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Agradecimientos: Son opcionales y tendrán un máximo de tres renglones para expresar agradecimientos a personas e instituciones que hayan contribuido a la realización del trabajo.
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Response of improved common bean (*Phaseolus vulgaris* L.) varieties to intermittent drought

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ABSTRACT

Objective: To evaluate the response of 16 improved bean varieties to intermittent drought.

Design/Methodology/Approach: A randomized complete block design was used, with four replications each under irrigated and drought conditions. The drought treatment consisted of suspending irrigation for 15 days in the flowering stage. The irrigation treatment consisted of maintaining available moisture above 60% throughout the cycle. The yield, its components, and days to physiological maturity were recorded. The drought tolerance of each variety was estimated using the drought susceptibility index, geometric mean, and productive mean.

Results: Drought reduced yield by 36%, the number of pods per plant by 28.5%, and days to physiological maturity by 0.7%. In contrast, the weight of 100 seeds increased by 4.9% and the number of seeds per pod was not affected. The Flor de Mayo Eugenia and Negro 8025 varieties were more tolerant to drought ($p \leq 0.05$) than the rest of the varieties analyzed. These varieties recorded yields of 2,768 and 2,854 kg ha^{-1} (irrigation) and 1,905 and 1,843 kg ha^{-1} (drought), respectively.

Study Limitations/Implications: The drought intensity applied was relatively low, which could reduce the visibility of the differences between treatments.

Findings/conclusions: The secondary attribute with more sensitivity to intermittent drought was the number of pods per plant. The varieties with highest tolerance to droughts were Flor de Mayo Eugenia and Negro 8025.

Keywords: Drought tolerance, water stress, yield components.

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) (Fabaceae) holds the second place in sown area in Mexico, 88% of which is grown under rainfed conditions (SIAP, 2021). In the north and center of the country, intermittent drought is the abiotic factor that most limits the production of this legume in the main producing regions (Acosta *et al.*, 2021). Yield losses between 12 and 92% have been recorded, depending on the duration and intensity of the drought, as well as the phenological stage and the genotype of the bean (Muñoz-Perea *et al.*, 2006). For example, Darkwa *et al.* (2016) found that some bean genotypes are not affected by a drought intensity of 30%, while others reduced seed yield a >60%. The phenological stage more sensitive to drought is flowering, followed by formation and filling pods (Beebe *et al.*, 2013).

Image by Jan Nijman at Pixabay

Developing drought-adapted bean varieties is essential to achieve a better understanding of the relationship between drought and yield, based on drought tolerance or susceptibility indices, yield components, phenology, and other attributes (Beebe *et al.*, 2013; Chaves-Barrantes *et al.*, 2018). The drought susceptibility index indicates yield stability and therefore is one of the soundest methods used to select drought resistant bean genotypes (Sánchez-Reinoso *et al.*, 2020); however, its capacity to detect potential high-yielding genotypes (which is also an important characteristic for selection) is known to be imprecise (Papathanasiou *et al.*, 2022). This deficiency is resolved through the use of complementary productivity indices, such as the geometric mean of yield and the productive mean (Rosales-Serna *et al.*, 2000). Chaves-Barrantes *et al.* (2018) pointed out that, due to the randomness of intermittent droughts throughout the cycle, the selected genotypes nevertheless show high performance under optimal and stress conditions. The analysis of the yield components (pods per plant, seeds per pod, and individual seed weight) helps to determine, in a given situation, which of them puts more limits on the seed yield and to choose the appropriate corrective measures (Kohashi, 1996). They are also useful as secondary criteria in the selection of varieties based on drought tolerance (Rai *et al.*, 2020). Phenological plasticity —which is the modification of the duration of the phenological stages, as a consequence of moisture availability— is a valuable drought tolerance mechanism in places with intermittent droughts (Beebe *et al.*, 2013), while greater precocity is essential in places where terminal drought prevails (Tosquy-Valle *et al.*, 2014).

In northern and central Mexico, few studies have been carried out on intermittent drought and its impact on bean seed yield, and they have mainly considered Flor de Mayo genotypes (Barrios *et al.*, 2011; Romero-Félix *et al.*, 2018; Romero-Félix *et al.*, 2019). Therefore, studies of genotypes with other seed types and market demand are required. The objective of this research was to evaluate the response of 16 improved varieties of common bean, with different seed coat colors, to intermittent drought in the flowering stage.

MATERIALS AND METHODS

Experiment location

The experiment was established in the San Agustín del Pulque ejido, in Cuitzeo, Michoacán (19° 57' 41" N, 101° 06' 4" W, 1,872 m.a.s.l.), with an average annual precipitation of 670 mm and an average annual temperature of 17.9 °C.

Evaluated varieties

Sixteen varieties were evaluated. Twelve were generated by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias: Alteño 2000, Azufrasin, Flor de Junio (FJ) Marcela, Flor de Mayo (FM) Anita, FM Dolores, FM Eugenia, FM M38, Negro (N) 8025, N Comapa, N Guanajuato, N Tacaná, and Pinto Saltillo. Three experimental varieties were obtained at the Universidad de Guadalajara (FM-98004, FM-98041 and MX-9065-4M). Finally, a local commercial variety known as Japonés was also used.

Experimental design and recorded variables

A randomized complete block experimental design was used, with four replications each under irrigation and drought conditions. The irrigation treatment consisted of maintaining available moisture above 60% throughout the cycle, while the drought treatment consisted of suspending irrigation for 15 days in the flowering stage. The experimental unit were four row 5.0 m in length and 0.8 m row width, and the two central rows 4 m long furrows as a useful plot. The following variables were recorded: days to physiological maturity, pods per plant, seeds per pod, weight of 100 seeds, and seed yield. In addition, drought tolerance was estimated based on the drought susceptibility index (DSI) (Fischer and Maurer, 1978), geometric mean (GM) (Samper and Adams, 1985), and productive mean (PM) (Rosielle and Hamblin, 1981).

The following equation was used to calculate the DSI (ISS):

$$ISS_i = \frac{1 - \frac{RS_i}{RR_i}}{1 - \frac{RS}{RR}}$$

Where ISS_i = drought susceptibility index of the *i*-th genotype; RS_i = average drought yield of the *i*-th material; RR_i = average irrigation yield of the *i*-th genotype; RS = general average yield in drought; and RR = general average irrigation performance.

The GM of yield was obtained as follows:

$$MG_i = \left(RS_i * RR_i\right)^{0.5}$$

Where MG_i = geometric mean of the *i*-th genotype; RS_i = average drought yield of the *i*-th material; RR_i = average irrigation yield of the *i*-th genotype.

The PM of a genotype is the arithmetic mean of its yield, based on its values under irrigation and drought. A variety is drought tolerant if its DSI value is less than the unit and it is relatively more tolerant to stress, to the extent that it has a higher GM and PM value (Rosales-Serna *et al.*, 2000).

Statistical analysis

The PM and GM were subjected to an analysis of variance and Tukey's mean comparison (MSD, $p \leq 0.05$), based on seed yield, days to physiological maturity, pods per plant, seeds per pod, and weight of 100 seeds, both under irrigation and drought conditions, with the SAS 9.2 statistical package.

Experimental management

The beans were sown on March 8, 2012, at a density of 180,000 plants per hectare. Two to three irrigations per week were applied with drip tape. Weeds were controlled with a hoe

25 days after sowing by hand 21 days later. A 18-46-00 fertilization dose was used, applying diammonium phosphate at the moment of sowing. In the middle of the cycle, whiteflies (*Bemisia tabaci*) were controlled with 375 g/L of Herald (Fenpropathrin).

RESULTS AND DISCUSSION

The analyses of variance reported significant differences ($p \leq 0.05$) in GM and PM between varieties. Significant differences were also reported between genotypes, in all the variables evaluated under irrigation, and in days to physiological maturity, seeds per pod and weight of 100 seeds, under the drought treatment, but not in pods per plant.

Drought tolerance

The drought intensity index was 0.36, equivalent to a moderate drought, according to Beebe *et al.* (2013), who recommend 0.6-0.8 indices. However, enough stress was applied to reach permanent wilting in all varieties.

Based on the DSI, the drought-tolerant varieties were Japonés, FM M38, Pinto Saltillo, FM Eugenia, FM Anita, N 8025, FM-98041, and N Guanajuato, with values between 0.44 and 0.98 (Table 1).

Table 1. Drought tolerance, pods per plant, and days to physiological maturity of 16 improved bean varieties.

Genotype	DSI	MG	MP	RS (%)	VPP			DMF		
		(kg ha^{-1})			R	S	RS (%)	R	S	RS (%)
FJ Marcela	1.18	2329 *	2428 *	43	15.3 *	7.3	52.3	90 *	89 *	1.1
N 8025	0.95	2287 *	2348 *	35	16.2 *	11.2	30.9	89 *	86	3.4
FM Eugenia	0.85	2292 *	2337 *	31	14.8 *	7.8	47.3	91 *	89 *	2.2
FM Dolores	1.23	2124 *	2234 *	45	13.0 *	9.7	28.7	90 *	89 *	1.1
FM Anita	0.94	2098 *	2156 *	35	13. 6 *	10.2	21.5	90 *	89 *	1.1
FM-98004	1.00	2073 *	2139 *	37	11.4 *	7.9	30.7	88 *	89 *	−1.1
FM-98041	0.96	2065 *	2118 *	35	14.9 *	10.0	32.9	90 *	89 *	1.1
Alteño 2000	1.09	2016 *	2090 *	40	14.5 *	10.8	25.5	90 *	90 *	0.0
N Guanajuato	0.98	1988 *	2046 *	36	16.7 *	10.3	38.3	89 *	87 *	2.2
Pinto Saltillo	0.82	1973 *	2010 *	30	13.9 *	9.9	28.8	79	78	1.3
N Comapa	1.03	1886 *	1944 *	38	15.4 *	11.8	23.4	88 *	86	2.3
FM M38	0.57	1927 *	1942 *	21	11.8 *	9.6	18.6	90 *	91 *	−1.1
N Tacaná	1.17	1746	1820	43	13.1 *	11.8	9.9	90 *	87 *	3.3
MX-9065-4M	1.40	1688	1805	51	13.7 *	10.4	24.1	88 *	90 *	−2.3
Japonés	0.44	1764	1772	16	7.5	7.9	−5.3	78	80	−2.6
Azufrasin	1.00	1713	1758	36	13.2 *	9.9	25.0	89 *	90 *	−1.1
Mean	0.98	1998	2059	36	13.7 a[§]	9.8 b	28.5	88 a	87 b	0.7
coefficient of variation (%)	__	9.9	12.8	__	20.7	28.9	__	1.5	1.6	__
MSD (0.05)	__	511.7	513.7	__	7.3	ns	__	3.4	3.5	__

* Statistically superior genotypes, according to the MSD; DSI=drought susceptibility index; GM=geometric mean; MP=productive mean; VPP=pods per plant; DPM=days to physiological maturity; R=irrigation; S=drought; RS%=percentage reduction due to drought stress; [§] statistically different pairs of means with different letter; FM=Flor de Mayo; FJ=Flor de Junio; N=Negro.

The genotypes that, due to their GM, are tolerant to drought were FJ Marcela, FM Eugenia, N 8025, FM Dolores, FM Anita, FM-98004, FM-98041, Alteño 2000, N Guanajuato, Pinto Saltillo FM M38, and N Comapa, with values between 1,886 and 2,329 kg ha^{-1}. The varieties with the highest PM were the same as those with the highest GM, with FJ Marcela being the most productive with 2,428 kg ha^{-1}, followed by N 8025, FM Eugenia, FM Dolores, and FM Anita, with averages of 2,348, 2,337, 2,234, and 2,156 kg ha^{-1}, respectively. Combining the three drought tolerance criteria, FM Eugenia, N 8025, FM Anita, FM-98041, N Guanajuato, Pinto Saltillo, and FM M38 were considered to be drought tolerant. Other authors (Barrios *et al.*, 2011; Romero-Félix *et al.*, 2019; Acosta-Gallegos *et al.*, 2020) have also documented that FM Eugenia, Pinto Saltillo, N 8025, N Guanajuato, and FM M38 have adapted to environments with water stress in the center and north of the country. In contrast with the findings of this study, Tosquy-Valle *et al.* (2014) report that N Tacaná is tolerant to water stress in the tropics. This discrepancy can be attributed to the relativity of the DSI of a given variety, which is influenced by the level of tolerance of the genotypes with which it is being compared (Fischer and Maurer, 1978). Except for N Guanajuato, the seven selected varieties were highly productive during drought (Figure 1), an essential attribute of water stress-tolerant varieties (Beebe *et al.*, 2013). However, in places with intermittent droughts, they should also show high yield under irrigation conditions (Chaves-Barrantes *et al.*, 2018), like FM Eugenia and N 8025, which recorded values of 2,768 and 2,854 kg ha^{-1} (irrigation) and 1,905 and 1,843 kg ha^{-1} (drought) (Figure 1). On the contrary, Azufrasin, N Tacaná, and Mx-9065-4M were the least productive varieties. In conclusion, FM Eugenia and N 8025 could potentially be grown in areas exposed to intermittent drought.

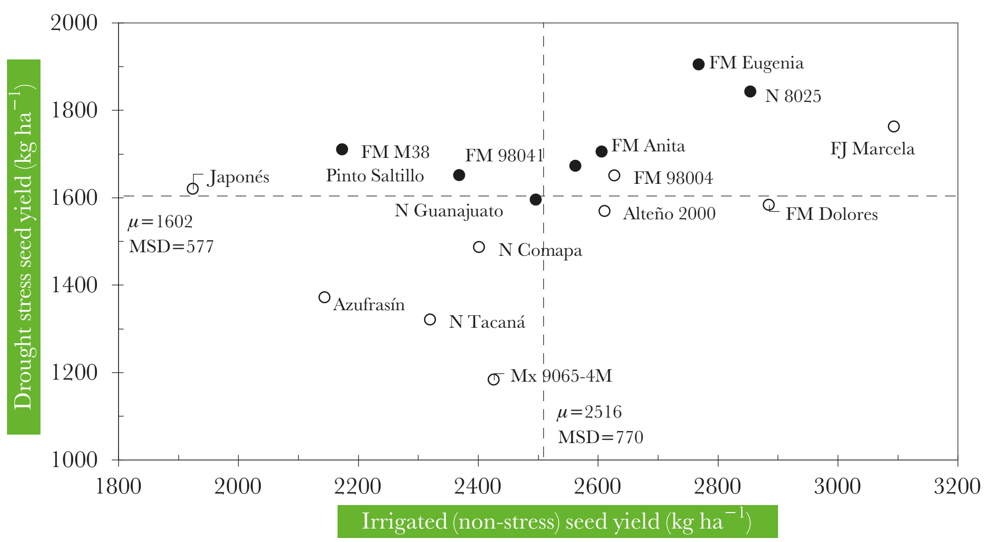

Figure 1. Seed yield of 16 improved bean varieties evaluated under irrigation and drought stress. Varieties with a full circle are drought tolerant, according to their drought susceptibility indices, geometric mean, and productive mean.

Yield components and phenology

Table 1 shows that the yield component most affected by drought was pods per plant (average reduction: 28.5%), followed by weight of 100 seeds (increase: 4.9%), and seeds per pod (unaffected by stress). The mean number of pods per plant was 13.7 and 9.8, under irrigation and drought conditions, respectively; there was a slight variability between varieties in the two moisture conditions. The reduced variability may be associated with the low drought intensity applied and with the low genetic variability of the materials evaluated (Tosquy-Valle *et al.*, 2014). Several authors have reported that pods per plant is the most drought-sensitive component, followed by weight of 100 seeds, and seeds per pod, especially when drought occurs in the reproductive phase (Tosquy-Valle *et al.*, 2014; Darkwa *et al.*, 2016; Romero-Félix *et al.*, 2018; Mazengo and Tryphone, 2019). The variable that was most associated with seed yield ($r=0.60$, $p\leq 0.01$) was pods per plant; therefore, it can be considered a reliable indirect criterion in the selection of species with tolerance to intermittent drought (Rai *et al.*, 2020). The weight of 100 seeds is highly affected by terminal drought (Mazengo and Tryphone, 2019); meanwhile intermittent drought during the flowering stage may not affect and, just as in the case of this research, it may even increase weight per 100 seeds (Nielsen and Nelson, 1998). This phenomenon was a response to the compensation of flower and pod abscission, caused by the reestablishment of the favorable moisture condition during the pod filling stage.

Days to physiological maturity were reduced by 0.7% (one day) due to stress, from a mean of 87 days in drought to 88 days in irrigation, with significant differences ($p\leq 0.05$) between varieties (Table 1). Pinto Saltillo was the more precocious variety, with 79 days in irrigation and 78 in drought, and FM M38 matured the latest, with 90 days in irrigation and 91 in drought. The rest of the varieties, with the exception of Japonés, took between 87 and 90 days to mature. The slight impact of intermittent drought on bean phenology in flowering was reported by Mazengo and Tryphone (2019), who concluded that, when the drought is not severe, genotypes can recover from stress (Beebe *et al.*, 2013).

CONCLUSIONS

Pods per plant was the secondary variable with the highest sensitivity to water stress and the one most associated with seed yield; consequently, it could be used as a reliable auxiliary attribute in the selection of bean genotypes with tolerance to intermittent drought.

Based on the drought susceptibility index, geometric mean, and productive mean, FM Eugenia, N 8025, FM Anita, FM-98041, N Guanajuato, Pinto Saltillo, and FM M38 were drought tolerant varieties. FM Eugenia and N 8025 also showed a remarkably high performance, under both irrigation and drought conditions, which makes them the most viable alternatives for places prone to intermittent droughts.

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Colegio de Postgraduados

Determination of geometric properties of cocoa beans (*Theobroma cacao* L.)

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ABSTRACT

Objective: To determine the linear physical dimensions of dried Mexican cocoa beans to estimate their geometric properties and differentiate them through a principal component analysis.

Design/Methodology/Approach: For the research, 51 dry samples of cocoa beans (*Theobroma cacao* L.) were collected from three producer states in Mexico. The physical properties of cocoa beans were determined based on their linear dimensions: length, width, thickness, geometric diameter, sphericity, volume, and shape factor. Moisture, average weight, and ether extract were also determined.

Results: The results revealed the relation between linear and geometric properties, particularly the fact that bean weight is significant ($p<0.001$) regarding all the properties evaluated, except moisture. Said relation explained 98.8% of the total variation in the first two components observed in the cocoa samples from the three states (Tabasco, Chiapas, and Oaxaca). Average bean weight, sphericity, and volume contributed the most to the total variation.

Findings/Conclusions: The only quantitative variable that showed significance was bean weight. The other measurements —length, width, and thickness— did not. However, there was significance when coupling the measurements in the expressions of surface area and volume.

Keywords: Geometric properties, Cocoa beans, Main components.

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INTRODUCTION

Cocoa beans are the prime raw material in the chocolate and confectionery industry. The preparation of cocoa beans depends on local customs and industrial processing. Based on the postharvest treatment, beans harvested in Mexico can be classified as washed-dried or fermented-dried (NMX-FF-118-SCFI-2014). Fermentation is the essential process to produce the particular sensory characteristics of cocoa. However, even today, the many factors involved in said process rely on traditional practices lacking controlled procedures (Saltini *et al.*, 2013; Alvarez-Villagomez *et al.*, 2022). Drying is the final stage of preparation,

its main objective being to reduce moisture to a microbiological and physicochemical safety value and to cut down the acidity generated during fermentation (Afoakwa, 2014; Saltini *et al.*, 2013; Arana-Sánchez *et al.*, 2015). Both fermentation and drying influence the original chemical composition through microbial, enzymatic, and chemical activity, also reflected in structural and physical changes in cocoa beans. According to García-Alamilla *et al.* (2012), in the case of cocoa, physical properties have great relevance, with the main characteristics that reflect postharvest phenomena being the length, width, thickness, and average weight of beans. In addition, these features are also indices of commercial importance and industrial processing.

Various studies have evaluated the size and shape of cocoa beans based on basic measurements (Bart-Plange and Baryeh, 2003; Aosiro *et al.*, 2020). Such data contribute to selecting and improving cultivars, managing the supply chain, and designing equipment. It also assists in the electrostatic separation of contaminants (Manfreda and Acosta, 2014; Aosiro *et al.*, 2020) and provides relevant variables for the bean drying unit operation. Although bean size has been a meaningful indicator for other crops, in the case of cocoa beans there is no adequate study defining the role of size. We therefore need more studies to evaluate the size-embryo ratio, just as Manfreda and Acosta (2014) reported for cereals. Bean size and its relation to surface area have an impact on fermentation due to the diffusion and hydrolysis processes that occur between the mucilage and the cotyledon. Moreover, roasting is the first step of industrial transformation after the postharvest stages, so that if bean size presents high variability and there is no adequate classification, there may be cocoa batches with under-roasted or over-roasted beans. Therefore, the objective was to determine the linear physical characteristics of dried Mexican cocoa beans to estimate their geometric properties and differentiate them through a principal component analysis.

MATERIALS AND METHODS

Study site selection and sampling

The study sites selected were representative of the states of Tabasco and Chiapas as the main cocoa producers in Mexico (SIAP, 2022). The state of Oaxaca was also included. The samples were collected from December 2021 to March 2022. Cocoa producers contributed samples of both washed-dried and fermented-dried beans. A total of 51 samples were collected: 36 from Tabasco (16 from Comalcalco, six from Paraíso, six from Huimanguillo, four from Cunduacán, two from Cárdenas, one from Jalapa, and one from Teapa); 12 from Chiapas (five from Pichucalco, two from Ostuacán, two from Tecpatán, one from Salto de Agua, one from Huixtla, and one from Ignacio Allende); and three from Oaxaca (two from Toltepec and one from Puerto Escondido). Each sample weighed approximately one kilogram.

Assessed variables

Moisture

An average of 10 cocoa beans were taken and placed in the Aqua Boy KPM202 moisture content meter (Germany) with direct reading.

Ether (fatty) extract

The ether extract was determined using the methodology according to the NMX-F-615-NORMEX-2018 Food –determination of ether extract (soxhlet method) standard.

Average bean weight

The average bean weight was determined based on the random quantification of 100 beans' weight, using a semi-analytical balance (Ohaus, Scout Pro SP202, China) with an accuracy of 0.01 g. To obtain the average weight, the recorded weight was divided by 100 (Afoakwa *et al.*, 2014).

Linear and geometric bean dimensions

The sample (60 grains) was taken at random to determine the length (L), width (W), and thickness (T) of each bean. The length was measured in millimeters using a digital vernier caliper (KNOVA, China) as per the description provided by Kaushik *et al.* (2007). Width and thickness were measured perpendicularly to the long axis (Bart-Plange *et al.*, 2003; Oyedokun *et al.*, 2011; García-Alamilla *et al.*, 2012; Jaiyeoba *et al.*, 2016; Sandoval *et al.*, 2019). The sphericity degree was calculated using equations (2) and (3) as described by Bart-Plange *et al.* (2003), Jaiyeoba *et al.* (2016), Kwino *et al.* (2017), and Sandoval *et al.* (2019).

$$\Phi = \left(\frac{WTL}{L}\right)^{1/3} \qquad (1)$$

$$\Phi = \left[\frac{B(2L-B)}{L}\right]^{1/3} \qquad B = (WT)^{0.5} \qquad (2)$$

Geometric diameter

The geometric mean diameter was calculated using the expression cited by Bart-Plange *et al.* (2003), García-Alamilla *et al.* (2012), Jaiyeoba *et al.* (2016), and Sandoval *et al.* (2019).

$$D_g = (WLT)^{1/3} \qquad (3)$$

Where: D_g is the geometric mean diameter (mm).

It becomes evident that the sphericity from equation (1) is a function of D_g and therefore:

$$\Phi = \frac{D_g}{L} \qquad (4)$$

Surface area

The surface area was calculated using the mathematical expression given by Bart-Plange *et al.* (2003), García-Alamilla *et al.* (2012), Jaiyeoba *et al.* (2016), and Sandoval *et al.* (2019).

$$S = \frac{\pi B L^2}{2L - B} \tag{5}$$

Where: S is the surface area.

The surface area was estimated based on the D_g and using the expression posited by McCabe, Smith, and Harriott (Bart-Plange *et al.*, 2003):

$$S = \left(\pi D_g\right)^2 \tag{6}$$

Volume

The volume was calculated using the mathematical expression given by García-Alamilla *et al.* (2012):

$$V = \frac{\pi B^2 L^2}{6(2L - B)} \tag{7}$$

Shape factor

Cocoa bean is considered an ellipsoid of revolution, and its shape factor is estimated as follows:

$$Fm = \frac{1}{2}\left(\frac{L}{D_g}\right)^{1/3}\left(\frac{D_g}{L} + \frac{L}{e} Arcsen(e)\right) \tag{8}$$

Considering the shape factor, the surface area is estimated as follows:

$$S = \frac{3(1-\varepsilon)}{D_g}\left(\frac{D_g}{L} + \frac{1}{e} Arcsen(e)\right) \tag{9}$$

$$e = \sqrt{\frac{L^2 - D_g^2}{L^2}} \tag{10}$$

And the estimated volume as follows:

$$V = \frac{\pi D_g^2 L}{6} \tag{11}$$

Data processing and statistical analysis

Descriptive statistics were conducted for all evaluated variables and a principal component analysis (PCA) was implemented. The PCA was performed using standard deviations, the Pareto scaling method, and a Pearson correlation analysis with MetaboAnalyst 5.0.

RESULTS AND DISCUSSION

The size and the number of beans per 100 g are two relevant indices for measuring the physical quality of cocoa beans (Kongor *et al.*, 2016). Another significant factor is bean moisture, which should be 7%, since a higher percentage leads to deterioration due to the presence of microorganisms (fungi), and less than 6% turns the beans brittle (Andrade-Almeida *et al.*, 2019). Table 1 shows the average values of all samples, of both washed-dried and fermented-dried cocoa beans. The general evaluation of physical measurements in all samples shows the variability between commercialized beans. These variations are associated with different factors, such as genetics, edaphoclimatic conditions, and postharvest treatment. As we already mentioned, the moisture variable is critical. Regarding the samples in this study, moisture reached 5.24% (Table 1) with a deviation slightly higher than 24%, which is above the permissible limits, making the beans susceptible to microbial contamination.

The visual appearance of cocoa beans is meaningful because it determines handling in the postharvest treatment (fermentation and drying), which will ultimately impact flavor, an essential quality in cocoa beans (Sánchez *et al.*, 2017). For example, authors such as Álvarez *et al.* (2007) argue that the average weight of fermented and dried cocoa beans is linked to their shell content (*testa*). Moreover, weight affects the fat yield. The results showed a variability of approximately 20% (Table 1) in the average weight, which has different causes. Among these are moisture (which is not homogeneous between the samples), genetics, and postharvest handling. Some samples were washed-dried, and others fermented-dried. When the fermentation process does not adequately degrade the mucilage, the weight of the beans is greater than that of a washed sample, which is reflected in the percentage of shell content (*testa*). We must consider that cell walls in fermented beans are destroyed, which exposes beans to chemical factors that affect their properties, including organoleptic ones (Vera *et al.*, 2014). The fat content was 49% on average, with moderate variation.

