Model for Generating and Utilizating Information in modernizing Societies. *In*: Beal, G., Dissanayake, W, & Konoshima, S. (Eds). Knowledge generation, exchange and utilization. Boulder, Colorado; Westview Press.

Manzo, R.F. (1994). The Role of education and Technological Change in Rural Development. Ph. D. Thesis. North Caroline State University. USA.

Munda, G. (2004). Multicriteria methods and multicriteria processes for social evaluation of public policy. REVIBEC. 1. 31-45.

Nahed, T.J., Palma, G.J.M., Aguilar, J.J.R., Grande, C.D., Valdivieso, P.I.A., Juárez, M.H.A., Trujillo, V.R., Sánchez, M.B., Ruíz, R.J.L., León, M.N.S., Parra, V.M.R. (2021). Índice de desarrollo tecnológico para la clasificación y análisis multicriterio de unidades de producción: Aplicación en la ganadería bovina de doble propósito convencional versus orgánica. *AIA*. 25(2). 97-118.

Rogers, M.E. (1983). Diffusion of innovations. Third Edition. Nueva York: The Free Press. 134-149.

Rojas-Soriano, R. (1979). Guía para realizar investigaciones sociales. Facultad de Ciencias Políticas y Sociales. Universidad Autónoma de México. México, D.F. 271 p.

Röling, N. (1988). Extension Science; Information systems in agricultural development. Cambridge University Press. Reprinted 1990. Great Britain. 233 p.

Röling, N. 1990. The Agricultural Research-Technology Transfer Interface: A Knowledge System Perspective: Kaimowitz, D. 1990. Making the Link: Agriculture Research and Technology Transfer in Developing Countries. ISNAR. West View Press. USA p 1-42

SAS. 2014. SAS- Statistical Analisis Softwer for Windows ver. 9.3. Cary, NC: SAS Institute Inc.

Uzcanga-Pérez, N; Cano-González, A.; Cadena-Iñiguez, P. 2021. Strategy to strengthen the traditional milpa family production systems. ISSN 2594-0252 *Agroproductividad*: Vol. 14, Núm. 4, abril. 2021. Pp: 119-125.