Various authors have evaluated the linear dimensions of cocoa beans (Bart-Plange and Baryeh, 2003; Álvarez *et al.*, 2007; Oyedokun *et al.*, 2011; García-Alamilla *et al.*, 2012; Andrade-Almeida *et al.*, 2019) using native or improved commercial varieties and different approaches in their research. Bart-Plange and Baryeh (2003) and Andrade-Almeida (2019)

Table 1. Physical properties of Mexican cocoa beans.

Physical characteristics of the grain	Means	Max	Min	±SD	CV (%)
Moisture (%)	5.24	9.1	2.4	1.30	24.93
Average weight 100 beans (g)	112.96	202.3	71.3	22.30	19.74
Butter (%)	49.66	75.13	27.34	6.70	13.48
Length (mm)	21.54	25.56	17.19	101.66	8.72
Width (mm)	12.65	21.27	8.72	1.22	9.64
Thickness (mm)	7.34	9.46	5.99	0.73	9.93
LB/TB	2.94	3.69	2.17	0.27	9.44

Source: compiled by authors.

worked with African and South American cocoa beans (ICS, CCN51) and obtained an average weight higher than the one registered in the present research. This research has no specific genetic variety, but rather commercial beans presenting native materials and unidentified crossbreeds. However, the evaluated samples included washed and dried cocoa beans, unlike those reported by the abovementioned authors, who used only fermented beans. As stated above, moisture is a critical value for the linear dimensions and the average weight. Vera *et al.* (2014) studied fermented samples from 15 clones in Ecuador and reported an average weight of 136 g for 100 beans, with a moisture of 6.49%. In the present study, the average moisture was 5.1%. Likewise, Álvarez *et al.* (2007) reported a moisture between 4.26% and 6.37% for the genotypes they evaluated and 5.17% for a commercial sample. Additionally, these same authors reported a fat content between 54.61% and 56.07% for the different genotypes and 56.01% for the commercial sample, higher values than those reported in the present study.

Running the linear dimensions of the cocoa beans through equations 1 to 11 allowed us to estimate the geometric diameter, sphericity, surface area, volume, and shape factor (ellipsoid of revolution). The results are presented in Table 2. The work conducted by Oyedokun *et al.* (2011) maintains that linear dimensions are linked to the cocoa variety and that morphology indicates quality, yield, and pest resistance or susceptibility while in storage. The shape, surface area, volume, and size of cocoa beans depend specifically on the amount of stored water —the more water contained, the greater the dimensions (García-Alamilla *et al.*, 2012). To determine sphericity, one must assess to what extent the object under observation resembles a sphere, which impacts its tendency to roll —something particularly relevant in agricultural products. This property is helpful in the design of hoppers and husking equipment (García-Alamilla *et al.*, 2012; Jaiyeoba *et al.*, 2016). To estimate sphericity and surface area, two sets of equations were used (equations 1 and 2; and equations 5 and 6, respectively). The results show that cocoa beans do not resemble a sphere. Still, regarding the shape factor (using equation 8), the cocoa beans coincided with an ellipsoid of revolution, as shown in Table 2. The geometric dimensions obtained from the linear dimensions were greater than those reported by Bart-Plange and Baryeh (2003). When compared to the results of García-Alamilla *et al.* (2012), the averages were lower for surface area (1006.99-890.48 mm^3) and volume (1280.17-873.76 mm^3). In the case of Sandoval *et al.* (2019), the geometric dimensions of fermented Trinitario beans are above those recorded in the present study, with a geometric diameter of 15.9 mm, a surface area of 742.4 mm^2, and a sphericity of 0.61. The results for each variable, one may infer, are related to very particular characteristics: edaphoclimatic conditions, plantation age, plantation management, distribution, and biodiversity. Concerning said characteristics, through an (unreported) questionnaire, for the most part, a density of 614.09 trees/ha, a productivity of 649-695 kg/ha, an average age of 31 years with a majority of young replantings, and biodiverse plantations were observed. The producers implement good practices, and few of them use agrochemicals. As reported by the producers, the genetic origin of the samples in ascending order was Trinidadian, foreigner, Creole, and clones.

Table 2. Average geometric properties.

Properties	Means	Max	Min	±SD	CV (%)
Sphericity (equation 1)	0.58	0.65	0.50	0.03	4.44
Sphericity (equation 2)	2.46	2.66	2.31	0.06	2.46
Dg (mm) (equation 3)	12.55	15.17	10.66	0.91	7.22
Surface area (mm^2) (equation 5)	420.23	609.51	306.99	59.70	14.21
Surface area (mm^2) (equation 6)	498.30	724.04	365.37	71.03	14.25
Volume (mm^3) (equation 7)	681.39	1195.50	436.77	146.20	21.46
Shape factor (equation 8)	1.04	1.089	1.022	0.008	0.83
Surface area (equation 9)	0.22	0.27	0.18	0.01	7.45
Volume (equation 11)	1816.35	3372.62	959.25	405.78	22.34

Source: Compiled by authors.

Table 3 shows the correlation between the physical variables of the commercial samples from the three states (Tabasco, Chiapas, and Oaxaca). The correlation analysis showed that the length of the dried beans was significant ($p \leq 0.0001$) regarding all the physical variables and the equations where it partakes, except moisture. The sphericity resulting from equations 1 and 2 indicates the extent to which the bean shape resembles a sphere —which was not the case for cocoa beans. Meanwhile, the shape factor obtained with equation 8 better represents the morphology of the bean as an ellipsoid, which was positively correlated ($p \leq 0.0001$) to bean length and negatively to bean thickness and width, which are part of the geometric diameter estimation. Bean weight was not dependent on moisture, shape factor, or sphericity (equation 1), but it was highly significant ($p \leq 0.001$), whether positively or negatively, for all other variables. The volume estimated through equation 9 was the variable most correlated to the rest, except for the bean's L/E ratio and the shape factor (equation 8). In all cases, moisture —already cited as a critical variable— did not show a correlation either with the linear measurements or with the results derived from the equations.

The PCA explained 98.8% of the total variation observed for the first two components in the samples from the three states (Tabasco, Chiapas, and Oaxaca). No outliers were observed.

The first principal component (PC1) explained 96.8% of the total variation, with bean weight, surface area (equations 5 and 6), and volume (equation 7) being the major contributors (Table 4). For this component, all significant variables presented a negative correlation. The second principal component (PC2) explained 2% of the total variation, with bean weight, bean length, surface area (by equations 5 and 6), volume (equation 7), and shape factor being the variables that described this variation. The positive contribution came from the area and volume equations.

Figures 1 and 2 contain the graphs from the PCA (scores and loadings), showing the weight of the two main components obtained with the standard deviations. The score graph shows the discrimination and classification of the assessed physical properties according to the data matrix of 51 samples. It also shows similarities between the groups. The pattern of

Table 3. Correlation of physical variables of cocoa beans from three Mexican states (Tabasco, Chiapas, and Oaxaca).

	LB	Eq. 7	Eq. 2	Eq. 6	Eq. 3	Eq. 5	ABW	Eq. 11	WB	TB	LB/TB	Eq. 8	Eq. 9	Eq. 1	Moisture
LB	1														
Eq. 7	0.7156*	1													
Eq. 2	0.7873*	0.9812*	1												
Eq. 6	0.7949*	0.9910*	0.995*	1											
Eq. 3	0.8075*	0.9839*	0.9982*	0.9983*	1										
Eq. 5	0.8075*	0.9887*	0.9938*	0.9996*	0.9982*	1									
ABW	0.7431*	0.7971*	0.8081*	0.8200*	0.8184*	0.8231*	1								
Eq. 11	0.8998*	0.9456*	0.9643*	0.9761*	0.9858*	0.9803*	0.8375*	1							
WB	0.4569*	0.8080*	0.7601*	0.7731*	0.7612*	0.7692*	0.5461*	0.7003*	1						
TB	0.4870*	0.8110*	0.8161*	0.8017*	0.8040*	0.7950*	0.6490*	0.7252*	0.03796*	1					
LB/TB	0.4322*	-0.1607**	-0.1062	-0.0844	-0.0747	-0.0657	0.0300	0.0910	0.0329	-0.5732*	1				
Eq. 8	0.5612*	-0.1601**	-0.0657	-0.0499	-0.0318	-0.0279	0.1120	0.1562	-0.2737*	-0.3085*	0.8464	1			
Eq. 9	-0.8864*	-0.9330**	-0.9792*	-0.9713*	-0.9815*	-0.9742*	-0.8137*	-0.9769*	-0.6931*	-07536*	-0.0524	-0.1264	1		
Eq. 1	-0.5691*	0.1545	0.0495	0.0400	0.0205	0.0198	-0.1146	-0.1654*	0.2745*	0.3007*	-0.8442*	-0.9953*	0.1418	1	
Moisture	-0.0433	0.0460	0.0192	0.0290	0.0202	0.0268	-0.0261	0.0149	0.0644	0.0221	-0.0647	-0.1051	0.0110	0.1009	1

ABW: Average Bean Weight; length (L), WB: Width Bean; TB: Thickness Bean; ratio LB/TB

Table 4. Principal component analysis of cocoa beans physical properties.

Variable	CP1	CP2	CP3
ABW (g)	-0.1494*	-0.1686*	0.9722*
LB (mm)	-0.0437	-0.1792*	-0.0426
WB (mm)	-0.0308	0.1062	-0.0277
TB (mm)	-0.0246	0.0671	0.0327
Ratio LB/TB	-0.00003	-0.1068	-0.0336
(eq 3)	-0.0359	0.0211	-0.0031
(eq 1)	0.0003	0.0434	0.0089
(eq 2)	-0.0091	0.0072	-0.0006
(eq 5)	-0.2967*	0.1699*	-0.0207
(eq 6)	-0.3230*	0.2299*	-0.0137
(eq 7)	-0.4629*	0.7107*	0.0574
(eq 8)	-0.0001	-0.0231	-0.0044
(eq 9)	0.0047	0.0023	0.0016
(eq 11)	-0.7523*	-0.5668*	-0.2116*
Moisture (%)	-0.0010	0.0319	-0.0346

ABW: Average Bean Weight; length (L), WB: Width Bean; TB: Thickness Bean; ratio LB/TB.

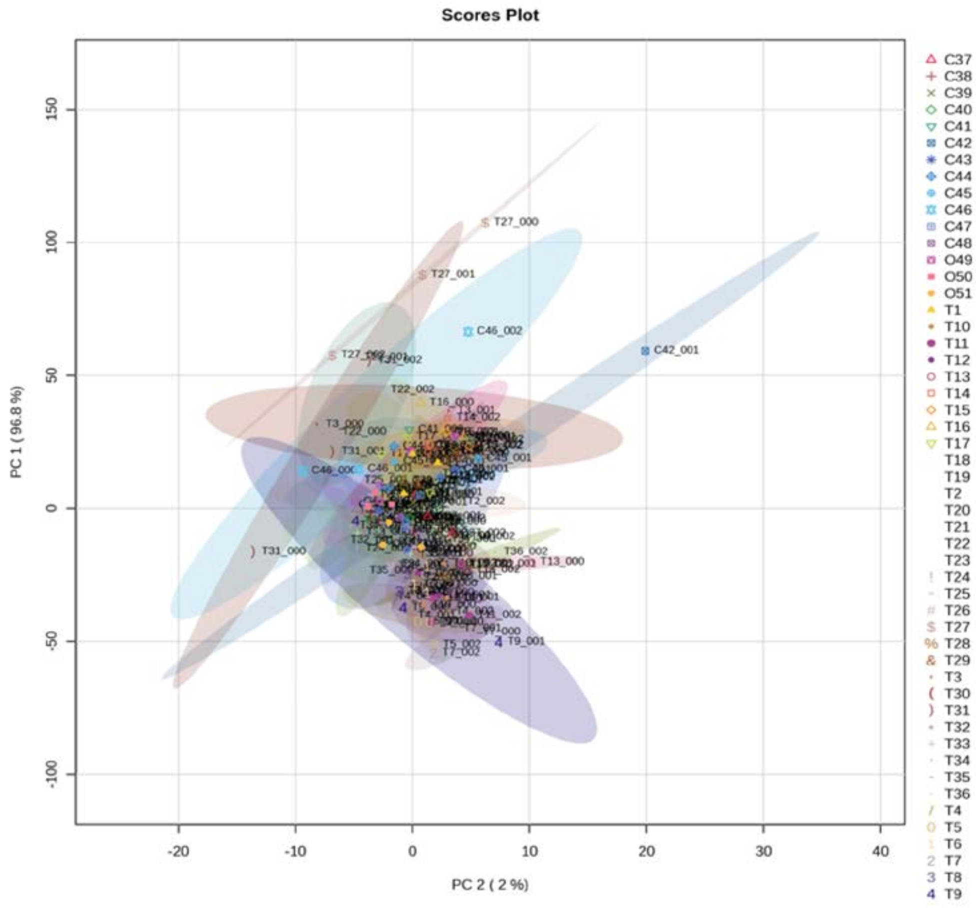

Figure 1. PCA spatial distribution scores for 51 samples of Mexican cocoa beans from different geographical origins (Tabasco=T1-T36; Chiapas=C37-C48; Oaxaca=O49-O51).

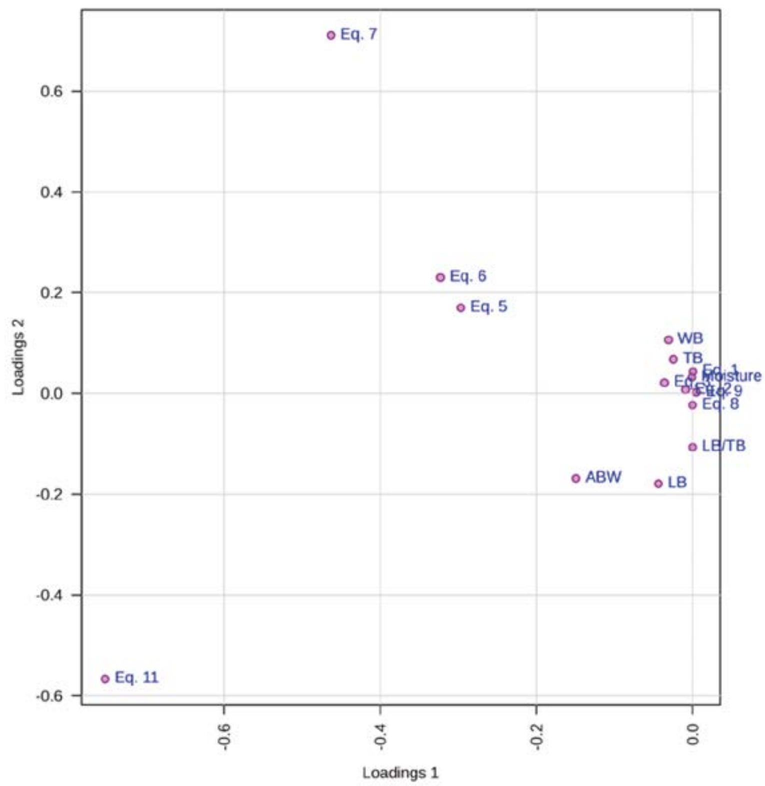

Figure 2. PCA loadings distribution for 51 samples of Mexican cocoa beans from different geographical origins.

groups and crossings is complex due to multiple factors associated with geographical origin, processing (washed and/or fermented cocoa beans), and edaphoclimatic conditions. In their PCA, Oyedokun *et al*. (2011) observed the formation of four groups where the determining factor was bean weight. They also reported that the highest correlation occurred between bean width and thickness, a characteristic feature of the grouping. Likewise, Jaiyeoba *et al*. (2016) observed an inverse relation between moisture and axial dimensions, geometric diameter, sphericity, and surface area. According to Figure 2, the physical parameters that describe the classification are grouped mainly into two domains: the first includes volume (equation 11), bean weight, and bean length, while the second considers equations 5, 6, and 7, corresponding to the expressions of surface area and volume. These results confirm which variables are of more consequence (Table 4).

CONCLUSIONS

Bean weight was the quantitative variable that presented significance. Other measurements, such as length, width, and thickness, were not significant on their own, but they were when combined in the expressions of surface area and volume. Moisture is a critical parameter in dried cocoa beans. However, it did not show significance regarding physical-geometric measurements. The shape factor to describe cocoa beans is an ellipsoid of revolution.

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Colegio de Postgraduados

Interspecific grafting of *Pinus patula*

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ABSTRACT
Objective: To evaluate the compatibility of *Pinus patula* grafts on rootstocks of seven pine species.
Design/Methodology/Approach: *P. patula* scions were grafted on *P. greggii*, *P. teocote*, *P. pseudostrobus*, *P. cembroides*, *P. ayacahuite*, *P. hartwegii*, and *P. patula* rootstocks. The seven treatments were established in a randomized complete four block design; survival and growth were evaluated and recorded during the experiment.
Results: At eight months, *P. patula* and *P. teocote* rootstocks recorded the highest survival rate (35%), while no *P. cembroides* graft survived. *P. patula* rootstocks recorded the highest graft growth, surpassing the grafts on *P. pseudostrobus*, *P. ayacahuite* and *P. hartwegii*, by 30, 78, and 90%, respectively.
Study Limitations/Implications: The rootstocks of *P. cembroides*, *P. hartwegii*, and *P. ayacahuite* were not compatible with *P. patula* grafting. This situation reduces the number of potential species that can be used to clone *P. patula* genotypes.
Finding/Conclusions: The survival and graft growth were more successful on rootstocks of species phylogenetically closer to *P. patula*.

Keywords: cloning, compatibility, rootstock, phylogenetic relationship.

INTRODUCTION

A graft is the union between two organs of plants from different origin, composed of a scion (aerial part) and a rootstock (root system) (Darikova *et al.*, 2011). The parts of the graft can come from the same (intraspecific) or different (interspecific) species. Interspecific grafts have been successfully used to increase productivity and resistance to adverse factors. In addition, they have great potential for forestry (Han *et al.*, 2019). Intraspecific grafts are usually more compatible (Hartmann *et al.*, 2014). However, variable results have been obtained with interspecific grafts (Melnyk, 2017). Pérez-Luna *et al.* (2020) grafted *Pinus engelmannii* Carr. with the same species and with the *P. engelmannii* × *P. arizonica* Engelm. var. *arizonica* hybrid. In this case, the survival of the first graft was higher on the hybrid.

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This type of grafts increases productivity, the capacity to adapt to different environments, and the resistance to pests, diseases, and stress caused by abiotic factors (drought, salinity, excess, or deficit of water); in addition, it accelerates or increases fruiting, improves graft compatibility, or promotes a smaller size that favors the harvesting of cones in asexual seed orchards (Castro-Garibay *et al.*, 2017; Han *et al.*, 2019). In Mexico, grafting has the potential to seek favorable combinations and to improve the compatibility between pine species, because 49 (40%) out of the approximately 120 species of the world can be found in its territory (Gernandt and Pérez-de la Rosa, 2014).

Grafting scions from superior genotypes of *Pinus patula* Schiede ex Schltdl. *et* Cham. var. *patula* on rootstocks of other pine species would increase the productive potential of the species, because it can be established in asexual seed orchards used for genetic improvement programs. Research is fundamental to overcome interspecies compatibility, one of the main problems in this practice. The aim of this study was to determine the initial compatibility of *P. patula* scions with rootstocks of *P. patula*, *P. greggii* var. *australis* Donahue *et* López, *P. teocote* Schiede ex Schltdl. *et* Cham., *P. pseudostrobus* Lindl., *P. cembroides* Zucc., *P. ayacahuite* Ehren., and *P. hartwegii* Lindl. The first three species belong to the Oocarpae subsection; meanwhile, *P. pseudostrobus* and *P. hartwegii* are part of the Ponderosae subsection of the *Pinus* subgenus. Finally, *P. ayacahuite* and *P. cembroides* are more taxonomically distant (subgenus *Strobus*) from the scion species (Gernandt *et al.*, 2005; Lira, 2020).

The objective of this study was to evaluate the initial compatibility of the *P. patula* graft on rootstocks of seven different pine species, in order to identify the most compatible rootstocks, regarding the survival and growth of the grafts.

MATERIALS AND METHODS

The experiment was established at the Colegio de Postgraduados, Campus Montecillo, located at 19° 27’ 34.8” N and 98° 54’ 15.8” W, at 2,249 m.a.s.l. Four months before grafting, the rootstock plants of the seven species were transplanted into 1 L plastic containers, using a substrate made of 60-20-20 peat moss, perlite, and vermiculite, with Multicote® 18-6-12+2MgO+ME at a dose of 8 g L^{-1}. The rootstocks were between 18 and 24 months old at the time of grafting and each species had its own morphological characteristics (Table 1).

Table 1. Origin, age, and morphological characteristics of the pine species used as rootstock plants in interspecific grafts of *Pinus patula*.

Rootstock species	Age (months)	Origin	Height (cm)	Diameter (mm)
P. patula	18	Chignahuapan, Puebla	58.7±1.1	7.1±0.2
P. greggii	18	Chignahuapan, Puebla	60.0±1.3	7.3±0.5
P. teocote	18	Acaxochitlán, Hidalgo	43.6±1.0	5.3±0.1
P. pseudostrobus	24	Tulancingo, Hidalgo	35.4±1.0	7.7±0.5
P. cembroides	24	Zimapán, Hidalgo	22.3±0.5	7.9±0.3
P. ayacahuite	24	Huayacocotla, Veracruz	51.8±2.5	8.4±0.3
P. hartwegii	24	Cofre de Perote, Veracruz	24.6±1.1	9.8±0.4

In March 2021, buds were collected from a 16-year-old select *P. patula* tree (G40). This tree had been chosen in 2018, because it stood up in the thinned sexual seed orchard, as a result of its height (16.9 m), normal diameter (26.8 cm), and stem straightness. This seed orchard is located at 19° 57' 36.09" N and 98° 06' 18.92" W, at 2,592 m.a.s.l., in the Peñuelas Pueblo Nuevo ejido, Chignahuapan, Puebla (Figure 1A). The buds were actively growing; nevertheless, they had not yet formed needles. They were vigorous and healthy, with a 20.0 cm average length and 4.7 mm average diameter (Figure 1B).

The buds were indistinctly collected from all over the tree crown. After their collection and until the moment of grafting, the buds were handled following the lateral technique described by González-Jiménez *et al.* (2022). However, depending on the morphology of the species evaluated, the height of the grafting varied from 5 to 10 cm from the base of the rootstock stem. Post-grafting management activities included: opening of the bag between weeks 3 to 5; removing the plastic at the junction point after two and a half months; pruning the aerial part of the rootstock at 2, 3, and 4 months after grafting, getting rid of approximately a third part of the crown each date, until only the grafted bud remained as the leader of the plant. The grafts were kept under 50% shade mesh. In average, irrigation was applied three times per week, using 1 g L^{-1} of Peters Profesional® 20-20-20 general purpose fertilizer.

The experiment was established under a randomized complete block design with four repetitions. Seven treatments were established in each block; the treatments matched the seven rootstock species used in this experiment (T1: *P. patula*, T2: *P. greggii*, T3: *P. teocote*, T4: *P. pseudostrobus*, T5: *P. cembroides*, T6: *P. ayacahuite*, and T7: *P. hartwegii*). The experimental unit consisted of five grafts, resulting in a total of 140 grafts. The variables evaluated were: 1) survival eight months after grafting (%); 2) growth of the length of the graft (cm); and 3) evaluation of the length of needles (cm). The growth of the length of the graft was determined based on the difference between the initial length of the scion (7 cm) and its length six months after grafting. The length of the needles was evaluated through the selection of four needles from each graft; afterwards, their length was measured from the union of the fascicle to the tip, obtaining the average per graft. Both evaluations were carried out on the same date.

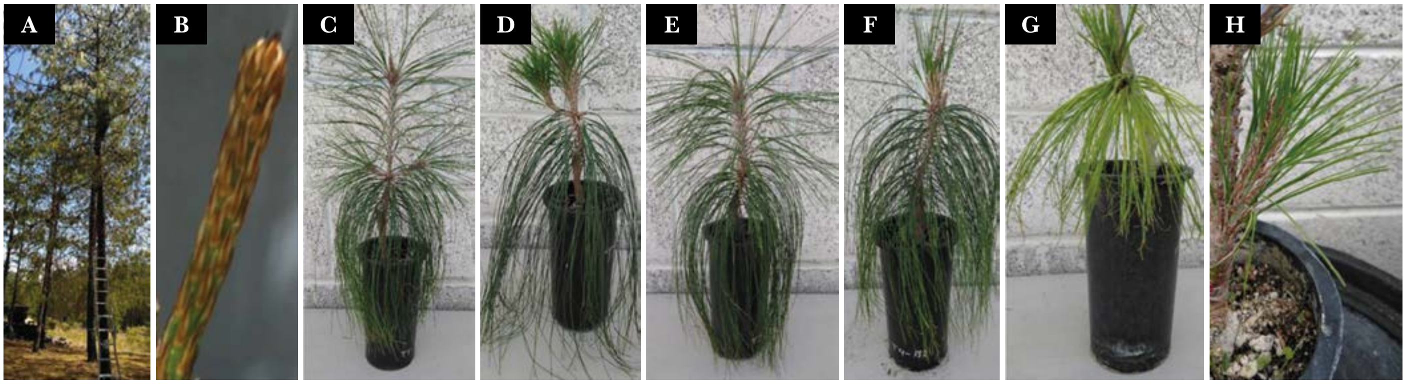

Figure 1. Interspecific grafts of *Pinus patula*. A: *Pinus patula* G40 scion donor tree; B: buds used as scions for grafting; C: eight-month-old grafts on *P. patula* rootstocks; D: *P. greggii*; E: *P. teocote*; F: *P. pseudostrobus*; G: *P. ayacahuite*; and H: *P. hartwegii*.

An analysis of variance was carried out to detect statistical differences between treatments; subsequently, the Tukey Multiple Comparison Test was performed, using the Mixed procedure of the Statistical Analysis System 9.4 software (SAS, 2013). The data of the variables (percentage) did not meet the assumption of normality; consequently, they were transformed using the $\left[T = \text{arcsine}\left(\sqrt{Y}\right)\right]$ function, before the analysis of variance was carried out. Subsequently, they were retransformed using the $\left[Y = 100\ \text{sine}^2(T)\right]$ function.

$$Y_{ij} = \mu + \tau_i + B_j + \varepsilon_{ij}$$

Where: i=1, 2,...t; j=1, 2,...r; t=number of treatments; j=number of blocks = number of repetitions; Y_{ij}=value of the response variable matching repetition j of the treatment i; μ=overall mean; i=effect of the treatment i; B_j=effect of the block j; ε_{ij}=experimental error, i=T1, T2, T3,...; j=1, 2, 3,... repetitions.

RESULTS AND DISCUSSION

Survival reduced to 22.5% by the final evaluation. The grafted scions recorded a length and needle growth of 2.1 and 8.7 cm, respectively. The analysis of variance recorded significant differences ($p \leq 0.05$) between treatments in the three variables (Table 2).

Graft survival

The highest graft mortality took place 60 days after the grafting. This situation could be the result of a possible physiological and anatomical incompatibility caused by the differences between rootstock species and the scions, as well as environmental factors. As a consequence of the atypically abundant rainfall in the area (May), excessive moisture inside the bag that covered the graft caused phytosanitary problems at the point of the union of the graft. However, survival remained relatively constant after this period. Likewise, Pérez-

Table 2. Mean values and standard error (±) in the experimental grafting of *Pinus patula* scions on rootstocks of seven different pine species.

Treatment	Survival (%)	Graft growth (cm)	Needle length (cm)
	0.0001*	**0.0001***	**0.0001***
P. patula	35±5.0 a	13±1.7 a	21.4±0.4 b
P. greggii	30±5.8 a	9.3±0.1 ab	29.9±1.2 a
P. teocote	35±5.0 a	9.5±0.7 ab	21.9±1.5 b
P. pseudostrobus	15±5.0 ab	9.1±0.4 b	22.5±0.6 b
P. cembroides	0.0±0.0 b	-	-
P. ayacahuite	5±5.0 b	2.8±0.5 c	11.9±0.3 c
P. hartwegii	15±5.0 ab	1.3±0.4 c	8.1±2.9 c
Average	22.5±4.6	7.5±1.5	1.3±1.6

*p≤0.05; means with different letters in each column indicate statistical differences between treatments (Tukey, 0.05).

Luna *et al.* (2020) recorded the stabilization of the survival of *P. engelmannii* × *P. arizonica* var. *arizonica* grafts on *P. engelmannii* rootstocks, 60 days after the grafting. Environmental factors are decisive during the "joint" period. Consequently, a special infrastructure is required for graft production. The nursery should be covered with shade mesh and a retractable plastic greenhouse roof, in order to protect the grafts from rain and hail. This roof can be opened or closed in order to keep the appropriate temperature, depending on the environmental conditions.

The percentage of graft survival variably reduced according to the rootstock species. *P. ayacahuite* recorded the highest mortality (95%), while *P. patula*, *P. teocote*, and *P. greggii* were less susceptible (65-70%) to the factors that influenced the mortality of the grafts during this period (Table 2). The grafts of *P. patula* on rootstocks of the same species recorded the highest survival value among the treatments (Table 2). Regarding the intraspecific grafts, a higher compatibility can usually be expected, as a result of their taxonomic affinity (Darikova *et al.*, 2011). Solorio-Barragán *et al.* (2021) grafted *P. rzedowskii* on five rootstock species, likewise achieving the highest survival of intraspecific grafts.

P. teocote and *P. greggii* had the grafts with the highest survival among scions grafted on rootstocks from a different species (Figure 1D and 1E). Gernandt *et al.* (2005) performed phylogenetic reconstructions with molecular data from DNA regions of chloroplasts and nucleus, placing *P. patula* in the same subsection as *P. teocote* and *P. greggii*; consequently these species are phylogenetically related. Therefore, the hypothesis is that the anatomical characteristics of these species may have evolved in a similar way. Consequently, there is a higher probability to obtain grafts compatible with these two pine species that have be used as rootstock plants for *P. patula* scions. In addition, Solorio-Barragán *et al.* (2021) reported a high compatibility when species of the same subsection (*Cembroides*) were grafted: scions of *P. rzedowskii* on *P. pinceana* Gordon & Glend.

On the one hand, *P. greggii* used as rootstock plant to graft scion from *P. patula* has some of the following characteristics: precocious flowering, high growth rates (height and diameter), and the potential to adapt to limiting humidity conditions and poor soils (Ruiz-Farfán *et al.*, 2015). On the other hand, *P. teocote* is another alternative that can be used as a rootstock for *P. patula*. This pine species is widely distributed in Mexico and could provide resistance to water deficit or tolerance to low-fertility soils. In addition, it is highly responsive when cut, which could favor the "succsess" percentage. Consequently, this species could be potentially used as rootstock for *P. patula*, as a result of the circular and continuous shape of its vascular cambium (Gernandt and Pérez-de la Rosa, 2014; Castro-Garibay *et al.*, 2017). No information on *P. patula* grafts has been documented on these two rootstock species; therefore, trials to determine its influence on the scion through their interaction must be carried out (Han *et al.*, 2019).

Studies aimed to determine the use of rootstocks of some species that are better adapted to adverse conditions have been carried out. Han *et al.* (2019) grafted *Populus cathayana* Rehder on *Populus deltoides* Bart. ex Marsh rootstocks which are more resistance to drought. The aim of that study was to increase the efficient use of water and soil nutrients. Guadaño *et al.* (2016) grafted *Pinus pinea* L. on *Pinus halepensis* Mill. because it is more resistant to drought and better adapted to limestone soils.

The rootstocks of *P. pseudostrobus* and *P. hartwegii* recorded a low survival rate (15%). Although the rootstock of these species had little compatibility, functional grafts were indeed obtained (Figure 1F and 1H). Although they belong to the same subgenus (*Pinus*), they are from a different subsection (Ponderosae), which can explain the results obtained (Gernandt *et al.*, 2005). Likewise, Solorio-Barragán *et al.* (2021) found compatibility and a higher survival rate (50%) when grafting *P. rzedowskii* scions on *P. ayacahuite* var. *veitchii*. Both species belong to the same subgenus, but to a different subsection. This situation shows that a successful graft is more difficult in some pine species, perhaps as a result of factors related to the cambium characteristics and their internal conduction structures (Castro-Garibay *et al.*, 2017). *P. ayacahuite* rootstocks recorded the lowest survival rate (Figure 1G). Meanwhile, *P. cembroides* rootstocks had no compatibility, probably because both rootstock species are the most taxonomically distant from *P. patula* and they belong to another subgenus (*Strobus*) (Gernandt *et al.*, 2005).

Graft growth

The scion successfully resumes its growth and development when the vascular connection is reestablished and consequently it can receive water and nutrients (Hartmann *et al.*, 2014). The highest average value was obtained with the rootstocks of the same species (13 cm), while with *P. pseudostrobus*, *P. ayacahuite*, and *P. hartwegii*, it recorded lower growth differences (30, 78, and 90%, respectively) (Table 2). These results match other intraspecific grafting experiments, where a better response was obtained, as a consequence of the anatomical and histological affinity of the parts of the graft (Castro-Garibay *et al.*, 2017).

Although, *P. patula* rootstocks obtained a higher value than *P. greggii* and *P. teocote*, there were not statistical differences between them, because their difference in growth fell into the range of 3.7 cm. The taxonomic closeness of these two species with *P. patula* (same subsection) could be related to their similar growth (Gernandt *et al.*, 2005). Since the evaluations of this study were limited to the first six months after the grafting took place, the growth of these scion-rootstock graft combinations should be evaluated after several years, in order to determine if the contrasts are permanent.

P. pseudostrobus is a relatively fast growing species and it is associated with the natural distribution range of *P. patula* (Perry, 1991). However, grafts on *P. pseudostrobus* rootstocks grew less than on the same species (*P. patula*). An example of the application of interspecific grafts is the use of dwarfing rootstocks (Gautier *et al.*, 2019). Low-height grafts can be kept in asexual seed orchards, facilitating their management and cone harvesting (Jayawickrama *et al.*, 1991). Grafting of *P. patula* on *P. radiata* resulted in a lower growth; however, strobile production did not diminish (Castro-Garibay *et al.*, 2017).

P. ayacahuite and *P. hartwegii* did not record a favorable graft growth (<3 cm) (Table 2). The particular growing habits of *P. hartwegii* may have influence this response: this pine species has one of the lowest growth rates. The growth of this species is mainly limited by the environmental conditions (low temperatures and low precipitation) that prevail in its native subalpine ecosystems. Additionally, after germination, the seedlings have a grassy state, they stop growing (height), and enter an apparent dormancy state that lasts between two and six years (Rivera *et al*., 2021). These characteristic may have influenced the growing habits of the grafted *P. patula* scion.

Needle length

The influence that the rootstock species exerts on the growing habits of the graft needles could be identified, because the scions belong to a single genotype. The use of different rootstocks may provide differences in phenotype or architecture of the scion (Gautier *et al*., 2019; Han *et al*., 2019). The development of needles is an indication of graft "succsess". When a functional xylem is formed, the growth of these structures begins, as a result of the great water demand involved in the process (Guadaño *et al*., 2016). In addition, these organs are responsible for all the photosynthesis and transpiration processes required by the new plant (González-Jiménez *et al*., 2022).

The grafts with *P. greggii* rootstocks recorded greater needle growth (29.9 cm) than both species. In ungrafted adult trees, *P. greggii* and *P. patula* develop 10-15 and 15-25 cm long needles, respectively (Perry, 1991). This combination recorded shorter length differences of 28, 27, 25, 60, and 73%, with *P. patula*, *P. teocote*, *P. pseudostrobus*, *P. ayacahuite*, and *P. hartwegii*, respectively (Table 2). Overall, larger needles have a greater photosynthetic area and increase productivity, which is related to plant growth (He *et al*., 2020). Consequently, the grafts on *P. ayacahuite* and *P. hartwegii* rootstocks recorded the lowest needle growth and lowest growth regarding graft length (Figure 1G and 1H), possibly because the rootstocks can alter the photosynthesis rate of the grafted scion (Han *et al*., 2019).

The length of needles in ungrafted plants of *P. ayacahuite* (10-18 cm) and *P. hartwegii* (8-16 cm) is shorter than the length of the needles of *P. patula* during the adult state of ungrafted trees (Perry, 1991). These morphological differences regarding the length of the needles must be a consequence of the phylogenetical distance between these two rootstock species and *P. patula*. Therefore, their different growing habits are assumed to be the result of their separate evolution (Gernandt *et al*., 2005). This same growing habit was observed in the scions of *P. patula* grafted on rootstocks of these two species, which developed shorter needles than those recorded on rootstocks of the same species.These two rootstock species exerted a stronger influence on the average growth of needles than the scion. Melnyk (2017) suggested selecting the appropriate rootstock that influences the desired size and vigor of the graft, because the rootstock exerts an important control over the scion.

CONCLUSIONS

The rootstock species with a closer phylogenetical relation with *Pinus patula* recorded a higher graft survival rate. Different rootstock species have particular effects on the growing habit of the grafted scions of *P. patula*.

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Colegio de Postgraduados

Impact of *Lippia palmeri* S. Watson during kid suckling and growth

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ABSTRACT
Objective: raising kids is a fundamental activity in goat production, since their development is the source of replacements for the dairy herd or the sale of meat. Therefore, the impact in the kids' growth of adding *Lippia palmeri* S. Watson oregano to the goat diet was evaluated.
Materials and methods: a total of 17 kids born from mothers who consumed the four diets (treatments) were evaluated. They were divided as follows: 6 kids in T1 (ASINC with ORE); 5 kids in T2 (ASINC without ORE); 3 kids in T3 (SINC without ORE); and 3 kids in T4 (SINC with ORE). The weight gain (kg) and daily milk consumption (kg) variables were evaluated for 60 days.
Results: no significant differences were found ($p>0.05$) between treatments, showing an average daily weight gain of 0.0895±0.018 kg in T1 (ASINC with ORE), 0.0892±0.026 kg in T2 (ASINC without ORE), 0.0934±0.035 kg in T3 (SINC without ORE), and 0.118±0.026 kg in T4 (SINC with ORE). Meanwhile, the average daily milk consumption was 0.509±0.240 kg in T1 (ASINC with ORE), 0.580±0.205 kg in T2 (ASINC without ORE), 0.553±0.190 kg in T3 (SINC without ORE), and 0.717±0.202 kg in T4 (SINC with ORE).
Conclusions: no treatment was significantly higher than the others. A significant and positive correlation ($r=0.879$, $p<0.0001$) was detected between the two variables under study.

Keywords: Growth, kids, *Lippia palmeri*, consumption, nutrient synchrony.

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INTRODUCTION

Maintaining a constant and increasing development is fundamental for raising goats, because it will allow them to reach the expected weight for sale or weaning. General considerations for weaning include objective of use, weight (8-10 kg), age (6-8 weeks), and the ability to consume solid foods. Therefore, management strategies that guarantee the achievement of these objectives allow the farm to get efficient economic results (Gómez-Gómez *et al.*, 2019). In livestock research, the growth analysis of ruminant offspring is

an important object of study. The diet received by developing animals is related to their genetic behavior and the efficiency of the foods used in their nutrition, which impact the growth rate and maximum use of food by the species (Galina *et al.*, 2009). The birth of kids is an important factor to predict the future performance of goat offspring and to decrease the herd mortality percentage; Abbas *et al.* (2020) have documented that the mother's diet before and after childbirth influences the development rate. Accordingly, this document shows the weight gain and milk consumption of kids from different treatments, resulting from the inclusion of oregano (*Lippia palmeri* S. Watson) in the diet and its nutrient synchrony in the feeding of mother goats.

MATERIALS AND METHODS

Study area

The research was carried out at "Rancho El Palmar de Abajo" (23° 38' 02" N and 110° 17' 05" W, 200 m.a.s.l.), located in the sub-delegation of La Matanza, municipality of La Paz, Baja California Sur, Mexico. The climate at the study site is desert arid with an average monthly temperature of 21.2 °C in summer and 9 °C in winter. The average annual precipitation is 200 mm and the average annual temperature is 28 °C. The main rainy season lasts from July to September, although occasional winter rains have been reported (Ramírez *et al.*, 2011). The dry season lasts from February to June (Troyo *et al.*, 2014).

Composition of the diet of mother goats

Table 1 shows the composition of the diet of mother goats, including the synchrony index of each ingredient. The protein-energy synchrony in the diet was determined, as described by Sinclair *et al.* (1993).

Table 1. Nutritional composition of diets.

Ingredient (% in Diet)	Treatment			
	T1	T2	T3	T4
	ASINC with oregano	ASINC without oregano	SINC without oregano	SINC with oregano
Alfalfa meal	0.5	9	1.1	4.9
Corn, ground	46	44	36.1	32.93
Soybean meal	0	0	9.1	7.6
Bean straw	49.5	45.9	53.6	51.8
Lippia palmeri (oregano)	2.6	0	0	2.6
Urea	1.4	1.1	0.1	0.17
Total	100	100	100	100
SI	0.69	0.689	0.71	0.71
DM (%)	89.21	89.44	90.59	90.57
CP (%)	11.0	11.1	11.0	11.1
ME (Mcal ME/kg)	3.38	3.38	3.33	3.21

SI = diet synchrony index: ingredient (%) = percentage of food in the diet; DM = dry matter (%); CP = crude protein (%); and ME = metabolizable energy (Mcal/kg, dry basis).

Kid growth evaluation

The growth and development of kids was analyzed using their weight as a reference. They were separated from the mother the previous night. Their empty weight was measured in the morning and they remained with the mother until the udder clearly had no more milk. After this step, the kids were weighed again to determine their milk consumption by weight difference; they were then separated again from their mother until the afternoon (approximately 9 hours). This procedure was repeated every day for 60 days (Benson *et al.*, 1999). Milk consumption (MC) and daily weight gain (DWG) were calculated with the following formulas (Benson *et al.*, 1999):

$$MC = EW - WAS \quad (1)$$

Where: MC = milk consumption (kg); EW = kid empty weight (kg); WAS = kid weight after suckling (kg).

$$DWG = EKW - EW \quad (2)$$

Where: DWG = daily weight gain (kg); EKW = empty kid weight day before (kg); EW = kid empty weight (kg).

Statistical analysis

A repeated measurements statistical model was used: a one-way analysis of variance (ANOVA) test was carried out for the milk consumption and weight gain variables (Galina *et al.*, 2009), carrying out the statistical analysis in the SPSS version 22.0 for Windows statistical package (IBM, 2013).

RESULTS AND DISCUSSION

Table 2 shows the results of the normality test of the data and the one-way ANOVA for each measured variable: the data meet the assumption of homogeneity of variances and normality. The ANOVA obtained non-significant values between treatments, for the milk consumption (kg) ($p=0.611$) and weight gain ($p=0.399$) variables. Therefore, oregano and nutrient synchrony do not have a significant impact on the development of kids.

Table 3 illustrates the results of average milk consumption per treatment and their totals. On the one hand, T4 (SINC with ORE) is observed with the highest milk consumption and

Table 2. Results of the analysis of the observation of the 17 kids.

Variable	Levene's test		Kolmogorov-Smirnov Test[(a)]		Shapiro-Wilk Test		ANOVA	
	Statistic	p Value	Statistic	p Value	Statistic	p Value	Statistic	p Value
Milk intake (kg)	0.345	.793	0.130	.200*	0.972	.846	0.626	.611
Weight gain (kg)	0.482	.701	0.090	.200*	0.982	.974	1.062	.399

[(a)] With Lilliefors correction. [(*)] This is a lower limit of true significance.

Table 3. Descriptive statistics of milk consumption and weight gain (kg), daily and total averages for the 60 days, per treatment (Trt) n = number of kids.

Variable	Treatment	n	Mean	Standard deviation	Standard error	Minimum value	Maximum value
Average daily milk intake (kg)	1	6	0.5096	0.24081	0.09831	0.2053	0.8576
	2	5	0.5809	0.20515	0.09175	0.3070	0.7528
	3	3	0.5534	0.19010	0.10975	0.3825	0.7582
	4	3	0.7178	0.20201	0.11663	0.5917	0.9508
	Total	17	0.5750	0.20926	0.05075	0.2053	0.9508
Average total milk intake (kg)	1	6	30.5750	14.44840	5.89853	12.3150	51.4580
	2	5	34.8528	12.30928	5.50488	18.4200	45.1670
	3	3	33.2060	11.40576	6.58512	22.9490	45.4890
	4	3	43.0703	12.12070	6.99789	35.4990	57.0500
	Total	17	34.5025	12.55560	3.04518	12.3150	57.0500
Average daily weight gain (kg)	1	6	0.0895	0.01815	0.00741	0.0675	0.1163
	2	5	0.0892	0.02683	0.01200	0.0543	0.1228
	3	3	0.0934	0.03507	0.02025	0.0592	0.1292
	4	3	0.1188	0.02632	0.01520	0.1005	0.1489
	Total	17	0.0952	0.02553	0.00619	0.0543	0.1489
Average total weight gain (kg)	1	6	5.3675	1.08915	0.44464	4.0500	6.9750
	2	5	5.3510	1.61011	0.72006	3.2600	7.3700
	3	3	5.6050	2.10404	1.21477	3.5490	7.7540
	4	3	7.1250	1.57898	0.91162	6.0300	8.9350
	Total	17	5.7147	1.53152	0.37145	3.2600	8.9350

greatest weight gain; on the other hand, treatments T1 (ASINC with ORE), T2 (ASINC without ORE), and T3 (SINC without ORE) had very close results in both variables.

Figure 1 shows the temporal evolution of the daily milk consumption of kids. Treatments with *Lippia palmeri* had similar initial values: 0.238±0.21 kg (T1), 0.347±0.41 kg (T4), and 0.282±0.135 kg (T3). Meanwhile, T2 had the highest value on day 1 of measurement (0.526±0.209 kg). T4 (SINC with ORE) showed similar values to the other treatments until day 31. In this period, its values were close to 0.617±0.298 kg and afterwards it had the highest levels, compared to the other treatments, reaching its maximum consumption on day 37 (0.995±0.293 kg) and a similar average value on day 49 (0.99±0.316 kg). The aforementioned high levels and low values fluctuated in this treatment (T4), varying from 0.70±0.175 kg on day 40 to 0.78±0.489 kg on day 54 and ending with the highest levels on the study: 0.703±0.234 kg and 0.667±0.205 kg on days 59 and 60, respectively.

Figure 2 shows the weight gain evolution of the kids per treatment during the 60 days of the experiment. Although no significant differences were recorded between treatments, the noticeable fluctuation caused some growth peaks, since T1 (ASINC with ORE) reported the lowest value per treatment on day 13 (0.012±0.018 kg), while T2 (ASINC without ORE) showed equally low values on day 34 and 38 (0.006±0.013 kg). For its part, T3 (SINC without ORE) had values of 0 on days 25, 41 and 45. Finally, T4 (SINC with ORE)

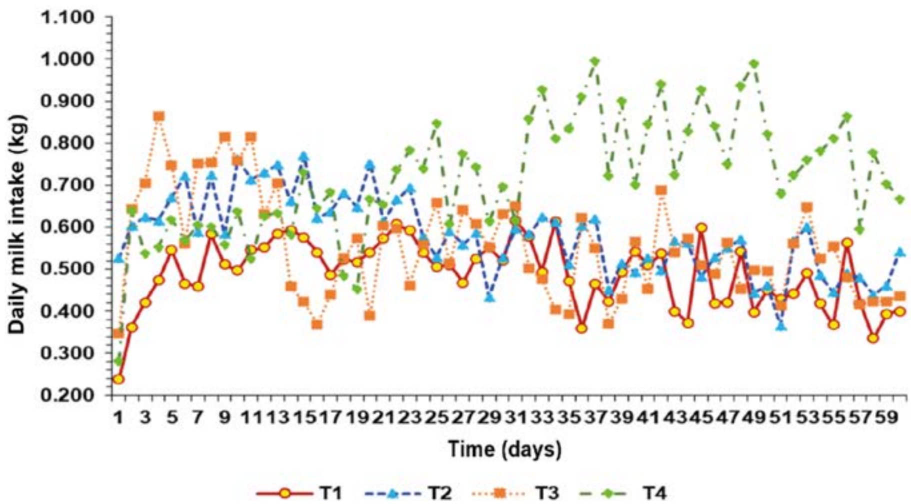

Figure 1. Average daily milk consumption (kg) per treatment.

recorded a value of 0 in weight gain on day 2. Meanwhile, the highest weight gain values in the experiment were, from lowest to highest: T1 on day 36 (0.187±0.129 kg); T2 on day 6 and 53 (0.24±0.212 kg); and, finally, T4 on day 3 (0.34±0.202 kg). At the end of the experiment (day 60), T1 showed the highest value (0.156±0.122 kg), followed by T2 (0.116±0.144). T3 was the treatment with the lowest value at the end of the experiment (0.017±0.004 kg). Finally, T4 recorded a final value of 0.032±0.05 kg.

Figure 3 illustrates the average weight gain and average milk consumption of all treatments. Both variables have a homogeneous behavior. They had an initial consumption of 0.348±0.127 kg on day 1. The values of this variable were then similar until day 13 (0.668±0.073 kg), when the peak of consumption was recorded. The two greatest drops in average consumption for the four treatments were recorded on day 51 (0.472±0.141 kg) and day 57 (0.478±0.083 kg). Likewise, the kid weight gain variable showed homogeneous values during the study, starting from an initial gain of 0.099±0.028 kg. The highest

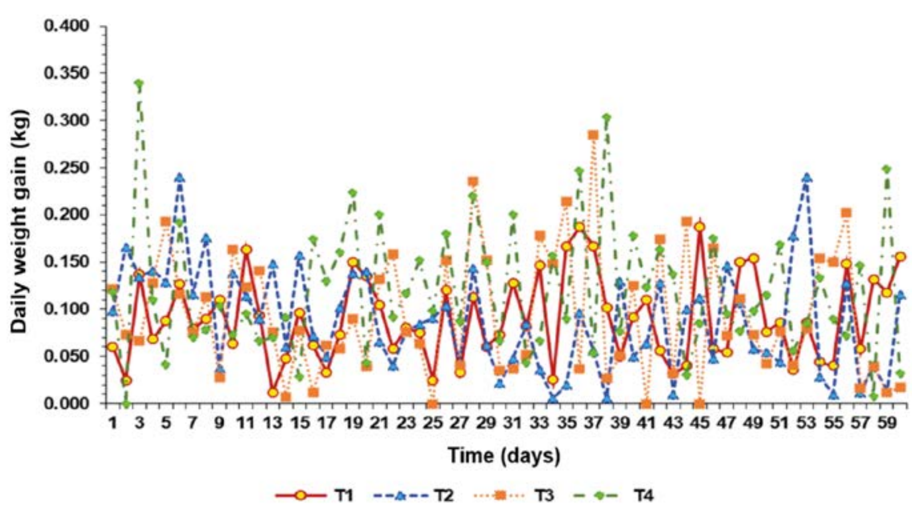

Figure 2. Average daily weight gain (kg) per treatment.

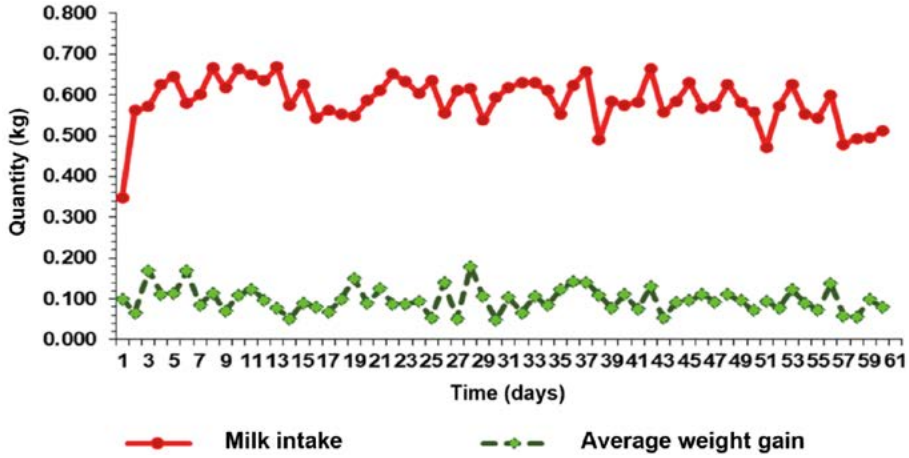

Figure 3. Average daily milk consumption and weight gain.

values were recorded on day 3 (0.169±0.118 kg), day 10 (0.109±0.049 kg), and day 19 (0.15±0.055 kg). The same behavior was reported during sampling, while similar low values were recorded on day 14 (0.052±0.035 kg), day 25 (0.054±0.049 kg), and day 58 (0.055±0.053 kg).

Figure 4 shows the results using a scatter diagram (n=17) prepared for the 4 treatments and its correlation (r=0.879) between the two daily weight gain and daily milk consumption variables. According to the analysis, these two variables have a linear and positive relationship, with a 0.103 slope and a 95% confidence interval (0.072 to 0.133), indicating that a 1.0 kg increase in milk consumption is expected to result in a 0.103 kg weight gain, when milk consumption ranges between 0.2 kg and 0.95 kg. Milk consumption accounted for approximately 77% (R^2=0.772) of the variability in weight gain.

Figure 5 illustrates the accumulated results for the weight gain (kg) variable in relation to milk consumption (kg) for each kid per treatment. On the one hand, T2-2-1 recorded

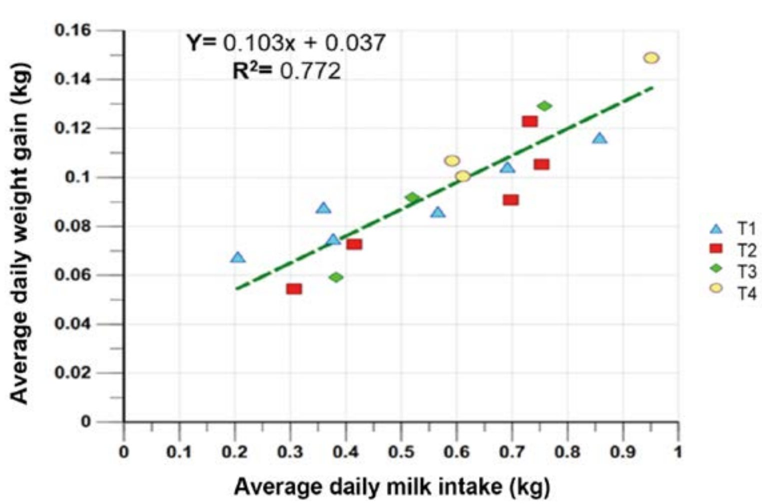

Figure 4. Scatter plot of the average weight gain and daily milk consumption, per kid (n=17), indicating the treatment to which it belongs.

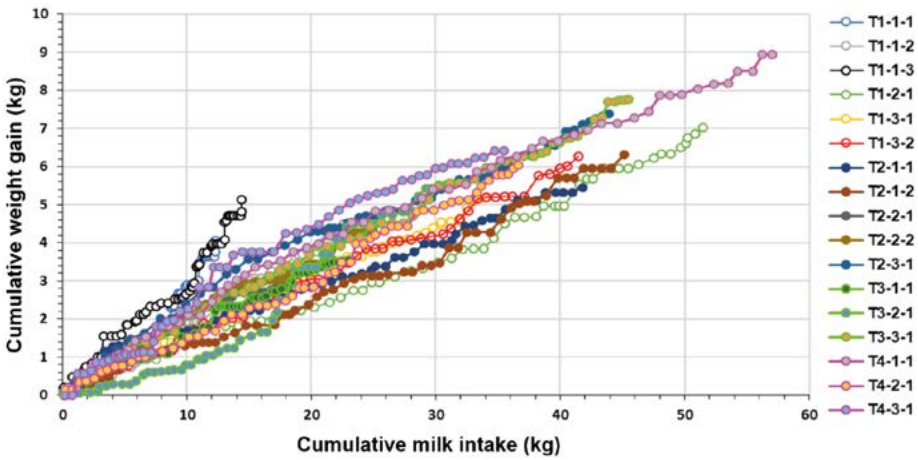

Figure 5. Accumulated weight gain (kg) and accumulated milk consumption (kg), for each kid in each treatment.

the lowest weight gain in the entire experiment (3.26±0.896 kg), with an accumulated consumption of 18.42±5.891 kg of milk and a feed conversion of 5.65±0.811 kg of milk consumption to gain 1 kg of weight during 60 days. On the other hand, T4-1-1 was the kid with the highest accumulated weight gain (8.935±2.603 kg) and the highest accumulated milk consumption (57.05±17.362 kg) during the experimental period. However, its feed conversion was higher than T1-1-3 —*i.e.*, it required a higher milk consumption to gain 1 kg of weight (conversion=6.385±0.636 kg).

No significant differences were found between treatments ($p>0.05$) for both variables (milk consumption and weight gain); however, certain behaviors which require further discussion can be perceived in the evolution of the two variables under study. The results match the findings of Galina *et al.* (2009), who supplemented the diet of kids with a lactic bacterium with probiotic benefits, recording an average daily weight gain of 0.169±0.018 and 0.129±0.022 kg/day in their treatment and in the control, respectively. These values are similar to those found in the evaluated treatments, especially in T1 (ASINC with ORE) and T3 (SINC without ORE). For their part, Williams *et al.* (2018) added dry oregano to the diet of growing kids and likewise found weight gains, both in the control treatment (0.146 kg/d) and in the tested treatments (0.126 kg/d and 122 kg/d), for which the doses of oregano in the diet were increased. The quantities evaluated in the said study were higher than in the present research, although the authors reported that the amount of oregano added did not significantly change food consumption and weight gain. As in the present study, these two authors used weaned kids. The results of this research do not differ from those observed by Avilés *et al.* (2019), who used the suckling-weighing-suckling method to measure the development of kids, using melatonin for the milk production of the mothers and obtaining average weight gains of 0.1-0.18 kg/d. These amounts are similar to those shown in Figure 3 for the total average weight gain. However, the said study reported statistical differences ($p<0.05$). Likewise, García and González *et al.* (2017) determined a positive correlation between milk consumption and weight gain among the kids in their study. A similar situation was observed in the present research.

CONCLUSIONS

In conclusion, including *Lippia palmeri* in the diet and the nutrient synchrony does not significantly impact the development and behavior of the kids under study. However, the results match the findings of other researchers about weight gain and milk consumption through the mothers, which resulted in normal growth values.

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Root density and accumulation of Myrobalan plum tree grafted with Methley Japanese plum

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ABSTRACT
The following variables were analyzed at different soil depths, during one phenological cycle: root density, root dry matter percentage, root accumulation, both growing and absorbing roots, and intermediate and conducting roots. The aim of the study was to determine the root phenology of Myrobalan plum tree grafted with Methley Japanese plum. A quota sampling was used to select five trees from the experimental orchard. Within the volume of soil adjacent to tree's roots, 330 cm^3 of soil were sampled and collected each month, in order to identify root type, quantify their fresh and dry weight, and carry out statistical analyses. The highest densities of growing and absorbing roots were observed at 0-25 cm soil depth during the phenological cycle. A higher density of intermediate and conducting roots was recorded at 25-50 cm soil depth, just at the beginning of the ecodormancy. The highest root accumulation was recorded when moisture and soil temperature were not optimal but the cultivar did not record a significant vegetative and reproductive growth.

Keywords: *Prunus cerasifera*, *Prunus salicina*, root density, root accumulation.

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INTRODUCTION

In the face of climate change and the limitation of natural resources, an interest has arisen to increase agricultural production without increasing crop areas (Lynch, 2007; Gregory *et al.*, 2013). Fruit trees have growing, absorbent, intermediate, and conducting roots (Kolesnikov, 1971), and some of the root functions include: water and nutrient uptake, reserve storage, and substance synthesis (Becerril *et al.*, 2009). Moreover, the roots of fruit species can be found at a soil depth of 10-90 cm, and 30 cm away from the trunk, depending on the vigor of the rootstock, biomass distribution (Ovando *et al*, 1993), rootstock-cultivar combination, soil characteristics, orchard management (Gutiérrez *et al.*, 2006), and mulching and irrigation system (Wei *et al.*, 2002). Furthermore, deciduous fruit trees have three fluxes of root growth in each phenological cycle: 1) before bud swelling; 2) during flowering; 3) and after leaf abscission (Ryugo, 1988; Shaw, 1998). Specifically, the roots of fruit trees from the *Prunus* genus requires between 40 and 50 cm of soil depth (Silva and Alonso, 1976), and they tend to grow right under soil surface (Agusti, 2004). As a result of its successful growth in a wide range of soils (including sandy soils), the Myrobalan

plum tree is frequently used as rootstock for plum cultivars (Popescu and Caudullo, 2016). Nevertheless, unlike the canopy management, the management of the root system of fruit trees is still limited, because of the roots biology has not been entirely understood, and sustainable soil management has not been adapted to improve media conditions related with plant development (Becerril *et al.*, 2009), to achieve an efficient use of soil resources (Thorup and Kirkegaard, 2016) and optimize fertilization (Salazar *et al.*, 2015). Therefore, the phenology of the root as well as the rootstock-cultivar interactions must be studied, in order to determine if root development is a limiting factor and, if that is the case, how to overcome it (Thorup and Kirkegaard, 2016). Consequently, the objective of this study was to determine the root phenology of the Myrobalan plum tree during a phenological cycle.

MATERIALS AND METHODS

Experimental site and plant material. The research was carried out from September 2015 to August 2016, in the Colegio de Postgraduados (19° 29' N, 98° 54' W, and 2,252 m.a.s.l.). The climate is semi-dry temperate, with warm summers, and a 15.2 °C mean annual temperature. The region has summer rains, with a 590 mm mean annual precipitation and a low percentage of winter rains (5%). It has a low thermal oscillation and its annual temperature progress is similar to the Ganges (García, 1988). The orchard is planted with Methley Japanese plum grafted on Myrobalan plum tree. The trees were 4 years-old (first year of a consistent fruit production, with a mean of 14.5 kg tree^{-1}). They were planted in a 4×4 m square planting pattern, with a Tatura trellis. The soil is sandy loam, with 1.95% organic matter, 6.68 pH, and a 46.05% total porosity. Its color is brownish-grey when it is dry and darker grey when it is wet.

Suckers were removed continuously, and weedings were made with a manual weed remover or a chain brush cutter. Empirical fertilizations were carried out evenly spreading granular N, P, and K fertilizer around the trunk of the trees and under organic soil covers. The last ones, were permanently maintained. Soil moisture was monitored using a HH2 Delta-T Devices® (UK); in addition, water was sprayed, using two mini sprinklers per tree (water use: 16 L h^{-1} 40 cm^{-1} h^{-1}), in order to maintain the moisture levels of the soil permanently close to field capacity (FC) (Figure 1).

Minimum temperature (MinT) and maximum temperature (MaxT) of the soil (Figure 2) were recorded daily, at 7:00 am and 3:30 pm, respectively. Both temperatures were measured at a depth of 25 and 50 cm, using a 1,450 mm digital thermometer with a fixed long probe (model 91000-021, Alla France®, France).

Treatments and experimental design. In the first study the treatments were the soil depths; a set of roots extracted at a depth of 0-25 and 26-50 cm, in the same sampling date, were analyzed. In the second study the treatments were certain phenological stages; a set of roots extracted at the same depth, during different phenological stages, were analyzed. A completely randomized experimental design was used. Through a quota sampling, five trees (EU) were selected for this study. The trees were 1.8 m tall and had a 1.7 m canopy, they were healthy and did not suffer nutritional disorders. Monthly, 330 cm^3 root samples were extracted with a California sampler (Figure 3A), at a depth of 0-25 and 26-50 cm, at 40 cm away from the trunk following the cardinal points (Figure 3B). The soil particles

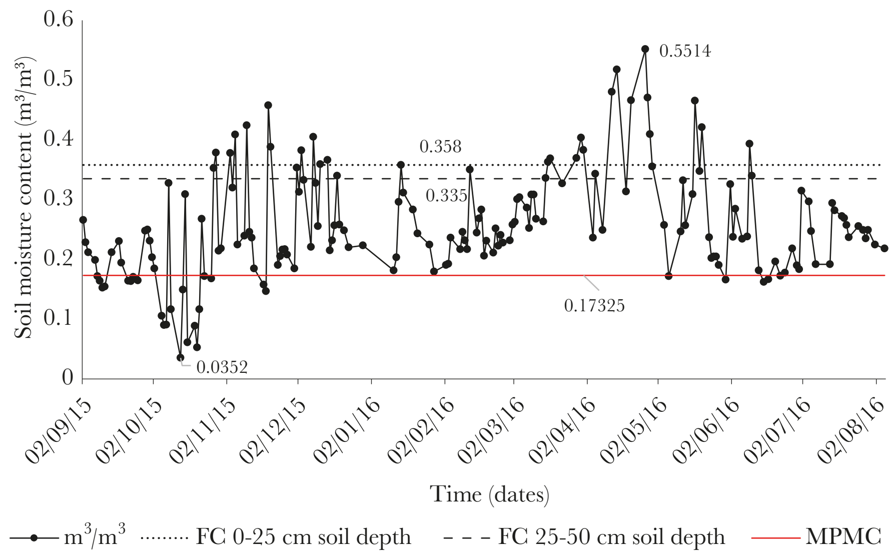

Figure 1. Soil moisture content of an orchard with drip irrigation system, planted with Myrobalan plum tree grafted with Methley Japanese plum.

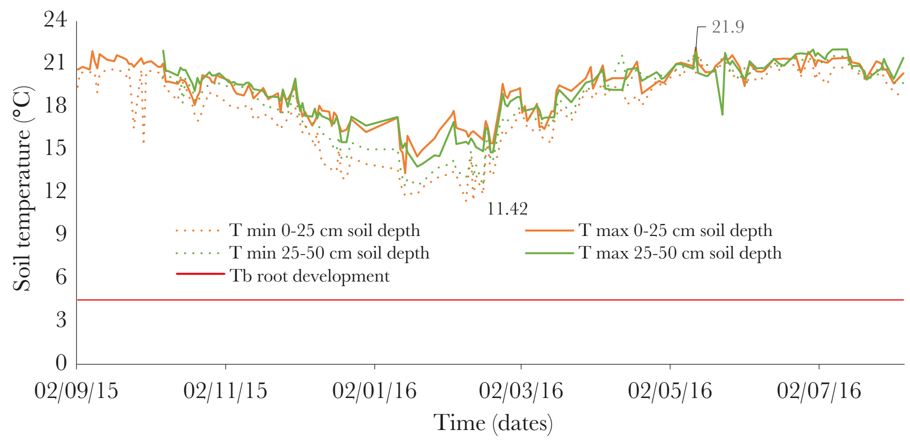

Figure 2. Temperature at a 25 and 50 cm depth in the soil of an orchard planted with Myrobalan plum tree grafted with Methley Japanese plum.

Figure 3. A) Sampling of the roots was made with a California Sampler; B) 330 cm^3 root sample extracted from a specific depth, at 40 cm away from the trunk following the cardinal points.

in the roots were separated using a #2 sieve, following a modified version of the method described by Cossio *et al.* (2008). Subsequently, the roots were washed with running water in the lab. Two groups of roots were created: a group of white growing roots and light brown absorbing roots (≤5 mm thick) (GA) and a group of intermediate and conducting roots (≥ 5 mm thick) (IC) (Becerril *et al.*, 2009; Kolesnikov, 1971).

Response of the variables

Thermal regime of the soil. Considering 4.5 °C as the root base temperature (Kolesnikov, 1971), the MinT and MaxT of the soil were replaced in the Residual Method proposed by Snyder (1985).

Root density. The GA and IC root groups were measured with an EY-2200a digital scale (Asleep®, USA); afterwards, they were dried in a 620 forced air stove (Napco®, USA) for 72 h, at 70 °C, in order to establish dry weight (g). Root density was equal to the g of root dry matter found in 330 cm^{-3}.

Root accumulation. Root accumulation was determined transforming the root densities into relative percentages (Cossio *et al.*, 2008).

Phenology of the cultivar. The phenology stages of the cultivar Methley used in this study were based on González-Pérez *et al.* (2018).

Statistical analysis

In order to determine potential significant differences, an ANOVA and a Student's t-test were used to analyze the depth of the extraction of the roots for the treatment. The data about the phenological stages treatment were analyzed with an ANOVA and a Tukey Multiple Comparison Test (P=0.05). The SAS 9.4 statistical package was used.

RESULTS AND DISCUSSION

The soil moisture content was influenced by the soil texture and the organic matter content of the soil, as well as the irrigation. It fluctuated between FC and MPMC (Minimum Permissible Moisture Content). This condition was appropriate for the growth of roots of fruit trees (Ley, 1994). On the other hand, the color of the soil affected its temperature: darker (wet) soils absorbed a higher solar radiation, and even more warmer as the dates in which sun rays hits the place in a perpendicular angle got closer (Forsythe, 2002). From May to September, soil temperature was closer to the optimum T shape of the root of the plum tree (23-25 °C) (Ley, 1994) and it was permanently higher than the Tb of the root (4.5 °C) (Kolesnikov, 1971). Consequently, the thermal regime of the soil during the study recorded 4,631.8 heat units. This physiological period allowed the formation of root mass and the monitoring of the development of the root during the phenological cycle. This thermal period can replaces the use of the calendar (Snyder, 1985), which does not accurately predict root development (Mendoza *et al.*, 2004; Slafer and Savin, 1991).

Root density. The density of the GA roots during the phenological cycle was higher at a depth of 0-25 cm than at a depth of 26-50 cm (Table 1), as a consequence of the tendency of the plum roots to grow right under soil surface (Agustí, 2004). In addition, mulching and irrigation promote the growth of the root in the upper layer of the soil (Wei *et al.*, 2002), through the increase of temperature and the reduction of water percolation (Zhang *et al.*, 2017). In fact, roots can adapt to the environment through the formation and elongation of lateral roots, which help them to survive changing nutritional conditions (Malamy, 2005). This difference in root density at both depths can be the consequence of the high variability of the vigor of the Myrobalan plum tree (Popescu and Caudullo, 2016) and the distribution and availability of nutrients in the soil (Hodge, 2006).

Meanwhile, the IC roots recorded the highest density at a depth of 0-25 cm, at the beginning of the ecodormancy (21/09/15) and leaf abscission (27/11/15) (Table 1). This biological response was consequence of the caulinar origin of the roots of the rootstocks (given their clonal propagation) and their tendency to develop horizontal roots (Barlow, 1986). In addition, the organic soil cover caused roots to grow closer to the surface (Gutiérrez *et al.*, 2006). During the rest of the sampling dates, similar root densities were recorded in IC roots, at both depths. Concerning these observations, a significant proportion of the total root system is composed of suberized roots (Azcón-Bieto and Talón 2000), which allow to maintain the fruit set (Becerril *et al.*, 2009; Gutiérrez *et al.*, 2006); this explains why the roots of fruit trees are found at a depth of 25-50 cm.

Although the Myrobalan plum tree can successfully grow in sandy soils (Popescu and Caudullo, 2016), no GA or IC roots were found at a depth of >50 cm. These data confirm that an appropriate development of the plum trees requires a depth of 40-50 cm (Silva and Alonso, 1976). Nevertheless, Gutiérrez *et al.* (2006) have reported a different scenario, in which the highest root densities of fruit trees are found at a depth of 10-90 cm.

Regarding root density affected by the phenological stage of the cultivar, GA recorded the greatest root densities during bud swelling (18/02/2016), at both depths (Table 2). These results are similar to root formation before budding (Ryugo, 1988; Shaw, 1998). Consequently, the maximum root growth rates recorded during flowering (Ryugo, 1988; Shaw, 1998) seem to be the result of the roots that appeared during bud swelling and budding. Although the Myrobalan plum tree can successfully grow in sandy soils

Table 1. Root density based on soil depth.

Date	Root density (g 330 cm^{-3})			
	GA 0-25	**GA 25-50**	**IC 0-25**	**IC 25-50**
21/09/15	0.199 a	0.031 b	1.735 a	0.415 b
26/10/15	0.143 a	0.264 a	1.642 a	0.939 a
27/11/15	1.286 a	0.173 b	2.706 a	2.845 a
28/12/15	0.747 a	0.09 b	2.421 a	2.938 a
18/02/16	1.479 a	0.658 a	1.592 a	1.655 a
21/03/16	0.56 a	0.046 a	1.399 a	1.569 a
6/06/16	0.877 a	0.169 b	0.517 a	0.714 a
11/07/16	1.422 a	0.48 b	1.496 a	0.59 b
4/08/16	1.161 a	0.454 b	0.357 a	0.988 a

GA 0-25 = growing and absorbing roots at a depth of 0-25 cm. GA 25-50 = growing and absorbing roots at a depth of 25-50 cm. IC 0-25 = intermediate and conducting roots at a depth of 0-25 cm. IC 25-50 = intermediate and conducting roots at a depth of 25-50 cm. According to the Student's t-test, the values with the same letter are equal for each group of roots (GA, IC) at both depths.

Table 2. Root density based on the phenological stage of the cultivar.

Phenological stage	Root density (g 330cm^{-3})			
	GA 0-25	GA 25-50	IC 0-25	IC 25-50
Beginning ecoletargy	0.199 ab	0.031 c	1.735 a	0.415 a
Beginning endoletargy	0.143 b	0.264 abc	1.642 a	0.939 a
Beginning endoletargy	1.286 ab	0.173 bc	2.706 a	2.845 a
Middle of endoletagy	0.747 ab	0.09 bc	2.421 a	2.938 a
Bud swelling	1.479 a	0.658 a	1.592 a	1.655 a
Full bloom	0.56 ab	0.046 bc	1.399 a	1.569 a
3rd stage of fruit development	0.877 ab	0.169 bc	0.517 a	0.714 a
Foliar abscission	1.422 ab	0.48 ab	0.59 a	1.496 a
Foliar abscission	1.161 ab	0.454 abc	0.357 a	0.988 a

GA 0-25 = growing and absorbing roots at a depth of 0-25 cm. GA 25-50 = growing and absorbing roots at a depth of 25-50 cm. IC 0-25 = intermediate and conducting roots at a depth of 0-25 cm. IC 25-50 = intermediate and conducting roots at a depth of 25-50 cm. Based on the Tukey's Test (P=0.05), values with the same letter are equal for both GA and IC groups, in each phenological stage.

(Popescu and Caudullo, 2016), the lowest root density rates were recorded during the ecodormancy (21/09/2015) and endodormancy (26/10/2015), at a depth of 0-25 and 25-50, respectively.

Meanwhile, no significant differences in IC root density were recorded at both depths (Table 2), during the phenological stages. Most of the root system of woody species is composed of suberized roots (Azcón-Bieto and Talón 2000), which are a permanent conducting structure of the plant (Esau, 1976; Robbins *et al.*, 1976). In addition, they store reserves and absorb water (Becerril *et al.*, 2009), which could have standardized their densities.

Root growth accumulation. From the beginning of both the ecodormancy (21/09/2015) and during the endodormancy (27/11/2015), a constant root accumulation took place in all the different types of roots (Figures 4-7). During full endodormancy (28/12/2015), the accumulation of GA roots decreased, while the IC roots recorded a considerable accumulation. During bud swelling (18/02/2016), the GA roots increased their root accumulation, while the IC roots decreased theirs. These tendencies were the result of the highly variable vigor of the Myrobalan plum tree (Popescu and Caudullo, 2016) and the capacity of the simultaneous primary and secondary growth of the different parts of the tree (Eshel and Beeckman, 2013).

Consequently, the overall maximum root accumulation was recorded from the beginning of the ecodormancy to the bud swelling, when the aerial growth and development decreased. During that period, soil moisture content fluctuated between MPMC and CF, while the temperature was closer to the Tb of the root (4.5 °C, Kolesnikov, 1971) and very distant from the optimal root temperature (23-25 °C, Ley, 1994). These results show that the dormancy of the cultivar is more influential on the root accumulation than soil temperature. These findings are different from those obtained by Ryugo (1988) and Shaw (1998), who pointed out that the root grows more during flowering, regardless of the type of fruit tree. In addition, both authors reported significant root growth fluxes before leaf abscission.

During flowering (21/02/2016), GA recorded a high and a medium root accumulation, at a depth of 0-25 cm and 26-50 cm, respectively (Figures 4 and 5). Meanwhile, the IC roots recorded a medium root accumulation at both depths. This difference in root accumulation is the result of the biomass distribution relationship between organs (Ovando *et al.*, 1993).

During fruit development (06/06/2016) until the harvest ripeness, a medium increase of root accumulation was recorded in GA roots, determined by the presence of fruits (Palmer *et al.*, 1991). These results differ from the findings of León (1994), who reported that the root development of the Japanese plum tree is highly limited by plant activity

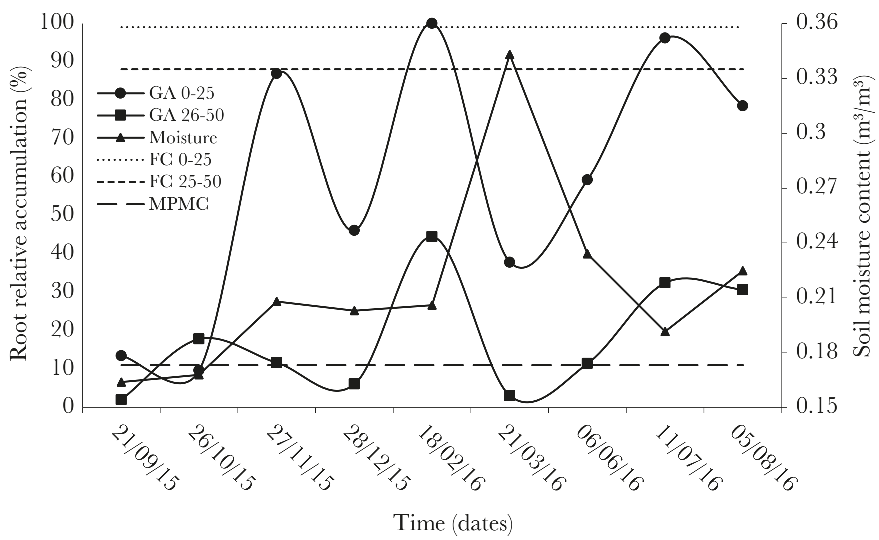

Figure 4. Accumulation of growing and absorbing roots and soil moisture content, from September 2015 to August 2016.

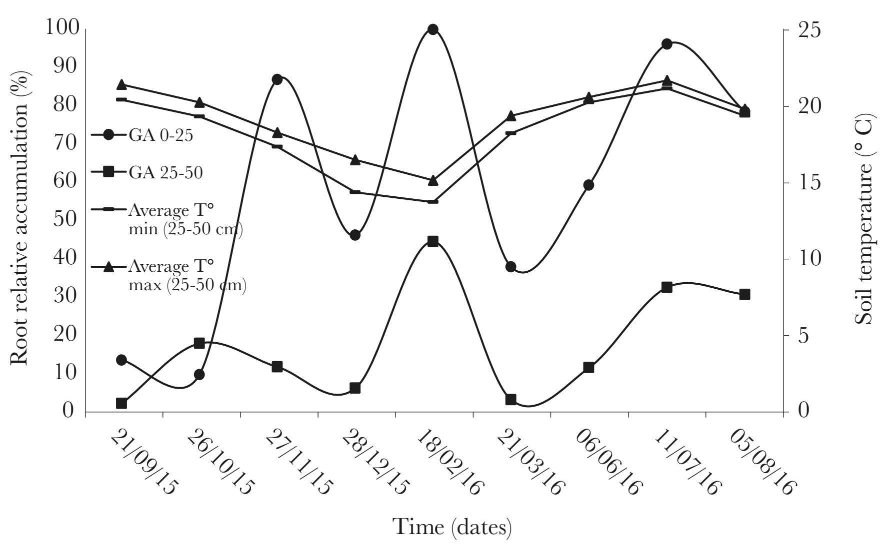

Figure 5. Accumulation of intermediate and conducting roots and soil temperature, from September 2015 to August 2016.

and reproductive processes, as well as root accumulation only occurs in the absence of canopy growth or reproductive stages, or when both have decreased. In this regard, the proportion of photo assimilates aimed to the roots diminishes as the tree bears a greater fruit load (Palmer *et al.*, 1991). This phenomenon was also recorded in this study, despite the appropriate water condition of the soil, which favored root accumulation (Shock *et al.*, 1998). The presence of intermediate roots during the whole phenological cycle (Figures 6 and 7), shows that the growing roots formed in previous phenological phases experienced a secondary growth (Esau, 1976; Robbins *et al.*, 1976). In addition, during the endodormancy, the trees generated the greatest portion of conducting roots, which is normal considering the high vigor of Myrobalan plum (Popescu and Caudullo,

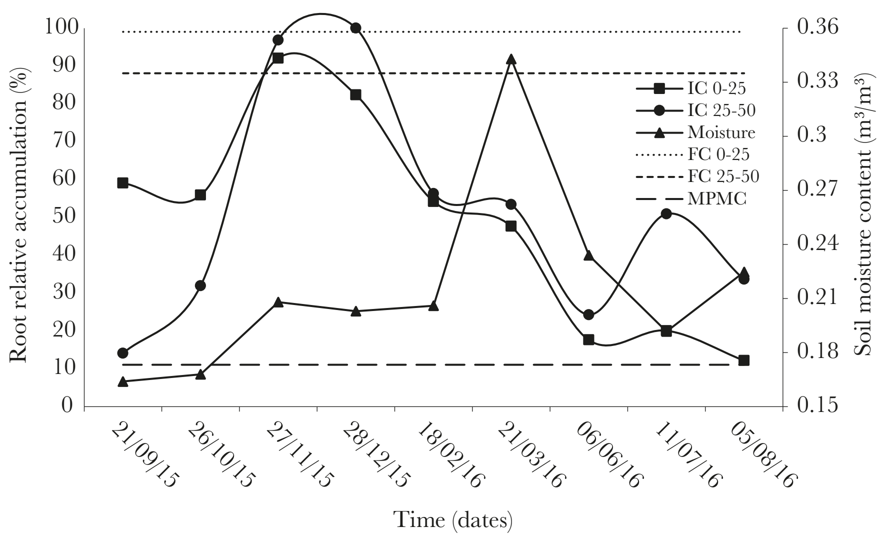

Figure 6. Accumulation of intermediate and conducting roots and soil moisture content, from September 2015 to August 2016.

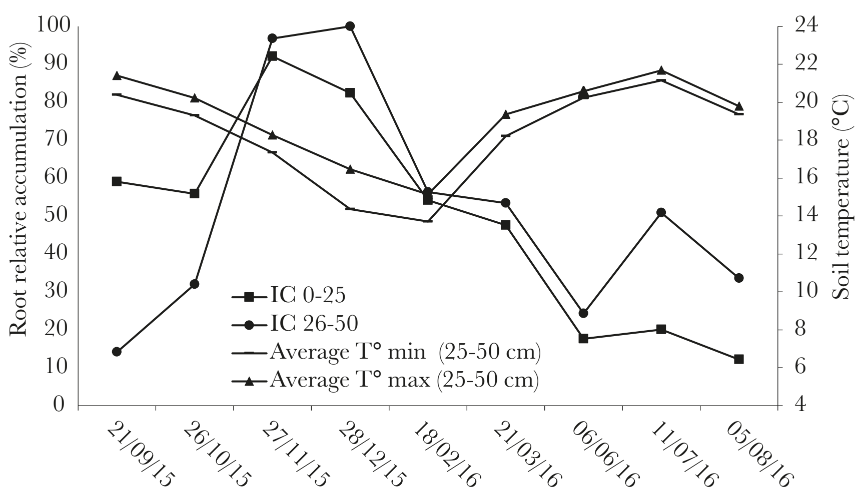

Figure 7. Accumulation of intermediate and conducting roots and soil temperature, from September 2015 to August 2016.

2016). These roots are the anchorage structure of the root system (Esau, 1976; Robbins *et al.*, 1976).

Finally, the uninterrupted generation of root material during the study period —even during the simultaneous phenological stages that took place in the canopy— is important, because roots synthesize cytokines, which carry information about the nutrient status of the root system (particularly nitrogen) to the aerial part of the tree (Sakakibara *et al.*, 1998; Yong *et al.*, 2000).

CONCLUSIONS

The results reported in this study are useful to plan agronomic activities such as fertilization and irrigation. This is due to the punctual identification of development stages where roots carry out primary and secondary growth (with their respective proportions) during the phenological cycle in certain soil depth. In conjunction with development stages of the canopy, for example, flowering or fruit development, allow to have a complete notion about the most appropriated moments to supply nutrients and water.

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Colegio de Postgraduados

Response of chickpea genotypes (*Cicer arietinum* L.) to the fungi complex that causes wilt

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ABSTRACT

Objective: The objective of the present study was to evaluate the response of 10 chickpea genotypes from INIFAP to the damage caused by the fungal complex.

Design/methodology/approach: Seedlings (15-day-old) of 10 genotypes (Blanco Sinaloa '92, Blanoro, Combo 743, CUGA2054, HOGA067, CUGA3168, CUGA08-1210, CUGA09-3160, R-12-1509 and R-12-1507) were inoculated by root immersion in a suspension of mycelial fragments of two isolates (high and low virulence) of each fungus: *Fusarium languescens*, *M. phaseolina*, *S. rolfsii* and *S. sclerotiorum*. Disease severity evaluation was performed 30 days after inoculation. The entire experiment was performed twice.

Results: The genotypes showed greater susceptibility to *S. sclerotiorum* and *S. rolfsii* compared to *F. languescens* and *M. phaseolina*. The highly virulent isolates caused a significant difference in the severity of the disease in the genotypes evaluated.

Findings/conclusions: All chickpea genotypes showed susceptibility to the fungal complex that causes wilt.

Keywords: *Fusarium*, *Rhizoctonia*, *Sclerotinia*, *Sclerotium*, genotypes.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important legume in the world, after bean and pea (Vishruta and Nath, 2021), and it is sown in more than 50 countries (Sunkad *et al.*, 2019). It is the only species of the *Cicer* genus that is apt for cultivation and has the capacity of increasing soil fertility, particularly dry soil through atmospheric nitrogen fixation (Jendoubi *et al.*, 2017). Nutritionally, chickpea is high in protein, dietary fiber and essential minerals, and therefore, it plays a critical role in the fight to reduce hunger and malnutrition in developing countries (Jha *et al.*, 2020; Mwape *et al.*, 2021a). Different biotic and abiotic factors negatively affect the global production of chickpea. Among the main limitations in the production of this crop, there is root rotting and wilting caused by a fungi complex which originates in the soil, including *Fusarium oxysporum*, *Macrophomina phaseolina*, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*, and these depend on the moment of infection and the amount of inoculum in the soil, reducing production in up to 100% (Rai *et al.*, 2022; Kamthe *et al.*, 2023). Wilting caused by *Fusarium* is one of the most severe diseases in this crop, causing losses in yield of up to 100% under favorable conditions for the infection (Rana *et al.*, 2023). The disease has been associated primarily with *Fusarium oxysporum*, although the phylogenetic evidence points to *Fusarium oxysporum* being a compound of cryptic species (Laurence *et al.*, 2014). It should be mentioned that the *Fusarium* genus includes at least 300 phylogenetically different species (Dongzhen *et al.*, 2020). On the other hand, *M. phaseolina* and *S. rolfsii* are present in the entire world, affecting more than 500 species of plants in more than 100 families (Marquez *et al.*, 2021; Napte *et al.*, 2021). Meanwhile, *S. sclerotiorum* is a necrotrophic fungus with a range of hosts of approximately 600 species of plants and can cause up to 100% of losses in chickpea (Mwape *et al.*, 2021b).

The management of root rotting and wilting in chickpea is difficult, since there is not an effective control measure and it should be done through an integrated management program, including the use of varieties that are tolerant to the disease (Khalifa *et al.*, 2022). Because of this, the objective of this study was to evaluate the response from 10 chickpea genotypes from the improvement program of the National Institute for Forestry, Agriculture and Livestock Research (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, INIFAP), to the damage caused by the phytopathogenic fungi complex.

MATERIALS AND METHODS

To prepare the inoculum, two fungal isolates of *Fusarium languescens* (belonging to the complex of the species *F. oxysporum*), *M. phaseolina*, *S. rolfsii* and *S. sclerotiorum* were grown in PDA medium at 25 °C for 10 days. The isolates from each species were distinguished previously according to their level of virulence. In this study, one with high virulence was used and another with low virulence, from each fungus species. The inoculate suspension was adjusted to a concentration of 1×10^5 mycelium fragments mL^{-1} and Tween 20® was added. The roots of the seedlings from the different chickpea genotypes at 15 days of age (Blanco Sinaloa '92, Blanoro, Combo 743, CUGA 2054, HOGA 067, CUGA 3168, CUGA-1210, CUGA09-3160, R-12-1509 and R-12-1507), were inoculated by immersion in the mycelium fragment suspension for 15 minutes. Once the time had passed, the seedlings of the different genotypes were transplanted into pots with sterilized substrate and kept in a greenhouse at temperature of 15 to 35 °C for 30 days. Assessment of the severity of the disease was conducted with a visual scale of 5 categories, where 0=healthy plant, 1≤25%, 2=26-50%, 3=51-75%, 4≥75%. The experiment had a completely random block design with arrangement in divided plots (4 pathogens × 10 genotypes) with 12 replicas. The data obtained were analyzed through analysis of variance and the means comparison was conducted with Tukey's test (P≤0.05) using the statistical package SAS (version 9.3). The varieties were classified through the statistical method of means cluster analysis, for which the fungi isolates and their level of virulence were used as clustering variables.

RESULTS AND DISCUSSION

All the chickpea genotypes evaluated showed susceptibility to the four species of phytopathogenic fungi, and they presented symptoms of yellowing and wilting. These symptoms have been reported in various studies with artificial inoculations of *Fusarium oxysporum* sensu lato, *M. phaseolina*, *S. rolfsii* and *S. sclerotiorum* in the chickpea crop (Manjunatha and Saifulla, 2018; Lamont and Bennett, 2019; Hale *et al.*, 2020; Babariya and Nath, 2021). A significant difference was found between the chickpea genotypes that were inoculated with highly virulent isolates, and no significant difference was observed between the genotypes evaluated with low virulence isolates (Table 1. Severity of the disease caused by isolates from four phytopathogenic fungi with different level of virulence in 10 chickpea genotypes). In addition, difference was found between the isolates of high and low virulence from each of the species of fungi inoculated (Table 2. Means comparison of severity of the disease caused by isolates of four highly virulent phytopathogenic fungi in 10 chickpea genotypes; Table 3. Means comparison of severity of the disease caused by isolates of four pathogenic fungi with low virulence in 10 chickpea genotypes). The difference between the two levels of virulence and between the fungi species evaluated can be because of the different toxins produced by the phytopathogens to invade their host in the different stages of the crop (Rampersad, 2020; Singh *et al.*, 2021).

Table 1. Severity of the disease caused by isolates from four phytopathogenic fungi with different level of virulence in 10 chickpea genotypes.

Genotype	Disease severity	
	Isolates with high virulence	Isolates with low virulence
Blanco Sinaloa'92	3.63 ab*	3.56 a
Blanoro	3.52 b	3.56 a
HOGA067	3.63 ab	3.54 a
Combo 743	3.65 ab	3.42 a
CUGA2054	3.66 ab	3.54 a
CUGA09-3160	3.88 a	3.68 a
CUGA-3168	3.81 a	3.68 a
CUGA08-1210	3.63 ab	3.54 a
R-12-1507	3.65 ab	3.68 a
R-12-1509	3.65 b	3.60 a

*=Means with the same letter in the same column are not significantly different according to Tukey's test (P>0.05).

Table 2. Means comparison of severity of the disease caused by isolates of four highly virulent phytopathogenic fungi in 10 chickpea genotypes.

Genotype	Disease severity			
	F. languescens	***M. phaseolina***	***S. rolfsii***	***S. sclerotiorum***
Blanoro	3.42 abcd*	2.83d	3.83 abc	4.00 a
Blanco Sinaloa'92	3.50 abcd	3.33 abcd	3.75 abc	3.92 ab
Combo 743	3.25 abcd	3.33 abcd	4.00 a	4.00 a
CUGA2054	3.33 abcd	3.50 abcd	4.00 a	3.83 abc
CUGA-3168	3.83 abc	3.58 abcd	3.92 ab	3.92 ab
CUGA09-3160	3.83 abc	3.67 abc	4.00 a	4.00 a
HOGA067	3.42 abcd	3.33 abcd	3.92ab	3.83 abc
CUGA08-1210	3.58 abcd	3.08 cd	3.92 ab	3.92 ab
R-12-1507	3.42 abcd	3.17 bcd	4.00 a	4.00 a
R-12-1509	3.42 abcd	3.17 bcd	4.00 a	4.00 a

*=Means with the same letter in the same column are not significantly different according to Tukey's test (P>0.05).

Table 3. Means comparison of severity of the disease caused by isolates of four pathogenic fungi with low virulence in 10 chickpea genotypes.

Genotype	Disease severity			
	F. languescens	***M. phaseolina***	***S. rolfsii***	***S. sclerotiorum***
Blanoro	3.42ab*	3.00 b	3.83 ab	4.00 a
Blanco Sinaloa'92	3.25 ab	3.75 ab	3.75 ab	3.50 ab
Combo 743	3.08 ab	3.00 b	3.67 ab	3.92 ab
CUGA2054	3.17 ab	3.67 ab	3.50 ab	3.83 ab
CUGA-3168	3.92 ab	3.17 ab	4.00 a	3.67 ab
CUGA09-3160	3.25 ab	3.58 ab	3.92 ab	4.00 a
HOGA067	3.58 ab	3.17 ab	3.67 ab	3.75 ab
CUGA08-1210	3.33 ab	3.50 ab	3.42 ab	3.92 ab
R-12-1507	3.33 ab	3.58 ab	4.00 a	3.83 ab
R-12-1509	3.00 b	3.42 ab	4.00 a	4.00 a

*=Means with the same letter in the same column are not significantly different according to Tukey's test (P>0.05).

The development of withering caused by *F. oxysporum* s. l., in chickpea can be influenced by the virulence, density of inoculate and environmental conditions. The high temperatures are critical for the development of wilting and the severity of the disease is higher at temperatures between 25-30 °C than 15-20 °C (Chen *et al.*, 2016). Regarding *M. phaseolina*, this fungus can cause losses in yields at high temperatures (30-35 °C) and low soil humidity (Rai *et al.*, 2022). Similarly, the disease caused by *S. rolfsii* is important in areas where the seedling is exposed by high temperatures and high soil humidity (Singh *et al.*, 2022). In the case of *S. sclerotiorum*, the disease is favored by temperatures <28 °C and conditions of high humidity (Willbur *et al.*, 2019).

Various studies mention the production of cell wall degrading enzymes by the fungi complex associated to chickpea wilting. In the case of *Fusarium* (FOSC), this production of enzymes allows the formation of a gelatinous mass that blocks the plant's nutrient and water transport system, causing the discoloration of the vascular system of the root (Sunkad *et al*., 2019). Similarly, *M. phaseolina* releases different toxins and enzymes inside the host, interrupting the plant's defense and resulting in dead cells and the establishment of the disease (Rai *et al*., 2022). In the case of *S. rolfsii*, this fungus produces oxalic acid, reacting synergistically with enzymes that cause maceration in the plant's tissue, in addition to inducing the production of oxygen reactive species including the radical superoxide, hydrogen peroxide, and hydroxyl radical, which damage the membrane and destroy cell organelles (Kumari *et al*., 2020b). Meanwhile, *S. sclerotiorum* secretes cell wall degrading enzymes that facilitate the penetration, maceration of the tissue, and degradation of components of the cell wall (Bolton *et al*., 2006).

According to the results, the isolate of *S. rolfsii* with high virulence caused the highest severity of the disease in the chickpea genotypes Combo 743, CUGA-2054, CUGA09-3160, R-12-1507 and R-12-1509, which is because of its prolific mycelium growth and its capacity to produce sclerotia (Shirsole *et al*., 2019). A similar behavior was observed with the isolates of *S. sclerotiorum* toward the genotypes Blanoro, Combo 743, CUGA09-3160, R-12-1507 and R-12-1509, which is due to its known aggressiveness and ability to attack, colonize and cause damage (Mwape *et al*., 2021a). In contrast, the isolate of *M. phaseolina* with high virulence caused lower level of severity of the disease in the genotype Blanoro; however, Sharma *et al*. (2016) mentioned that the pathogenic variability of *Macrophomina* is due to mutations, hyphae fusions, and mitotic recombination (Manjunatha and Saifulla, 2018). Various studies have reported the production of sclerotia by *S. rolfsii* (Shirsole *et al*., 2019), *S. sclerotiorum* (Bolton *et al*., 2006) and *M. phaseolina* (Mirchandani *et al*., 2023), which are resistance structures, as well as primary source of inoculum for the development of the disease. Sharma *et al*. (2016) reported for *M. phaseolina*, a significant relationship between the formation of sclerotia, the density and the severity of the disease. In the case of *F. oxysporum* s. l., it produces chlamydospores, and the soil infested by these structures is a source of primary inoculum for the development of withering (Jiménez-Díaz *et al*., 2015).

Based on the cluster analysis integrated by genotypes in function of each species of phytopathogenic fungus evaluated and their degree of virulence, three groups were observed: cluster 1 which grouped 2 genotypes (Blanoro and Combo 743), cluster 2 which grouped 7 genotypes (BS'92, CUGA-2054, HOGA-067, CUGA08-1210, R-12-1507, R-12-1509 and CUGA09-3160), and cluster 3 which grouped only the genotype CUGA-3168. In this analysis, Blanoro and Combo 742 were the genotypes that presented the lowest degree of susceptibility to phytopathogens, while the genotype CUGA-3168 presented the highest degree of susceptibility (Figure 1).

The management of wilting caused by *Fusarium* (FOSC) is difficult despite crop rotation or application of fungicides, because of the nature of this pathogen which originates in the soil. In the case of *M. phaseolina*, *S. rolfsii*, *S. sclerotiorum*, something similar happens to what has been described for *Fusarium*, because this group of fungi cannot be controlled efficiently through the use of chemical, physical and cultural control due to its wide range

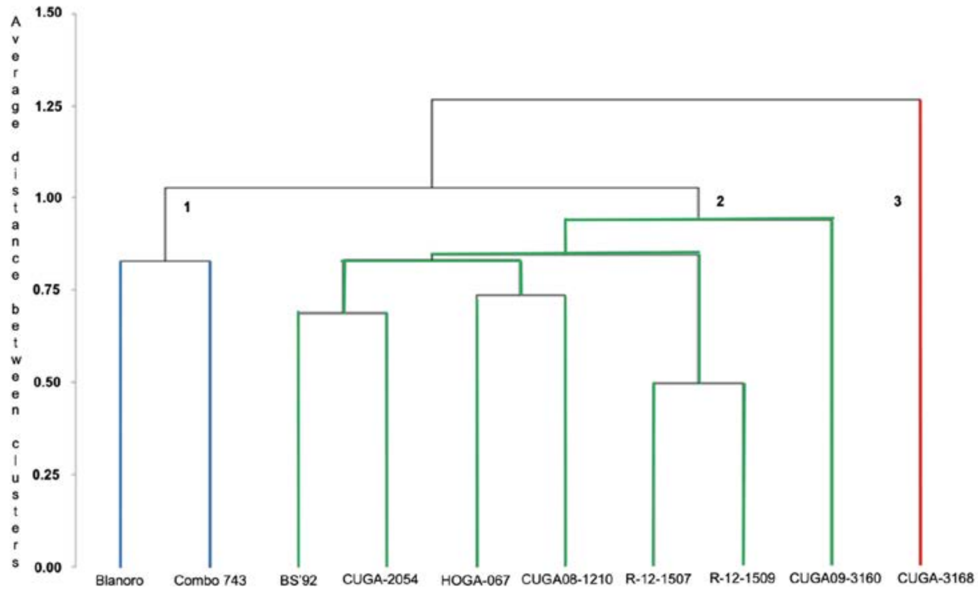

Figure 1. Number of groups integrated between varieties in function of the species of fungi and isolates evaluated.

of hosts and its survival in the soil for long periods of time in form of sclerotia. Therefore, the method with greatest feasible effectiveness and economically viability is the use of tolerant chickpea genotypes (Khalifa *et al.*, 2022; Sheshma *et al.*, 2022).

Diverse studies have conducted the search for sources of resistance to *F. oxysporum* s. l. (Rana *et al.*, 2023), *M. phaseolina* (Mirchandani *et al.*, 2023), *S. rolfsii* (Vishruta and Nath, 2021) and *S. sclerotiorum* (Mwape *et al.*, 2021a) in genotypes of *Cicer* spp.; however, only some genotypes of chickpea resistant to *F. oxysporum* s. l. (Rana *et al.*, 2023) have been found. Therefore, there is a need to continue with the search for sources of resistance to the diverse fungi which originate in the soil that affect the chickpea crop, with the aim of including this resistance to the future chickpea varieties to develop in Mexico.

CONCLUSIONS

The highly virulent isolates of *F. languescens*, *M. phaseolina*, *S. rolfsii* and *S. sclerotiorum* caused higher degree of severity of the disease in the different chickpea genotypes, compared to the damage caused by the isolates with low virulence.

The isolates of *S. rolfsii* and *S. sclerotiorum* caused greater severity of the disease in the different genotypes of chickpea compared to the isolates of *F. languescens* and *M. phaseolina*.

The genotypes of Blanoro and Combo 743 were the ones that presented lower level of susceptibility in response to the infection caused by the four species of phytopathogenic fungi, while the genotype CUGA-3168 was the most susceptible among the 10 genotypes evaluated.

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Colegio de Postgraduados

Effect of spontaneous micro-fermentation on the physicochemical characteristics of *Theobroma cacao* L. beans

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ABSTRACT

Objective: To evaluate the physicochemical characteristics of cacao beans during the spontaneous fermentation process in tepemixtle wooden boxes of three different sizes.

Design/Methodology/Approach: Trinitarian-type cacao (*Theobroma cacao* L.) fruits were collected in a commercial orchard. The beans were fermented in boxes with the following operating conditions: volume of cacao with pulp, box size, a six-day period, and uncontrolled temperature and pH. The experiments were carried out in triplicate and an analysis of variance and a multiple comparison of means were performed with the Tukey test ($p<0.05$) using the R program.

Results: The parameters evaluated had the same behavior in the 4 kg box than in the 25 kg box and the 60 kg box, which indicates that the physicochemical and quality characteristics of cacao beans are not impacted by the decrease in the box size. Therefore, smaller boxes are viable for small-scale fermentations.

Study Limitations/Implications: Only cacao beans from the Trinitarian genetic group were evaluated.

Findings/Conclusions: Small-scale fermentations can be an option for the evaluation of varieties when the beans must be fermented for the sensory analysis of their qualitative attributes. This micro-fermentation methodology can also be incorporated into genetic improvement programs or the evaluation of the processes aimed at their optimization.

Keywords: color, quality, small scale.

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INTRODUCTION

Chocolate, cocoa, nibs, and other products are result of the fermentation of cacao (*Theobroma cacao* L.) beans. The flavor of cacao depends on multiple intrinsic and extrinsic factors that are involved before and after its harvest (Engeseth and Ac Pangan, 2018). Cacao beans are composed of an embryo and two cotyledons, they are surrounded by a mucilaginous pulp that can vary depending on their origin, and they have a bitter and astringent taste after being harvested (Dominguez-Perez *et al.*, 2020; Alexander J. Taylor, 2022; Rawel *et al.*, 2019; Santander *et al.*, 2019). Cacao pulp is made up of sugars (glucose, fructose, and sucrose), citric acid, and pectin, which serve as substrates

for the microorganisms involved in fermentation. The high polyphenol content of cacao beans causes an astringent, bitter, and unpleasant flavor, which decreases to 5% during the fermentation process (Afoakwa *et al.*, 2008; Dominguez-Perez *et al.*, 2020). Therefore, cacao beans must undergo a post-harvest processing that comprises the fermentation, drying, and roasting stage to develop precursors that provide desirable aroma and flavor profiles (Rawel *et al.*, 2019; Dominguez-Perez *et al.*, 2020).

Spontaneous fermentation accomplishes three main tasks: (i) decomposition and reduction of the pulp surrounding the beans; (ii) formation of volatile organic compounds, which reduce bitterness and astringency; and (iii) hydrolytic reactions within the cotyledons (Amoa-Awua, 2015).

Traditionally, the fermentation method implemented by producers involves two transformation phases: (1) anaerobic and (2) aerobic. The length of these phases and the frequency of aeration of the mass in the aerobic phase varies depending on the producer (Escobar *et al.*, 2019). All the biochemical reactions that can take place inside the beans in both phases are determined by the action of microorganisms, whose biocatalytic activity generates physical and chemical transformations in the cacao beans. These microorganisms are significantly influenced by the transfer of oxygen through the aeration of the cacao bean mass, the transfer of the mass from the acids and ethanol (metabolites produced by microorganisms) to the bean, and the heat transfer caused by the exothermic reactions; together, these phenomena modulate the formation of flavor precursors (Santander Muñoz *et al.*, 2020). This process is conventionally called spontaneous fermentation and is carried out by cacao producers.

Nowadays, most cacao is fermented spontaneously by producer cooperatives as an uncontrolled and non-industrialized bioprocess (John *et al.*, 2020, Rottiers *et al.*, 2019). A prolonged process can produce unpleasant flavors, as a result of the increase in bacilli and filamentous fungi (Moreno-Zambrano *et al.*, 2018; Mota-Gutiérrez *et al.*, 2018). Fermentation takes place for 5 to 7 days, depending on the variety and the climate of the place (Schwan and Fleet, 2014; Schwan and Wheals, 2004). Overall, spontaneous cacao fermentations take place in approximately 100-kg wooden boxes, in containers made from some unusual materials (such as buckets or plastic trays), or in piles on the ground. Each producer cooperative has its own practices and preferred styles, which differ by region, culture, and fermentation, drying, and roasting times (Levai *et al.*, 2015). Nevertheless, cacao fermentation remains a successful strategy for the improvement of product quality (J. Taylor *et al.*, 2022). However, most small-scale producers in southern Chiapas, whose main product is chocolate, do not ferment the cacao beans; consequently, they must add three times more sugar to reduce the bitter and astringent taste of the washed cacao bean. This problem is caused by the producers' lack of knowledge about the fermentation processes and the small size of their plots. Some cooperatives work with 100 kg of cacao, which could hardly be fermented in such plots.

Meanwhile, breeding programs that use individual plants of the varieties under study must ferment the grains for the sensory analysis of their qualitative attributes (Clapperton *et al.*, 1994). Often, only small quantities of cacao beans are available in evaluation, validation, or conservation plots (approximately 3 to 4 kg per plant). Micro-fermentation methods are particularly useful to research fermentation process variables (*e.g.*, temperature, pH, moisture), as well as to identify microorganisms with biotechnological potential. These types of processes facilitate the evaluation work, because they do not require large quantities of cacao. Therefore, the objective of this research was to evaluate the physicochemical characteristics of cacao beans during the spontaneous fermentation process in *tepemixtle* (*Ocotea veraguensis* (Meisn) Mez) wooden boxes of three different sizes.

MATERIALS AND METHODS

Raw material

Cacao fruits from the Trinitarian genetic group were harvested ripe, without mechanical or biological damage, in the plots of producers from Tuzantán, Chiapas, Mexico. They were subsequently transferred to the post-harvest laboratory facilities, where they were washed with water and sprayed with 70% alcohol, before they were opened and the cacao beans with pulp were removed.

Fermentation and drying

The beans with pulp were fermented under a completely randomized design, using *tepemixtle* wooden boxes of three sizes. Box 1 measured 15×15×15 cm and had a capacity of 4 kg; box 2 measured 30×30×30 cm and had a capacity of 25 kg; and box 3 measured 45×45×45 cm and had a capacity of 60 kg. The boxes were covered with a black polyethylene sheet and tied with rope to avoid temperature losses. The operating conditions over the course of 6 days were uncontrolled temperature and pH. Sampling was always carried out at the same time (every 24 h) for each fermentation time (24, 48, 72, 96, 120 and 144 h), the mass was turned over and a 50 g sample was extracted to carry out the physicochemical analyses. The evaluations were carried out in triplicate.

After fermentation, the cacao beans were spread on wooden trays for drying, moved approximately every 4 h each day to facilitate evaporation, until they reached 7% water content. Samples were stored in hermetically sealed containers.

Fermentation monitoring

During fermentation, the temperature was measured every 24 h, placing the thermometer at half the height of the box and taking data at five points. The following data were likewise recorded for the cacao beans with pulp: change in pH (AOAC 981.12), total titratable acidity (AOAC 939.05), moisture, and ash (AOAC 972.15).

Moisture determination

Three g of bean with pulp were weighed and placed in a Yamato® convection drying oven for 24 h at 60 °C. Subsequently, they were weighed again and the moisture content of the grain and pulp was determined by gravimetric analysis.

Determination of oil content

To determine the oil content, 3 g of dried and ground cacao beans were weighed and placed in a cartridge to start the extraction process. The Soxleth method was followed

for this purpose, using hexane for 4 h at 70 °C. Subsequently, the excess hexane was removed with a Heidolp® rotary evaporator and the oil sample was poured in a flask and subsequently placed in an oven at 60 °C for 24 h to eliminate hexane traces. The oil content of the sample was determined by gravimetry. The experiments were carried out in triplicate. The analysis of variance and multiple comparison of means were carried out with the Tukey test ($p \leq 0.05$), using the R program (Enio *et al.*, 2014; R Core Team, 2018).
2.6 Colorimetric determination

The color of the cacao beans was observed throughout the process. Color measurements were taken from three experimental units, from which three cacao beans were extracted at random. A longitudinal cut was made to examine and grade their internal state (Chire *et al.*, 2016). The L*, a*, and b* readings were recorded using a colorimeter. The ΔE* color changes were calculated by the equation:

$$\Delta E^* = \left[(\Delta L^*)2 + (\Delta a^*)2 + (\Delta b^*)2\right]^{1/2}$$

In each experimental unit, the average color (L*, a*, and b*) was measured every 24 h throughout the fermentation process.

Statistical analysis

All analyses were performed in triplicate. An analysis of variance and a Tukey's mean comparison test were applied to the results with the R statistical program (Enio *et al.*, 2014; R Core Team, 2018).

RESULTS AND DISCUSSION

Physicochemical characterization of cacao beans during the spontaneous fermentation process

Figure 1 shows the behavior of temperature and pH of the three box sizes throughout time. The initial average temperature of spontaneous fermentation was 32 °C and gradually increased until it reached its maximum level at 72 h: 40.3 in box 1, 42.6 in box 2,

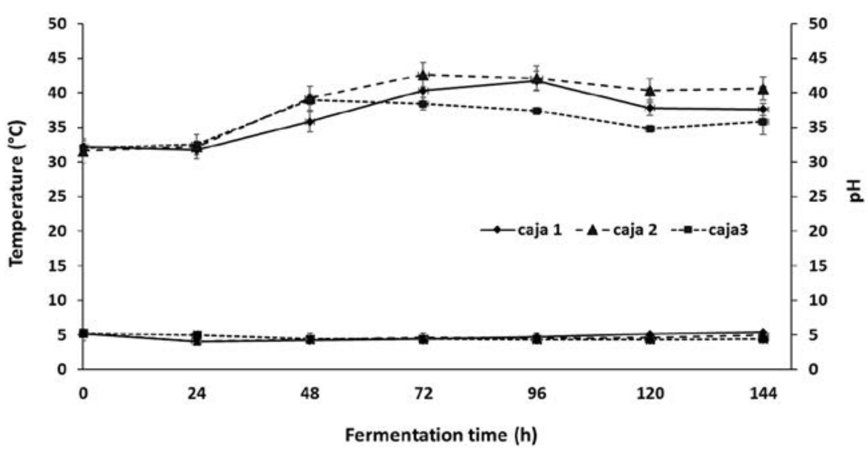

Figure 1. Behavior of temperature (°C) and pH of grains with pulp, during 6 days of spontaneous fermentation, in three wooden boxes of different sizes.

and 38.4 °C in box 3. Although the three boxes behaved similarly during the spontaneous fermentation process after 72 hours, box 1, despite having the smallest dimensions, reached higher temperatures than box 3. The constant increase in temperature may be associated with the release of heat throughout the whole process. This release may be caused by the conversion of the available fermentable substrate (sugars) into the desired metabolite (Melo *et al.*, 2020). With an adequate fermentation, the temperature of the bean mass should reach 45 to 48 °C in approximately 72 h (Melo *et al.*, 2020). The temperatures throughout the process favor the production of ethanol and the death of the bean embryo (Crafack *et al.*, 2014). The production of ethanol allows the succession of microorganisms (such as acetic acid bacteria), responsible for oxidizing ethanol into acetic acid. According to García *et al.* (2019), low temperatures during fermentation can be caused by the type of bioreactor material and the amount of grain with pulp. These authors recommend filling the fermentation boxes to the brim to avoid heat losses.

In the three box sizes evaluated, a stable pH was observed, with similar behavior during the sampling days. The initial pH was 5.17±0.057 for box 1, 5.2±0.01 for box 2, and 5.27±0.057 for box 3. The pH decreased as the days of fermentation increased, although box 1 recorded a slight increase towards the end of the fermentation (5.42±0.01). This phenomenon can be correlated with the production of organic acids (lactic acid, acetic acid, citric acid, among others) by fermenting microorganisms (Balogu *et al.*, 2014). These organic acids decreased the pH of treatment 2 (box 2) and treatment 3 (box 3), which could potentially vary the microbial profile and the population dynamics of the fermentation system and possibly prevent the microorganisms from generating the appropriate acids and conditions that enable them to enter the grain (Balogu *et al.*, 2014). Santos *et al.* (2020), M. D. P. López *et al.* (2019), and Balogu *et al.* (2014) recorded a dynamic pH range (6.64-3.52), which was similar to the ranges reported in this study. The variations observed are perhaps due to the different treatments, geographical areas, and varieties of cacao beans.

Figure 2 shows the dynamics of the oil content and ash content throughout time. The oil content started at 36.45±0.165 in box 1, 40±0.09 in box 2, and 39.22±1.15 in

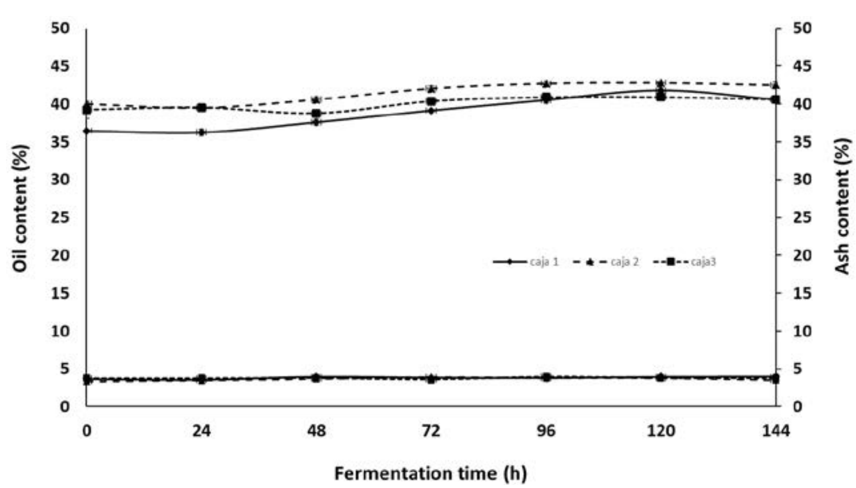

Figure 2. Behavior of oil (%) and ash (%) content, during 6 days of spontaneous fermentation, in three sizes of wooden boxes.

box 3. Throughout the days of fermentation, the three treatments had a similar behavior and had a slight increase in their oil content, resulting in 40.57±0.2362 in box 1, 42.49±0.358 in box 2, and 40.62±0.158 in box 3 at the end point of fermentation (144 h). In relation to this variable, Guehi *et al.* (2007) mention that the fat content of cacao is often quantified and assumed to be stable based on two facts: 1) that, with the exception of lipase activity, fermentation only affects hydrophilic compounds or bonds; and 2) the low risk of oxidation of cacao butter, as a consequence of the low content of unsaturated free fatty acids. This oxidation stability has been confirmed during the shelf life of cacao butter (Hu and Jacobsen, 2016). However, fermentation leads to modifications such as temperature increase, alcohol formation, grain acidification, or modification of enzymatic activities (that can lead to lipid degradation), enzyme breakdown, hydrolysis, or oxidation. Regarding modifications to fats during fermentation, Ndife *et al.* (2013) described a decrease in fat content, while Krähmer *et al.* (2015), Servent *et al.* (2018), and K.T. Dewandari *et al.* (2021) reported an increase in fat content. In terms of plant tissue, lipases are also known to degrade triglycerides, while the optimal pH of cacao lipase activity is reached during cacao fermentation, due to the production and spreading of acetic acid (Servent *et al.*, 2018).

Meanwhile, the ash content showed a stable behavior during the fermentation of the three boxes, with a slight increase. The starting values were 3.67±0.40 in box 1, 3.34±0.11 in box 2, and 3.76±0.31 in box 3. Finally, after 144 hours of fermentation, the final values were 3.93±0.163 in box 1, 3.54±0.38 in box 2, and 3.78±0.03 in box 3. This change is perhaps due to the formation of bioactive compounds that are triggered during the spontaneous fermentation process (K.T. Dewandari *et al.*, 2021).

The moisture percentage of fresh beans with pulp does not have statistically significant differences ($p \leq 0.05$) between treatments, during the 48 h of fermentation. Box 3 and boxes 1 and 2 have differences at 72, 96, and 120 h (Figure 3). Furthermore, at the end of fermentation, a 16.83%, 23.68%, and 16.60% moisture loss can be observed in box 1, box 2, and box 3, respectively (Figure 3). Similar results were obtained by García *et al.*,

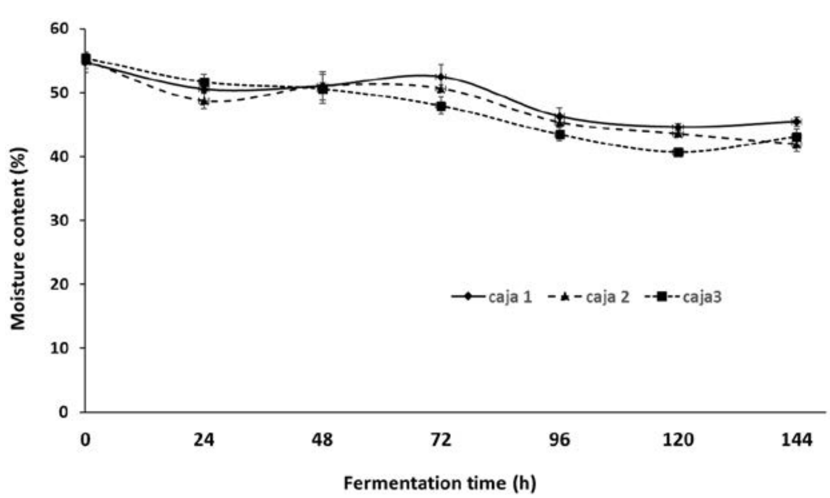

Figure 3. Behavior of moisture content (%), during 6 days of spontaneous fermentation, in three sizes of wooden boxes.

(2019), who reported a 59.5 to 53.8% moisture decrease —*i.e.*, 10% at the end of the 96 h fermentation in wooden boxes.

Color analysis

The relationship of color change (ΔE) of cacao beans in the three treatments during the spontaneous fermentation process was studied. Table 1 shows no significant difference ($p>0.05$) between the treatments; however, a progressive increase in color change (Table 1) and a decrease in luminosity were recorded, from non-fermented samples to completely fermented samples. The cacao beans turned darker as fermentation increased. According to Afoakwa *et al.* (2011), these changes may be caused by the degradation of anthocyanins resulting from enzymatic hydrolysis, which is accompanied by the bleaching and subsequent darkening of the cacao beans. The color change in the cotyledons of cacao beans during fermentation is a response variable that indicates the degree of fermentation (Marciano *et al.*, 2021). The cut test is the simplest and most widely used method to visually evaluate the quality of a random sample of the beans from a plot (Kongor *et al.*, 2013). However, even if it is carried out by trained personnel, it is considered a subjective method and, despite its ease of use, it is difficult to standardize (Kongor *et al.*, 2013). An accurate measurement technique is the CIELAB L*, a*, and b* system. Chire *et al.* (2016) reported color changes of 15.6 after three days of fermentation; those results are similar to those identified in this research.

Table 1. Color change (ΔE) every 24 hours, during 6 days of fermentation.

Treatments	Hours					
	0	24	72	96	120	144
Box 1	7.945±1.155ab	13.644±2.754a	12.407±1.819a	14.570±1.211b	17.129±0.783a	17.269±2.248a
Box 2	6.057±0.855b	15.758±1.743a	14.014±3.236a	18.992±1.556ab	15.138±1.889a	14.089±3.040a
Box 3	10.933±2.450a	14.780±1.224a	16.332±3.061a	16.961±1.089a	17.098±0.956a	17.969±1.455a

CONCLUSIONS

The use of small-scale spontaneous fermentations can be an option for varieties whose beans must be fermented for the sensory analysis of their qualitative attributes. Additionally, this micro-fermentation methodology can be incorporated into genetic improvement programs or in the evaluation of the processes aimed at its optimization.

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Colegio de Postgraduados

Assessment of tepojal as a support medium in *in vitro* germination of *Barkeria whartoniana* (Orchidaceae) and subcultures of *B. uniflora* and *B. scandens*

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ABSTRACT

Objective: We evaluated the use of tepojal as a substitute for bacteriological agar for *in vitro* culture of three species of Mexican orchids of the genus *Barkeria*. We had three objectives: (1) evaluate the use of tepojal for the *in vitro* germination of *B. whartoniana* seeds; (2) compare the growth and survival of *B. uniflora* and *B. scandens* plants in *in vitro* culture on bacteriological agar and tepojal, and (3) compare the early *ex situ* survival of the *B. uniflora* and *B. scandens* previously cultivated *in vitro* on bacteriological agar and tepojal.

Design/Methodology/Approach: For the growth experiment, 1,050 seedlings of *Barkeria scandens* and *B. uniflora* were used. Two types of culture medium were prepared: (1) Liquid with tepojal and 40% MS medium with dextrose, yeast, coconut water, activated carbon and refined sugar, and (2) solid at 40%, with the same elements as the liquid one, but with 6 g of bacteriological agar. For the germination experiment of *Barkeria whartoniana* in tepojal we use seeds from a mature (open) capsule. After disinfection, seeds were sown in tepojal with liquid medium and in a solid (agar) culture using a syringe technique. All the seeds were sown in 100% p668 MS medium with 100 mL/L of coconut water and 15 g/L of refined sugar and using in both the MS medium.

Results: *Barkeria uniflora* seedlings had a greater growth than in *B. scandens* regardless of the type of treatment. When comparing within each species, we found that the two treatments (tepojal *vs.* agar) did not produce differences in the growth of the shoot of both species. Roots growth was influenced by both the effect of the species and the treatment, as the longest roots were found in tepojal medium. Seed germination observed in the liquid medium with tepojal was not apparently different from that in agar. In both cases the entire surface of the jar was covered with green protocorms.

Findings/Conclusions: Tepojal did function well as a substrate to germinate *Barkeria whartoniana*, but tests must continue to be carried out to evaluate its effectiveness. As no differences were found for aerial growth between agar and tepojal in *Barkeria scandens* and *Barkeria uniflora*, we consider this substrate as a good substitute for agar because it can produce more biomass per gram of culture medium used; therefore, if we consider the costs the use of tepojal is much cheaper and much easier to get.

Keywords: Agar substitution, *in vitro* culture, *ex vitro* survival, orchid.

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INTRODUCTION

Many efforts have been made in the last 30 years to find a suitable substitute for bacteriological agar due to its high cost, low accessibility, and high demand (Jain-Raina and Babbar 2011). However, a noneffective substitute has been found (Khoobbakht *et al.*, 2024), particularly as a support medium for *in vitro* orchid cultivation. Bacteriological agar is a material that is not easy to find in the market and expensive for small producers (McGuffey *et al.*, 2018), for which there is a continuous search for alternatives to replace it (Gordo *et al.*, 2012; Herrera-Toledo *et al.*, 2013; Sánchez-Cardozo, 2019).

Agar is mainly extracted from marine Rhodophyta; however, populations of these wild algae are vulnerable due to massive extraction (Porse and Rudolph, 2017). Agar is composed by agarose and agaropectin, and it is highly prized because it remains gelled at room temperature and it has high clarity and is harmless to organisms being cultivated (Jain and Babbar, 2005). Some commercial products, such as Phytagel, Gelrite and Natugel, have been used as substitutes for agar. Nevertheless, these fail to equalize the firmness that bacteriological agar achieves. Also, plants being cultivated in them have greater oxidation problems and higher mortality. Other substances with gelling properties such as starches, gums, and polymers, both from plants and bacteria have also been tested (Ozel *et al*., 2008; Gordo *et al*., 2012). These substances have reduced production costs of *in vitro* culture media up to 70% but fail to reproduce the same results as agar (Fujiwara *et al*., 1993).

Because suitable gelling agents have not been found, some culture media made with materials that make a solid structure, but which have the liquid nutrient medium, have been tested. For example, culture media have been made with coconut, cotton and betel nut fibers, starches like sweet potato, corn and potato, polyurethane foam, chopped litter leaves, isubgol, some gums such as guar, gellan, katira and cassava flour have shown good results (Moraes-Cerdeira *et al*., 1995; Afreen-Zobayed *et al*., 2000; Jeyaseeli and Raj, 2010). For orchids, some of these substrates have been used for in vitro germination for some genera such as *Cymbium*, *Cattleya* and *Stanhopea* (Deb and Pongener, 2010; Aggarwal and Nirmala, 2012). Recently, tezontle has been used together with coconut fiber in *in vitro* cultivation of orchids (Flores-Hernández *et al*., 2017).

Tepojal is a vitreous extrusive volcanic rock. It is a light inert mineral, with a neutral pH. In agriculture it is widely used in the preparation of substrates for various crops since it facilitates aeration and produces light substrates. Tepojal, as an inert rock, does not generate changes in the chemical composition of the culture medium and it does not weather easily either with heat or with water. This is important, as it remains intact during sterilization procedures (even autoclaved) and during the time it is used as an *in vitro* culture medium.

In this study, we evaluated the use of tepojal as a substitute for bacteriological agar for *in vitro* culture of three species of orchids of the genus *Barkeria*. We had three objectives: (1) evaluate the use of tepojal for the *in vitro* germination of *B. whartoniana* seeds; (2) compare the growth and survival of *B. uniflora* and *B. scandens* plants in *in vitro* culture on bacteriological agar and tepojal, and (3) compare the early *ex situ* survival of the *B. uniflora* and *B. scandens* previously cultivated *in vitro* on bacteriological agar and tepojal. We expected that, due to the properties of tepojal, there would be no differences between the cultures of both species in tepojal and bacteriological agar.

METHODS

Study species

The genus *Barkeria* has 16 species of which 12 are endemic to Mexico. It is distributed along the Pacific coast and their main habitat is deciduous forests (Soto-Arenas, 2005). Of the 16 species of the genus, seven are within a category of NOM-059, among them *B. scandens* and *B. whartoniana* are in special protection. This genus is composed by herbs with thickened stems or small pseudobulbs, they have thick roots that tend to form dense masses, they are epiphytes or lithophytes with cespitose or scandent growth. Their inflorescence is terminal, flowers are 2 to 5 cm in size, and their leaves are deciduous.

Selection and sterilization of tepojal

Tepojal was purchased at a gardening store in the Madreselva market in Xochimilco, Mexico City. For subcultivation in tepojal, particles the size of 2 mm in diameter was used, while for the germination experiment in fine tepojal, smaller particles were used. The 2 mm thick tepojal was washed in running water until the water was clear and dried by placing it under direct sunlight in porous, plastic trays. Once dry, the tepojal was placed in 90 g glass jars with plastic screw caps and 300 ml of distilled water. The jars with tepojal were autoclaved two times (one with only tepojal and other with liquid culture medium) (Felisa) at 120 °C and a pressure of 1.5 kg/cm^2 for 20 min. After that, they were sealed with self-adhesive plastic and stored on shelve.

Preparation of solid culture medium for subcultures

To make a solid culture medium, a commercial preparation of Murashige and Skoog medium (M 519) at 40%, 1 g/L of dextrose, 1 g/L of yeast, 100 ml/L of coconut water, 2 gr/L of activated carbon, 15 gr/L of refined sugar was used. The medium was prepared on a shaker rack in beakers using distilled water. At the end of this process the pH was adjusted to 5.6±0.2, either with HCL (hydrochloric acid) or NaOH (sodium hydroxide) both at 1 N. Once the culture medium was prepared and the pH adjusted, the solution was heated to the boiling point and 6 g of bacteriological agar were added little by little until it was completely diluted. 50 mL of this culture medium were immediately poured into 90 g glass bottles with plastic screw caps. The vials with the culture medium were sterilized the same way as tepojal. After that, jars were cooled outdoors, shaking them from time to time so that the carbon remained uniformly suspended in the medium. Once the culture media had gelled, the glass bottles were sealed with plastic wrap and stored until use (less than a week).

Liquid medium with tepojal for subcultures

The liquid culture medium was prepared in the same way as the solid culture medium, except that it was not brought to a boil because bacteriological agar was not added. In 90 g jars with 75 g of sterilized 2 mm diameter tepojal, 35 ml of the liquid medium and sterilized in an autoclave at 120 °C and a pressure of 1.5 kg/cm^2 for 17 min. During the pouring process, the culture medium was stirred to keep the activated carbon suspended. Once the vials came out of the autoclave, they were sealed with plastic wrap and stored.

Vegetative material

Seeds from *Barkeria scandens*, *B. uniflora* and *B. whartoniana* were obtained from a mature capsule cultivated in the "Miguel Ángel Soto Arenas" orchidarium at the Faculty of Sciences, UNAM. Propagules from *Barkeria scandens* and *B. uniflora* were germinated in 40% solid MS medium and allowed to grow for 6 months before the experiment. For *B. whartonianan* seeds were stored for two months until the germination experiment. A total of 1,050 seedlings of *Barkeria scandens* and *Barkeria uniflora* were used for the growth experiment.

Seed disinfection

Disinfection was performed with a 15% (v/v) sodium hypochlorite solution (domestic bleach) for 15 minutes, stirring the seeds constantly under aseptic conditions in a laminar flow hood, 3 rinses were performed with sterile distilled water.

Germination of *B. whartoniana* seeds

After disinfection, seeds were sown in liquid and solid culture using a syringe technique. 1 ml of the seed solution was placed in each vial. All the seeds were sown in 100% p668 MS medium with 100 mL/L of coconut water and 15 g/L of refined sugar. 6 g of agar were used, and 30 ml of medium were occupied in each bottle for the solid culture medium. For the liquid culture medium with tepojal, 25 mL of the nutrient solution and 15 g of fine-grained tepojal were used. The seeds were incubated at 25 °C, under a photoperiod of 16 h light/8 h dark.

Growth experiment

Two types of culture medium were prepared to evaluate the use of tepojal as a substitute for bacteriological agar in the development (growth and survival) of *Barkeria scandens* and *B. uniflora* seedlings. The first was liquid with tepojal and 40% MS medium with dextrose, yeast, coconut water, activated carbon and refined sugar. The second was solid at 40%, with the same elements as the liquid one, but with 6 g of bacteriological agar. Seedlings of both species were planted in solid culture medium (with agar) and in liquid culture medium with tepojal as a substrate. In total, 15 randomly selected plants were taken from a previous culture in solid MS medium. At the beginning of the experiment 140 seedlings were randomly selected inside the laminar flow hood and measured using a laminated millimeter paper (previously superficially sterilized with 70% alcohol). The seedlings were measured at the beginning and at the end of the experiment, taking two growth measures: (1) length of the shoot from the base of the stem to the apex of the longest leaf and (2) the length of the root from the base of the stem to the apex of the longest root. Once the six months of *in vitro* culture had elapsed, the plants were taken out for their *ex-vitro* culture and sown in small baskets (1 inch in diameter) in a mixture of tepojal and bark (2:1), and after six months their survival *ex vitro* was evaluated.

Statistical analysis

For growth of the aerial part of the plant and root growth, we fitted a set of linear models that included the initial size of the plant, the species, and the treatment in which the seedlings were found (agar or tepojal) as independent variables. The dependent variables were log transformed to be normalized. In total, 15 different nested models were obtained, which included all possible combinations with the variables, as well as the interactions between them and a null model where no variable has an effect. For probability of survival, 5 different nested linear mixed effects models were fitted. We converted the response variable with the arc sine of the square root to normalize it. These models included the treatment, the species identity, and their interaction as well as a null model. The jar used for germination of each seed was included as a random effect. All the analyzes were performed in R (R Core Team 2018) and the mixed effects models of fitted using the statistical package "lme4" (Bates *et al.*, 2015). Model with the lowest Akaike information criteria (AIC) value was accepted as the best model, and models that did not differ by more than two units (Δ AIC) from the best model were considered equally acceptable.

RESULTS AND DISCUSSION

Evaluation of the longitudinal growth of *Barkeria uniflora* and *B. scandens*

Barkeria uniflora seedlings shoots were bigger than in *B. scandens* regardless of the type of treatment. When comparing within each species, we found that the two treatments did not produce differences in the shoot growth (Figure 1). According to the fitted linear models, the best model included the initial size and the species as factors that influence the final size of the shoot (Table 1). It is important to note that the next best model did not differ by more than two AIC units, as was the case for the rest of the models in the set. Thus, the treatment may be slightly affecting the final size of the aerial part of seedlings, particularly for *B. uniflora*.

Root growth

Final root growth was influenced by both the species and the treatment (Table 2). Longest roots were found for *Barkeria uniflora* in the tepojal treatment, followed by those of *B. scandens* also in tepojal, while the smallest were those of *B. scandens* in agar (Figure 2). The best model found included all the independent variables without their interactions (Table 2). This means that the initial size of the seedlings, the species, and the treatment in which they were found determined the growth of the roots. It should be noted that there are four other linear models that could not be ruled out because their AIC did not differ in more than two units (Table 2). In general, these four models that had a good fit included

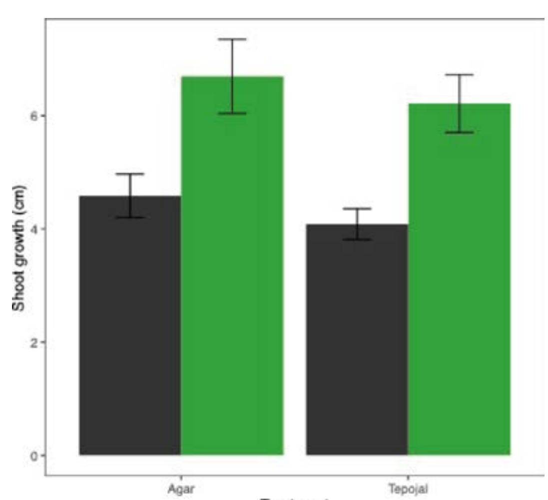

Figure 1. Shoot growth of *Barkeria uniflora* (green) y *B. scandens* (gray) cultivated *in vitro*.

Table 1. Linear mixed effect models used to evaluate shoot growth.

Shoot growth models	df	AIC	Δ AIC
Initial size+Species	4	133.68	0
Initial size+Species+Treatment	5	134.17	0.49
Initial size+Treatment+Species+Initial size×Treatment	6	135.83	2.14
Initial size+Treatment+Species+Initial size×Species	6	136.16	2.47
Initial size+Treatment+Species+Species×Treatment	6	136.17	2.48
Initial size+Treatment+Species+Species×Treatment+Initial size×Treatment	7	137.80	4.11
Initial size+Treatment+Species+Initial size×Species+Initial size×Treatment	7	137.83	4.14
Initial size+Treatment+Species+Initial size×Species+Initial size×Treatment	7	137.83	4.14
Initial size+Treatment+Species+Species×Treatment+Initial size×Species	7	138.15	4.46
Initial size+Treatment+Species+Species×Treatment+Initial size×Species+Initial size×Treatment	8	139.80	6.11
Initial size	3	142.24	8.55
Initial size+Species	4	143.24	9.55
Species	3	151.69	18.00
Treatment+Species	4	153.49	19.80
Null	2	164.98	31.29
Treatment	3	166.97	33.28

df=degrees of freedom, AIC=Akaike information criteria, Δ AIC=difference in AIC between each model and the best one.

Table 2. Linear mixed effect models used to evaluate root growth.

Root growth models	df	AIC	Δ AIC
Initial size+Treatment+Species	5	27.07	0
Initial size+Treatment+Species+Species×Treatment	6	27.31	0.23
Initial size+Treatment+Species+Initial size×Treatment	6	28.04	0.96
Initial size+Treatment+Species+Species×Treatment+Initial size×Treatment	7	28.30	1.22
Initial size+Treatment+Species+Initial size×Species	6	29.02	1.94
Initial size+Treatment+Species+Species×Treatment+Initial size×Species	7	29.08	2.00
Initial size+Treatment+Species+Initial size×Species+Initial size×Treatment	7	29.89	2.81
Initial size+Treatment+Species+Initial size×Species+Initial size×Treatment	7	29.89	2.81
Initial size+Treatment+Species+Species×Treatment+Initial size×Species+Initial size×Treatment	8	30.21	3.13
Initial size+species	4	31.06	3.98
Initial size+treatment	4	40.68	13.60
Initial size	3	44.10	17.02
Treatment+species	4	129.32	102.25
Treatment	3	134.15	107.07
Species	3	162.28	135.20
Null	2	165.77	138.69

df=degrees of freedom, AIC=Akaike information criteria, Δ AIC=difference in AIC between each model and the best one.

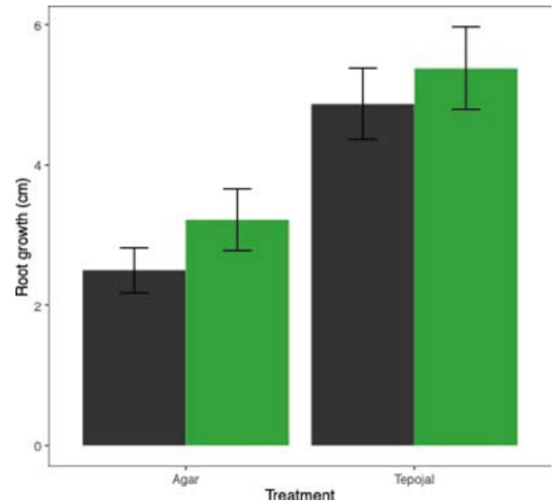

Figure 2. Root growth of *Barkeria uniflora* (green) y *B. scandens* (gray) cultivated *in vitro*.

all three independent variables that are present in the best model and differed only in the inclusion of the interaction of the variables.

Evaluation of the *ex-vitro* survival of *Barkeria uniflora* and *B. scandens*

Regardless of the species, survival at six months of culture *ex vitro* was higher in the seedlings that were cultivated in tepojal in *in vitro* conditions compared to those that were cultivated in agar. On average, the survival of the seedlings in tepojal was almost double compared to those from agar (Figure 3). The difference in survival of the treatment was greater in *Barkeria uniflora*, since it was almost three times greater for the seedlings that were previously cultivated *in vitro* in tepojal. According to the mixed effects linear models, using the jar as a random factor, we found that the best model is the one that only includes the treatment (Table 3).

Seed germination of *Barkeria whartoniana* in tepojal

Germination of *B. whartoniana* seeds were evaluated both in agar and in tepojal. In both culture media, *in vitro* seed germination occurred asynchronously, mainly between day 25 and day 28. At that time, green protocorms could already be recognized. The first leaves and roots emerged around day 42 after seed sowing. Seed germination observed in the

Table 3. Linear mixed effects models used to evaluate survival.

Survival Models	df	AIC	Δ AIC
Size	4	793.43	0
Treatment+Species	5	798.89	5.45
Treatment+Species+Treatment×Species	6	799.95	6.51
Null	3	840.74	47.30
Species	4	843.53	50.09

df=degrees of freedom, AIC=Akaike information criteria, Δ AIC=difference in AIC between each model and the best one.

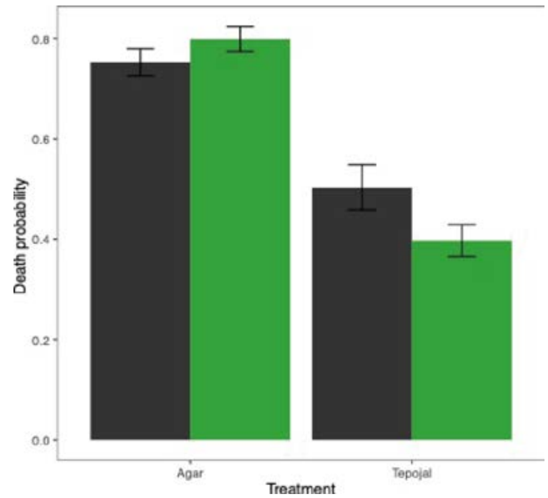

Figure 3. *Ex vitro* death probability of *Barkeria uniflora* (green) y *B. scandens* (gray) from plants cultivated *in vitro* with tepojal and agar.

liquid medium with tepojal was not apparently different from that in agar. In both cases the entire surface of the jar was covered with green protocorms.

DISCUSSION

This is the first study were the effect of tepojal *vs*. agar has been evaluated. At first, we considered that tepojal would not have better results than agar, since the latter is the most widely used gelling agent worldwide. But, considering the low cost and accessibility of tepojal, it would compensate for lower effectiveness compared to agar. Contrary to our expectations, tepojal produced better-than-expected results.

Effect of tepojal on growth of *Barkeria uniflora* and *B. scandens*

No differences were found in growth between agar and tepojal in both species (Figure 1 and 2). This result would be enough to consider tepojal as a good substitute for agar for aerial growth. But if we consider that cultivation in this volcanic rock uses a much less nutritious culture medium, it turns out that it is more efficient. Greater vegetative growth was obtained per gram of culture medium used; therefore, if we consider the cost of agar and the nutrient medium, the use of tepojal with liquid medium is much cheaper and much easier to obtain, especially for hobby *in vitro* growers.

Although it was found that the initial size of the plants and the species are decisive in the final length of the root, an important finding was that plants grown in tepojal had the most robust and longest roots. For *Barkeria* species, developing large roots is important because they do not have pseudobulbs and they can not store. It should be noted that even though the plants grown in tepojal had a liquid nutrient medium, the roots did not present hyperhydratability. Likewise, it was possible to observe that the seedlings grown in agar had less robust and shorter roots, their color was light green, somewhat transparent, somewhat slimy, and very fragile or brittle. In contrast, seedlings grown in tepojal, had roots with whitish in color, not very viscous and less brittle. The whitish color of the root suggests that that they may already have a well-differentiated canopy.

Barkeria uniflora and *B. scandens* produced longer roots in the tepojal. Although, the models detected that the interactions between independent variables may be important in determining the length of the roots. This suggests that cultivation in tepojal can produce different effects between species and be influenced by the initial size of the plants. Thus, it is necessary to carry out more studies that involve other species of the genera, as well as plants of different sizes to evaluate the effect of cultivation in tepojal on root development.

Use of tepojal in the germination of *Barkeria whartoniana*

Originally, tepojal was not considered as a possible substitute for agar for the germination process of orchid seeds. A concern we had for the use of tepojal was that the coarse grain could cause the seeds to migrate to the bottom of the jars, causing seedlings to drown. For this reason, we initially used very fine tepojal sand, but the material turned out to be very compact. All plants growth in these jars occurred only at the surface of the culture medium, producing a cluster of roots and stems that could be detached very easily from the culture medium. Because of these issues, we performed the experiment again using slightly larger grains (2 to 3 mm in diameter). Even though the seeds could have gone to the bottom of the jar given the size of the tepojal particles, this did not occur very often because of the liquid culture medium that cause the seeds to float. Plants in this experiment produced roots that managed to penetrate the substrate. As a result, we obtained vigorous plants with well-developed roots, which developed for 3 to 4 months. Subsequently, a small additional experiment was carried out to see if it was possible to subcultivate seedlings that had been germinated in tepojal to a new jar with this same material but with a coarser grain, as well as to a culture medium with agar. We found that both subcultures are feasible, and that the seedlings develop normally. Most of the seeds that successfully germinated using this method are currently already growing *ex vitro* in the greenhouse.

An unexpected result of the use of tepojal sand for the germination of *Barkeria whartoniana* was found when we removed the plants for their subsequent subculture. It was observed that there were still some seeds that had not developed as protocorms. So, with a shaker, the surface of the sand was slightly stirred and rehydrated with liquid culture medium. After a few days, a second wave of protocorms, which grew and generate new seedlings, were observed. This is an additional advantage as more plants can be obtained with fewer steps. This contrasts with germination carried out in agar, where at the time of subculturing, the plants are removed with fragments of agar and in the end the flasks are left with almost no culture medium and are unusable for the development of the seeds that did not germinate.

Ex vitro* survival of *Barkeria uniflora* and *B. scandens

Survival after six months of *ex vitro* culture was almost twice as high in seedlings that were previously cultivated *in vitro* in tepojal than in agar (Figure 3). Apparently, this is because the seedlings cultivated in tepojal had longer and less brittle roots. Thus, they may be more viable from the moment they come out of the jar. In addition, roots from the agar culture were more fragile. Also, it was much easier to extract from tepojal the seedlings with all their roots compared to agar. This was because agar adheres and wraps more firmly around the root, so it is necessary to remove it to avoid the growth of fungi and bacteria. In addition, although the roots may remain attached to the tepojal, it was not necessary to remove this substrate completely.

Survival was greater in *Barkeria uniflora* than *B. scandens* on tepojal. This result implies that species can respond differently to subcultivation after growing in tepojal. This finding was the result of using two species at the same time for the experiment, a situation that is rare in *in vitro* culture experiments. It is not clear why this difference was found between the two species. There may be some explanations of the higher survival in initial stages of *B. uniflora* compared to *B. scandens*. One of them is that *in vitro* development is more vigorous in *B. uniflora*, and it has previously been observed that larger plants are more successful in surviving, even under natural conditions (Segovia-Rivas *et al.*, 2018). The faster growth of *B. uniflora* could be associated with its life history strategy (Halbinger 1977), since it behaves like a species capable of colonizing slightly disturbed environments (ruderal), such as guava crops, while *B. scandens* can be considered a stress tolerant plant. These differences in life histories were reflected by several *B. uniflora* plants flowering during *in vitro* culture, while none of the *B. scandens* plants did. Therefore, it is important to understand the biology associated with the species used, since this can affect the results obtained in the acclimatization of the plants.

Because with the use of tepojal a little water stress can be applied before removing the plants from the jar, it is possible that the orchid seedlings are more "hardened" and therefore may have greater ex vitro survival compared to plants grown on agar. The development of this process can substantially increase the survival of the plants in one of the most critical steps in the production of orchids where mortality is higher (Suárez-Quijada *et al.*, 2007). This experiment helped to obtain experiences in subcultivation and techniques have subsequently been developed that have allowed for greater survival of Barkeria seedlings and other groups of orchids.

FINAL CONSIDERATIONS

The use of tepojal is novel because it does not involve the gelation phase, and this apparently generated some additional advantages in *ex vitro* survival. This technic could increase *in vitro* culture of orchids, since it is easier to obtain for hobbyists who do not have agar suppliers, especially in small quantities and in remote places. In addition, tepojal reduces production costs because less nutrient solution is used per volume of culture medium and has similar or even better results than agar (Romay *et al.*, 2006). Another advantage is that time in laboratory facilities is reduced because it is not necessary to wait for the media to gel and resuspension. When using tepojal, it is enough to wait for the medium to cool before it can be used. It is also possible to reduce costs in subcultures because it is enough to hydrate the medium with a nutrient solution when it is cultivated in tepojal without having to repeat the entire sowing procedure. Additionally, during *in vitro* culture, it is possible to redistribute the nutrients and conditioners in the culture medium by gently decanting the flasks, particularly for the less soluble ones such as activated carbon.

This study is a pioneer in the use of tepojal as a substitute for agar. It has given us very encouraging results for its widespread use but there is still much to be investigated. Particularly, it will be necessary to evaluate how other species and other genera react to *in vitro* culture in tepojal. It is also necessary to evaluate the entire rehydration process with nutrient solutions because the adequate amounts of nutrients and conditioners of the culture medium that must be incorporated are unknown. An aspect that needs to be resolved is the process of cleaning and sterilization of tepojal to reduce the use of water and energy. In this study, the tepojal was autoclaved two times in total to avoid contamination. The use of energy and time in this process is still high and other alternatives will have to be found in the future. However, despite all these possible drawbacks, we may conclude that the use of tepojal as a substrate in *in vitro* culture is highly recommended.

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Colegio de Postgraduados

Physical and physiological quality of oat (*Avena sativa* L. cv. Turquesa) seeds

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ABSTRACT

Objective: The objective of this study was to determine the physical and physiological quality of a batch of oat (*Avena sativa* L. cv. Turquesa) seeds.

Design/Methodology/Approach: The following physical quality variables were evaluated: seed purity, weight of a thousand seeds, volumetric weight, and moisture content. Physiological quality was evaluated through a germination and emergence speed test, which also was used to measure seed vigor. A completely randomized experimental design, with factorial arrangement, and four repetitions was used. The factors analyzed were seed size (small and large) and aging (with and without aging).

Results: The following results were recorded: 99.52% seed purity; 34.31 g weight of a thousand seeds; 54.80 kg hl^{-1} volumetric weight; and 6.50% moisture content. Regarding treatment germination, no significant differences were found between the seed size and the size × aging interaction ($P=0.422$). The aging treatment reduced germination from 96.50% (unaged seeds) to 89.25% (aged seeds). The emergence speed did not show significant differences regarding seed size ($P=0.066$) and size × aging interaction ($P=0.868$). The aging treatment had a negative impact on the emergence rate. The aged seeds emerged at a 15.55 plants d^{-1} speed, while unaged seeds reached a 17.88 plants d^{-1} speed.

Study Limitations/Implications: This study only evaluated one batch of oat seeds.

Findings/Conclusions: The seeds have an adequate physical and physiological quality to establish oat crops. In addition, the seed batch was highly vigorous, because it maintained >80% germination rate after the aging treatment.

Keywords: germination, grain, speed of emergence, vigor.

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INTRODUCTION

In Mexico, oat (*Avena sativa* L.) is the most popular grain used as forage among the small grain cereals. From 1980 to 2021, the oat sowing area increased from 300,000 to >640,000 ha $year^{-1}$. Approximately 94% of this sowing area is used for forage production. The average yield during the same period reached 9.8 t ha^{-1} and Chihuahua, Durango, and Mexico were the main producer states (SIAP, 2022). Oat cultivation has mainly increased as a result of its adaptability to different agroclimatic conditions and the quality of its forage (Espitia-Rangel *et al.*, 2012).

The seed is a key supply for vegetal production and no agricultural practice can improve crops beyond the limits established by their germplasm (Camargo and Vaughn, 2021). A high-quality batch of seeds has a high germination capacity, vigor, size uniformity, and purity (Sabry, 2018).

Forage crops should be established as soon as possible. Consequently, the soil should be quickly covered to increase the efficient use of the water available in the soil (Rebolledo *et al.*, 2015) and the capacity of the plants to compete with weeds (Zhao *et al.*, 2006). Plants that grow from high vigorous seeds emerge faster and successfully establish themselves during the first development stages (Finch-Savage and Bassel, 2016).

Seed development comprises a series of ontogenetic stages, including fertilization, nutrient accumulation, seed drying, and dormancy. Each stage represents a change in the ontogenetic and physiological morphology that can alter the performance of the seed (Copeland and McDonald, 2001). The moment in which the seed reaches it maximum dry matter accumulation is known as physiological maturity (Copeland and McDonald, 2001). At this point, the seed reaches its maximum germination potential and records its highest vigor (Delouche, 1974). However, Copeland and McDonald (2001) determined that seeds reach their physiological maturity with a high moisture content (>12%); consequently, their crops should be harvested when they reach "harvest maturity" (≤12%). At this moment, the moisture level is appropriate for the storage of the seeds without incurring mechanical damages (Copeland and McDonald, 2001). Between the physiological and the harvest maturity, the grain is stored in the plant, where it can be exposed to adverse environmental conditions that can impact its quality (Copeland and McDonald, 2001). Seeds are not always sown immediately after the harvest, without being stored for a certain period; consequently, the storage time, the type of seed, and the environment of the warehouse (relative humidity, temperature, and oxygen levels) can impact the vigor of the seeds (Copeland and McDonald, 2001).

Determining the physical and physiological state of the seeds after a storage period is fundamental to guarantee the success of forage crops. Therefore, the objective of this study was to determine the physical and physiological quality of a batch of Turquesa oat seeds.

MATERIALS AND METHODS

This study was developed in the Laboratorio de Análisis de Semillas of the Postgrado en Recursos Genéticos y Productividad - Producción de Semillas, located in the Campus Montecillo of the Colegio de Postgraduados. The area is located at an altitude of 2,250 m and has a sub-humid warm climate, with summer rains. The mean annual temperature is 15 °C and the average precipitation is 645 mm (García, 1998).

A batch of *Avena sativa* L. cv. Turquesa seeds was acquired from the Bajío region, in Guanajuato, Mexico. The seeds were produced during the 2020 spring-summer cycle.

Four primary samples were taken from the batch of seeds, and they were mixed to obtain a composite sample (∼1 kg). At the same time, a 300 g sample was taken to determine the moisture content of the seeds. The composite sample was homogenized in the lab and ∼500 g was weighted and used as work sample. This latter sample was used to determine the physical and physiological quality of the seeds.

Following the methodology established by the ISTA (2018), the physical quality of the seeds was determined based on the quantity of pure seeds (PS), the weight of a thousand seeds (W1000S), the volumetric weight (VW), and the moisture (M) percentage.

Germination percentage (ISTA, 2018), accelerated aging (Delouche and Baskin, 1973, with some modifications by Huber *et al.* (1982) and Kim *et al.* (1985)), and speed of emergence (Maguire, 1962) were used to determine the physiological quality and vigor of the seeds.

During the physical quality analysis, a 2.8-mm sieve was used to separate large (L) from small (S) seeds; all the seeds that went through the sieve were considered S, while the seeds that remained in the sieve were classified as L. Four hundred seeds of both sizes were subject to accelerated aging (AA), while another 400 seed remained without the accelerated aging treatment (WAA). As a result of the combination of both factors (seed size and preparation), four treatments were established, with four repetitions each.

The sowing was carried out in a 2.5×1 m seedbed, with a substrate prepared with previously sterilized river sand. Twenty-five seeds were sown in each of the four rows of each repetition. The separation between the seeds and the rows was 3 cm. The distribution of the treatments and their respective repetitions was randomized within the seedbed.

The analysis of variance of the germination and the emergence speed was carried out using a completely randomized experimental design, with a factorial arrangement and four repetitions. Tukey's test ($P<0.05$) was used to compare the means. All the data were analyzed using the SigmaPlot 12.3 statistical package (Systat Software Inc. San Jose, CA, USA).

RESULTS AND DISCUSSION

The results of the physical analysis of the seed indicated a purity of 99.5% and an inert material content of 0.5%. The weight of a thousand seeds reached 34.31 g, 7.22 g more than the 27.09 g reported by Rodríguez-Herrera *et al.* (2020). That study recorded the average weight of a thousand oat seeds, with different doses and sources of fertilization. The variation in the nutrient supply impacts the development of the plant and the filling of the grain: larger seeds frequently produce better developed embryos and have more reserves for their maintenance (Carvalho and Nakagawa, 2000). In conclusion, the objective should be to obtain larger grains. The results obtained in this study fall within the range reported by Bobadilla *et al.* (2013), who recorded 33.80-38.34 g values in four oat varieties sown at different dates.

The volumetric weight obtained in this study was 54.8 kg hL^{-1}. Bobadilla *et al.* (2013) reported a volumetric weight between 42.63 and 46.19 kg hL^{-1} for four oat varieties, sown at different dates. A higher volumetric weight is associated with heavier, larger, and wider seed. This phenomenon could be related to late sowings because low temperatures during the flowering stage impact the development of the grain (Bobadilla *et al.*, 2013). The batch of seeds used in this study was produced during the 2020 spring-summer cycle, while the abovementioned study used seeds produced in winter.

The evaluated seeds recorded a 6.50% moisture, which is lower than the 12% recommended to prevent microorganism proliferation during the storage period (ISTA, 2018). A high moisture content at the time of arrival to the warehouse is the main cause of seed deterioration. Temperature and relative humidity in the warehouse are secondary factors; however, they become relevant if the seeds have a high moisture content (Pomeranz, 1992). The longevity of seeds with 5-14% moisture content increases by 50% per each 1% of lost moisture (Chala and Bekana, 2017).

The analysis of variance of the germination data did not record statistical differences between L and S seeds ($P=0.422$). There were no significant differences between seed size and aging interaction ($P=0.422$). However, the germination percentage between aging treatments (Figure 1) recorded significant statistical differences ($P<0.005$).

The WAA seed recorded a higher germination percentage than the AA seed. These results match the findings of Bobadilla *et al.* (2013), who reported that the aging treatment reduces 50-71% of the germination of four oat varieties, at different sowing dates. Tekrony (1995) mentioned that the germination percentage after the aging treatment can be used to classify the vigor of soy seeds: high vigor seeds have a >80% germination rate, medium vigor seeds have a 60-80% rate, and low vigor seeds have <60% rate. In addition, Nagel and Borner (2010) pointed out that 80% is a high germination percentage. In this study, >89% of the seeds subjected to the aging treatment germinated. Consequently, the batch of seeds evaluated in this research is considered as a highly vigorous batch.

The emergence speed did not record significant differences based on seed size ($P=0.066$) and the size × aging interaction ($P=0.868$). There were statistical differences between the different aging treatments (Figure 2).

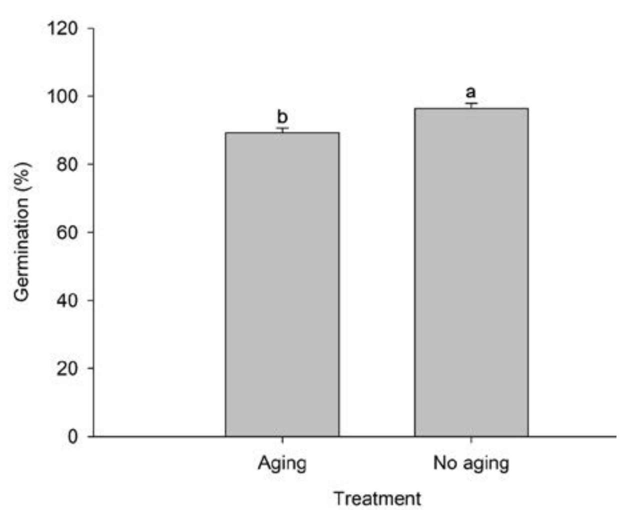

Figure 1. *Avena sativa* L. cv. Turquesa seed germination subjected to different accelerated aging treatments. Each bar shows the average of eight repetitions, with their respective standard error.

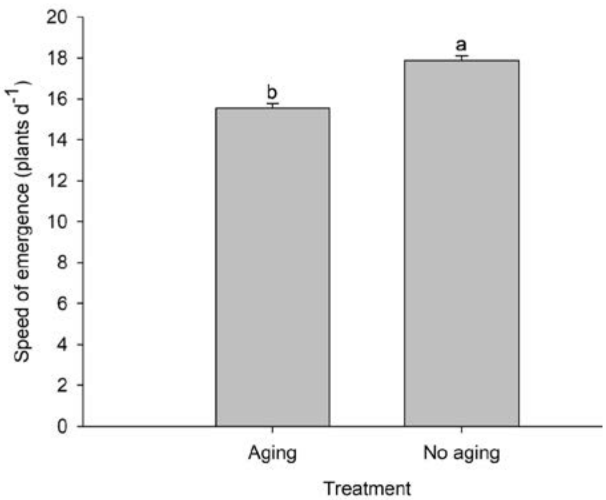

Figure 2. Emergence speed of *Avena sativa* L. cv. Turquesa seeds, using different accelerated aging treatments. Each bar shows the average of eight repetitions and their respective standard error.

Maguire (1962) defined emergence speed as a methodology used to evaluate seed vigor, under lab and field conditions. High values obtained with this expression indicate that one sample is more vigorous than the other (Ranal and Santana, 2006). The results of this study showed that, compared with the WAA, the AA treatment reduced the emergence speed of the plants by 13%. Based on the statistical differences, the seed used in this research can be classified as a highly vigorous seed.

The principle of the aging treatment is to increase the deterioration speed of the seeds. This process takes place through the 72 h exposition of the germplasm to a high temperature (40 °C) and a high relative humidity (100%). These two factors determine the intensity and the speed of the detriment of the seeds (Ohlson *et al.*, 2010). The main components of the reserve of seeds are lipids, proteins, and carbohydrates. The aging of seeds impacts the reserves at the cellular level. This phenomenon results in the degradation of the cell membrane, metabolic changes, reduction of the use of reserves, modification of proteins and enzymes, degradation of lipids and carbohydrates, and production of reactive oxygen species and other toxic compounds (Onder *et al.*, 2020). The loss of germination and vigor is the consequence of cell damages caused to the seed (Onder *et al.*, 2020).

The small seeds without aging treatment (SWAT) reached their emergence peak (74 plants) five DAS. Compared with SWAT, large seeds without aging treatment (LWAT) did not record such a high emergence peak (Figure 3); however, 47 and 46 plants emerged during the fifth and sixth DAS, respectively. During the sixth day, 91 plants had already emerged in SWAT, while LWAT recorded 93 plants.

Additionally, the small (SAA) and large (LAA) seeds subjected to the aging treatment started to emerge from five DAS. Nevertheless, these treatments reached their emergence peak during the sixth DAS (Figure 1): LAA recorded an accumulated emergence of 77 plants, while SAA recorded 82 plants. The SAA and LAA groups recorded 100% emergence (89.25 plants) until 12 DAS. For their part, SWAT and LWAT recorded their total plant emergence between 9 and 11 DAS.

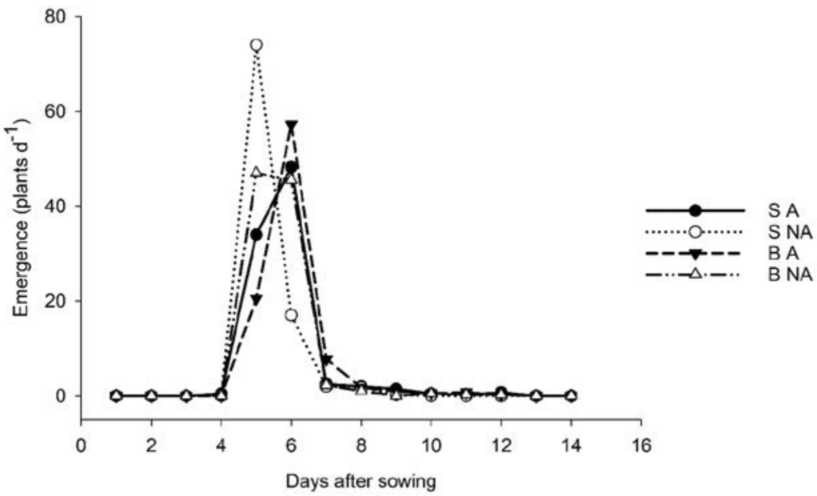

Figure 3. Emergence of *Avena sativa* L. cv. Turquesa plants grown from small (S) and large (L) seeds, subjected to aging treatment (AA) and without aging treatment (WAT), during 14 DAS. Each bar shows the average of four repetitions per treatment.

According to the proposal of Delouche and Baskin (1973), the aging treatments significantly reduce the germination and emergence speed of Turquesa oat seeds.

CONCLUSIONS

The batch of Turquesa oat seeds had the appropriate physical and physiological quality to establish oat crops for forage production. However, seed size did not record any effect on the germination percentage and the emergence speed. The aging treatment significantly reduced germination and emergence speed. Nevertheless, the seed recorded >80% germination after the aging treatment. Consequently, the evaluated seed is highly vigorous. Finally, the interaction between the study factors was not significant for germination and emergence speed.

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Colegio de Postgraduados

In vitro, *in situ*, and *in vivo* evaluation of a sesame paste supplement for grazing calves

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ABSTRACT

Objective: To carry out an *in vitro*, *in situ*, and *in vivo* evaluation of soybean paste (T1) and sesame paste (T2) supplements for calves under grazing conditions.

Methodology: Partial (24, 48, and 72 h) and accumulated biogas and methane production, as well as dry matter degradation (DMD), were measured in the *in vitro* analysis. The digestibility kinetics parameters (a, b, a+b, c, k, ED) were estimated in the in situ test. Feed supplement consumption (SC), daily weight gain (DWG), bacterial and protozoan populations, acetate, propionate, and butyrate were evaluated in the *in vivo* test. A completely randomized experimental design was used.

Results: T1 produced a higher amount of partial and accumulated biogas and methane at 24 h, as well as the highest values in the b, a+b, k, and ED ($p \leq 0.05$) parameters. Meanwhile the rest of the *in vitro*, *in situ*, and *in vivo* variables did not record any differences between treatments ($p > 0.05$).

Study Limitations/Implications: The production of sesame paste is seasonal; consequently, its harvest limits its year-round availability.

Conclusions: Based on the *in vitro*, *in situ*, and *in vivo* evaluations, sesame paste is a feasible option to replace soybean paste as a source of protein, in the supplementation of calves in the tropics.

Keywords: grazing, tropics, protein supplementation, alternatives.

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INTRODUCTION

Cattle raising is the main agricultural activity worldwide (Gutiérrez *et al.*, 2018). In the tropical regions of Mexico, cattle raising is practiced in extensive systems, in which vast extensions of land are used to grow native and introduced grasses (REDGATRO, 2018). This system accounts for 78.5% of the domestic production (García *et al.*, 2018). Given their high cell wall levels, low digestibility, reduced protein content, and high nitrogen compound degradability, the quality of tropical grasses is associated with animal productivity (Rojo *et al.*, 2000). This situation delays the growth of calves, reducing productivity and fertility (Rodríguez, 2011). Since feeding accounts for 70% of their total production costs, production units frequently seek to improve their feeding efficiency. Therefore, finding new feeding alternatives —which produce a biological and economic efficiency improvement— is fundamental (García-Balbuena *et al.*, 2022).

Oleaginous fruits, oilseeds, or their by-products are a feasible alternative for bovine feeding systems, because they provide proteins to the animals (Aguilera *et al.*, 2018). In most of the producing countries, sesame (*Sesamum indicum*) is grown by small producers. Few of these producers have technified systems or are aware of the chemical components of the industrial sesame by-products (paste), which leads to the underutilization of this species (Mussi *et al.*, 2016). Consequently, the producers of these regions are limited to subsistence animal production systems (Velázquez *et al.*, 2005). Aguilera (2014) compared the inclusion of sesame paste with soy paste in the diets of pigs, recording a higher ileal digestibility of crude protein (CP) in pigs that were fed with the former diet. Although Cuca and Ávila (1978) evaluated the inclusion of sesame paste in feeding portions for broilers, information about the use of that paste in the diet of ruminants is still scarce. García-Balbuena *et al.* (2022) evaluated the effect of the addition of sesame paste to the integral diets of Simbrah calves; however, they did not record significant differences between treatments. The hypothesis of this research was that replacing soy paste with sesame paste, as source of protein, did not modify the characteristics of *in vitro* and *in situ* fermentation and the productive response of F1 calves (under grazing conditions and fed a protein supplement). In conclusion, the objective of this study was to carry out an *in vitro*, *in situ*, and *in vivo* evaluation of sesame and soy paste supplements, in F1 calves under grazing conditions.

MATERIALS AND METHODS

Location

The *in vitro* and *in situ* evaluations were carried out in the facilities of the Facultad de Medicina Veterinaria y Zootecnia No. 2, Universidad Autónoma de Guerrero, located in km 197 of the Acapulco-Pinotepa Nacional highway. The *in vivo* evaluation was carried out in El 29 ranch, located in the community of Cuajinicuilapa, Guerrero. The experiment was conducted from February to April, 2022. Cuajinicuilapa is located at 16° 58' N and −98° 45' W, at 30 m.a.s.l. The climate is sub-humid warm, with summer rains, an annual precipitation of 1,200 mm, and an annual average temperature of 25 °C (INAFED, 2022).

Treatments

The treatments were based on the soy paste (T1) and sesame paste (T2) content used to prepare feed supplements (Table 1).

In vitro evaluation

The culture medium was prepared according to the method proposed by Cobos and Yokoyama (1995) and modified by Cañaveral-Martínez *et al.* (2020). The culture medium was made up of 50.9% distilled water, 30% clarified ruminal fluid, 5% mineral solution I, 5% mineral solution II, 0.1% resazurin (Sigma-Aldrich®), 4% reducing agent, and 5% buffer solution. The clarified ruminal fluid contained fresh ruminal bovine liquid, centrifuged for 10 m at 12,857 × g and was then sterilized at 121 °C, for 15 m, 15 psi (All American® 1941X, USA); the whole process was carried out twice. Mineral solution I contained 6 g of K_2HPO_4 (J. T. Baker®) in 1,000 mL of distilled water. Mineral solution II was made up of 6 g KH_2PO_4 (J. T. Baker®) + 6 g $(NH_4)_2SO_4$ (J. T. Baker®) + 12 g NaCl (Meyer®)

Table 1. Feed supplement composition with different sources of protein for grazing calves.

Ingredient (%)	T1	T2
Soybean paste	35	-
Sesame paste	-	30
Molasses	10	10
Salt	20	20
Mineral mixture	10	10
Urea	5	5
Ground maize kernel	20	25
Chemical composition (%)		
Crude protein	30.63	30.11
Dry matter	96.37	96.27
Ash	33.40	34.71
Neutral detergent fiber	26.01	32.46
Acid detergent fiber	6.48	10.02

+ 2.45 g $MgSO_4$ (Meyer®) + 1.6 g CaCl-2H_2O (Meyer®) in 1,000 mL of distilled water. The reducing agent contained 3.125 g L-cysteine (Sigma-Aldrich®), its pH was adjusted to 10 using NaOH (Meyer®) + 3.125 g Na_2S-9H_2O (Meyer®), it was gauged with 250 mL of distilled water, and 0.1 mL of resazurin were added, with a low CO_2 flow and heat, in order to obtain anaerobiosis. Finally, the buffer solution consisted of 80 g of Na_2CO_3 (J. T. Baker®) in 1,000 mL of distilled water.

The biodigesters were placed in 120 mL serological pipettes, to which 0.5 g samples of T1 or T2 (particle size: 1 mm), 40 mL of culture medium, and 10 mL of fresh ruminal fluid (inoculum), under a continuous CO_2 flux, were added. There were 10 independent repetitions per treatment. A neoprene cap and an aluminum ring with a removable center were used to seal the pipettes. The biodigesters were incubated for 72 h, at 39 °C, in a bath Marie. In order to precipitate food and protozoan particles, the fresh ruminal fluid was centrifuged at 1,137 × g for 3 m. The ruminal fluid was obtained from a bovine with a ruminal fistula, previously grazed with Pangola grass. The bovine was handled according to the bioethics and welfare internal regulations of the UAGro and based on the NOM-062- ZOO-1999 Mexican official standard. The biodigesters were incubated for 72 h at 39 °C (Cañaveral-Martínez *et al.*, 2020).

Biogas production was measured displacing the plunger of a 50 mL glass syringe (BD Yale®, Brazil). This process was measured at 2, 4, 6, 8, 10, 12, 24, 48, and 72 h of incubation (Hernández-Morales *et al.*, 2018). Partial biogas production was reported at 24, 48, and 72 h; accumulated biogas production was recorded at 72 h.

Methane (CH_4) production was measured using a Taygon® hose (internal-Ø: 2.38 mm; length: 45 cm), with 20 G × 32 mm hypodermic needles at each end. The needles were used to couple the biodigesters with measurement vials. These vials were filled with 80 g of NaOH (2N) solution (Merck®) in 1,000 mL of distilled water. The procedure was carried out following the method proposed by Stolaroff *et al.* (2008), as described by Torres-Salado *et al.* (2018). Partial methane production was considered as the mL of the NaOH (2N) solution displaced at 24, 48, and 72 h of incubation; meanwhile, the accumulated production was recorded at 72 h. Dry matter degradation (DMD) was estimated after 72 h, following the methodology proposed by Hernández-Morales *et al.* (2018).

In situ evaluation

Two cows with ruminal fistula under stabling conditions were used for the *in situ* test. As part of the adaptation of the microbiota to the rumen, the DM consumption of each cow was estimated at 3% LW, divided as follows: 70% forage and 30% commercial feeding. Cows had *ad libitum* access to water. In order to guarantee that the sample stayed in the ventral part of the rumen, a 1.5 cm wide × 1 m long galvanized iron chain, modified with a security hook at one end, was attached to the cap of the ruminal fistula (Cañaveral-Martínez *et al.*, 2020).

Five-gram samples from each treatment (T1 and T2) were placed in 10 × 20 cm poly-silk bags, with a 40 μm average pore, and were kept at constant weight. The bags were sealed with 100 mm long × 2.5 mm wide plastic belts. The bags were incubated in triplicate for 0, 2, 4, 8, 12, 20, 32, 48, and 64 h, in the rumen of each cow. All the bags were inserted in the rumen in the reverse order of their incubation time (64, 48, 32, 20, 12, 8, 4, 2, and 0 h); therefore, all the samples could be removed simultaneously, after the incubation period. Once the bags were extracted from the rumen, they were immediately washed with cold running water, until the outcoming water was clear. The 0 h bags were not incubated in the rumen: they were just washed, using the same protocol followed for the bags incubated in the rumen.

The bags with residues were dried at 55 °C for 72 h; subsequently, they were weighted to determine DM disappearance. The *in situ* DM disappearance kinetic was estimated with a non-linear regression procedure in SAS (2011), using the equation described by McDonald (1981):

$$P = a + b\left[1 - e - c\left(t - l\right)\right]$$

Where: P=ruminal digestibility in time (t; %); a=rapidly soluble digestible fraction (%); b=low or potentially digestible fraction (%); $a+b$=maximum potential digestibility; c= speed at which b is digested (% h^{-1}); t=incubation time (h) in the rumen; and l=delay time (h).

DM effective digestibility (ED) was estimated using the equation described by Ørskov and McDonald (1979):

$$ED = a + \left[b * c / \left(c + k\right)\right] e - \left(c + k\right) L$$

Where: a, b, c, and L are used as described in the previous paragraph and k=rumen exit rate (% h^{-1}).

In vivo evaluation

The experiment lasted 56 days. Ten F1 calves (*Bos taurus* × *Bos indicus*) of 200 ± 20 kg LW were used in the evaluation (five calves per treatment). Based on the number of their SINIGA earring, the calves were assigned to each treatment. A prophylactic treatment consisting of a 5 mL animal^{-1} subcutaneous selenium injection was applied to the calves. In addition, they were injected a subcutaneous vaccine with bacterin (Exgon 10®; 5 ml animal^{-1}). The calves were periodically bathed with tickicides and insecticides (Bovitraz®; 2 mL L^{-1}), using a 20 L sprinkler pump (maximum coverage per pump: 5 calves). Afterwards, a cosproparasitoscopic analysis was carried out. The calves were no dewormed, considering the negative result of this analysis.

The calves grazed together in a rotational arrangement, in three paddocks with cross 1 Bermuda grass predominance (Table 2). Before the experiment started, the calves were subjected to the following supplement adaptation procedure for 10 d: the calves were placed into 2 m^2 individual stables, where they were initially provided 100 g of the supplement at 08:00 am and, subsequently, the supplement amount increased 100 g at a time, until it reached 500 g d^{-1}. Starting from the first day of the experiment, the calves spent 2 h in the stables, where they fed on 500 g of one of the treatments. The dry matter consumption (DMC) of each treatment was measured as the amount of the feeding offered to the calf minus the amount rejected per day by the calf. The calves had ad libitum access to water. In order to determine the daily weight gain (DWG), they were weighted at the beginning and at the end of the experiment, using a digital livestock scale.

Twenty mL of ruminal fluid were extracted at day 56, with an esophageal catheter, 2 h after the supplement consumption. A doble-gauze layer was used to filter the fluid. Immediately after, a SA 210 portable potentiometer (ORION®, USA), with a 7-4 pH calibration, was used to measure pH. A micro-pipette (Corning®, USA) was used to extract a 1 mL sample of the ruminal fluid, in order to carry out the total bacteria counting (TBC). Afterwards, the sample was poured into a test tube (Pirex®, México), with 0.25 ml of 10% formaldehyde (Sigma-Aldrich®). The total bacteria were counted with a Petroff Hausser bacteria counting chamber. A BX31 microscope (Olympus®, USA) with a 1,000 X magnification, was used for the recounting. The number of bacteria was calculated using the following formula:

$$number\ of\ bacteria = (average)(dilution\ factor,\ 2\times10^7)$$

Table 2. Bromatological composition of the forage found in the paddocks and used in the *in vivo* evaluation.

Variable (%)	Paddock 1		Paddock 2		Paddock 3	
	Home	End	Home	Final	Home	End
Crude protein	7.07	6.2	9.23	5.19	9.94	5.47
Dry matter	94.9	95.8	95.15	95.46	94.77	95.74
Ash	8.87	8.01	8.85	8.24	8.8	7.89
Neutral detergent fiber	72.42	71.6	70.74	71.29	72.91	74.23
Acid detergent fiber	36.62	36.5	36.87	36.81	39.68	38.83

The same technique used for the total bacteria counting was used for the protozoa counting (PC); however, the recounting was carried out in a Brand® bright-line Neubauer chamber with a 400 X magnification. The number of protozoa was calculated using the following formula:

$$\textit{protozoa counting} = (\textit{average})(\textit{dilution factor},\ 2\times10^4)$$

(Espinoza-Sánchez *et al.*, 2020).

In addition, 1 mL of ruminal fluid was poured into a 2 mL tube, which was then placed inside a Neptune microcentrifuge (Mexico). The content of the tube was mixed with metaphosphoric acid at 25% (4:1 ratio), before the tubes were centrifuged at 18,800 × g for 10 minutes. The supernatant was placed in 1.5 mL vials (Perkin Elmer®, USA) to carry out a chromatography. A Claurus 580 gas chromatograph (Perkin Elmer®, USA), equipped with a flame ionization detector and a 30 m × 0.25 mm capillary column (Elite FFAP, Agilent®), was used to determine the VFA concentration. Helium (with a constant pressure of 10 psi) was used as gas carrier for this procedure, along with H_2 and air to generate a flame with a 40- and 400-mL min^{-1} flux, respectively. The oven, the injector, and the column temperatures were 80, 240, and 250 °C, respectively; 1 μL of the sample were injected. Acetic acid, propionic acid, and butyric acid recorded retention times of 1.22, 1.54, and 2.01, respectively.

Bromatological analysis

The 967.03, 920.105, and 942.05 methods of the AOAC methodology (2007) were used to determine dry matter (DM), crude protein (CP), and ash (A), respectively. Following the recommendations of Van Soest *et al.* (1991), the ANKOM Technology® methodology was used to determine the neutral detergent fiber (NDF) and acid detergent fiber (ADF) content. Organic matter (OM) was determined subtracting the ash percentage from 100.

Statistical analysis

The *in vitro*, DMC, and DWG variables and the ruminal fermentation characteristics were analyzed using a completely randomized design, with the GLM procedure of SAS (2011). The MIXED model of SAS (2011) was used for the *in situ* evaluation.

RESULTS AND DISCUSSION

The gas production technique is an indirect measurement of the ruminal microbial fermentation and is based on the ratio between the microbial digestion of food and the production of volatile fatty acids and gases (CO_2 and CH_4), as the final metabolites of the fermentation (Amanzougarene and Fondevila, 2020). Consequently, no differences were recorded ($p>0.05$) between treatments, regarding biogas and methane production at 24-48 h and at 48-72 h (Table 3). T1 biogas production obtained higher values from 0 to 24 h (41.82%) and from 0 to 72 h (34.95%) than T2 ($p\leq0.05$). Likewise, T1 produced more CH_4 than T2, at 0-24 h (34.32%) and at 0-72 h (22.65%). These results indicate that the soy paste supplement (T1) had more non-structural carbohydrates (Table 1) —which ferment during the first 24 h (Texta *et al.*, 2019). The biogas accumulated production is a consequence of fermentation. Meanwhile, no differences were recorded in the biogas production after 24 h, which indicates that the fermentation of the structural carbohydrates was similar between treatments (Texta *et al.*, 2019). Additionally, methanogenic archaea use CO_2 and H_2 in their metabolic route, producing CH_4 as a fermentation product. The stoichiometric reactions indicate a higher CO_2 and H_2 production when the final products are acetate and butyrate. Table 3 shows that, if these ratios are analyzed (Blümmel and Ørskov, 1993)two varieties of spring barley and one variety of winter wheat either untreated or treated with anhydrous ammonia, were examined. *In vitro* gas production was compared with in vivo results and with nylon bag degradabilities; the sources of the gas were determined. Total gas production a+b as described by the exponential equation were correlated with intake (0.88, the higher CH_4 production could be associated with a higher acetate and butyrate production, as a result of the use of the soy paste supplement (T1).

The *in vitro* technique is used to predict digestibility when the animals consume DM (Gosselink *et al.*, 2004). Consequently, the digestibility of the evaluated treatments should record similar results, because DMD did not show differences ($p>0.05$) between treatments (Table 3). In this study, the biogas production values for T2 were lower than those reported by García-Balbuena *et al.* (2022), who carried out a research about a 10% sesame paste diet and recorded 117.02, 37.14, 18.10, and 172.32 mL g^{-1} DM, at 0-24 h, 24-48 h, 48-72 h, and 0-72 h, respectively. Methane production and DMD recorded higher values in T2 than those reported by García-Balbuena *et al.* (2022) who obtained 30.39, 8.14, 4.86, 43.39, and 26.22 mL g^{-1} DM, at 0-24 h, 24-48 h, 48-72 h, 0-72 h, and DMD, respectively.

Table 3. *In vitro* fermentation characteristics of supplements made from soybean paste or sesame paste for grazing calves.

Variable	T1	T2	SEM
Production of biogas *in vitro* (mL g^{-1} DM)			
0-24 h	141.4^{a}	99.7^{b}	7.7
24-48 h	19.7	17.6	1.1
48-72 h	13.8	12.2	1.1
0-72 h	174.9^{a}	129.6^{b}	6.7
Production of methane (mL g^{-1} DM)			
0-24 h	54.0^{a}	40.2^{b}	1.9
24-48 h	11.5	10.3	0.6
48-72 h	6.5	8.2	0.5
0-72 h	72.0^{a}	58.7^{b}	1.7
Dry matter degradation (%)	87.7	83.0	2.1

^{a,b}: Means in a row with different letter are different ($p \leq 0.05$).
T1=protein source with soybean paste; T2=protein source with sesame paste; SEM=Standard error of the mean.

The *in situ* digestibility technique is used to measure digestion and apparent digestibility at a ruminal level (Torres *et al.*, 2009). *In situ* DM digestibility at 2, 4, and 48 h did not record differences between treatments ($p>0.05$). T1 recorded a higher *in situ* digestibility at 8, 12, 20, 32, and 64 h than T2 ($p\leq0.05$) (Table 4). Regarding kinetics, both treatments showed the same content of digestible soluble fraction (a) ($p>0.05$). However, T1 had a higher potentially digestible fraction (b) ($p\leq0.05$), although its fermentation rate was similar to T2 (c) ($p>0.05$). Consequently, T1 reported higher potentially digestibility rate ($a+b$), rumen exit rate (k), and effective digestibility (ED) than T2 ($p\leq0.05$) (Table 4). These digestibility differences between supplements can be the result of the sesame paste content in T2, which has 5.4% phytic acid, whose chelating ability reduces dry matter digestibility (Cesária *et al.*, 2022). Aguilera *et al.* (2018) reported lower digestibility values at 64 h. These authors recorded a 60.4% value in a sheep diet prepared with 60% sesame paste.

Initial and final weight, DWG, and SC did not record differences ($p>0.05$) between treatments (Table 5). García-Balbuena *et al.* (2022) reported higher results than T2. They recorded a 238.75 kg initial weight and a 268.75 kg final weight, as well as a 0.77 kg d^{-1} DWG, in calves fed on a diet prepared with 10% sesame paste, 48% corn ensilage, 12% star grass hay, 28% grounded corn grain, and 2% mineral mix. In this study, the average supplement consumption was 324.93 g d^{-1} —this figure is 55% lower than the findings of Rojo *et al.* (2000), who reported a consumption of 730 g d^{-1} in young bulls fed on a feed supplement prepared with 84% corn, 12% molasses, 4% urea, 0.05% mineral salt, and 10 g of *Saccharomyces cerevisiae* animal^{-1} day^{-1}.

The ruminal variables —such as pH and total bacteria and protozoa counting— did not record differences between treatments ($p>0.05$); in average, the following results were recorded: pH, 6.74; total bacteria, 1.82×10^9 bacteria mL^{-1}; and total protozoa, 2.41×10^5 protozoa mL^{-1} (Table 5). Consequently, according to Cardona-Iglesias *et al.* (2016), these variables fall within the optimal intervals for the induction of a fast growth of cellulolytic bacteria (Borroto, 2015): 6.2-7.0 pH and 1010 cells mL^{-1} ruminal bacteria, and 10^5 cell mL^{-1} protozoa concentrations. García-Balbuena *et al.* (2022) reported a higher pH (7.47), bacteria (4.67×10^9 cell mL^{-1}) and protozoa (11.02×10^5 cell mL^{-1}) than this study. In addition, Carbajal-Márquez *et al.* (2021) reported similar pH (6.6), bacteria (4.27×10^9 cell mL^{-1}), and protozoa (2.5×10^5 cell mL^{-1}) values for Suiz-Bu calves that fed on Mulato II grass and were supplemented (1% of LW) with increasing levels of Earpod tree (*Enterolobium cyclocarpum*) pods. The concentration of acetate, propionate, and butyrate in the rumen depends on the diet composition, the microbial activity, the pH, the frequency with which the diet is consumed, the texture of the ingredients, and the prevailing substrate of the portion (Carbajal-Márquez *et al.*, 2021). Consequently, the evaluated treatments did not have an impact on the production of these volatile fatty acids ($p\leq0.05$) (Table 5). For their part, Carbajal-Márquez *et al.* (2021) reported higher acetate values (24.63 Mmol L^{-1}) and lower propionate and butyrate values (9.00 Mmol L^{-1} and 5.60 Mmol L^{-1}, respectively).

Table 4. *In situ* dry matter digestibility of supplements made from soybean paste or sesame paste for grazing calves.

Variable	T1	T2
Dry matter digestibility (%)		
0 h	57.75	59.21
2 h	65.98	63.54
4 h	73.22	65.25
8 h	79.20[a]	75.44[b]
12 h	86.05[a]	82.07[b]
20 h	91.94[a]	87.00[b]
32 h	91.01[a]	87.26[b]
48 h	90.43	84.93
64 h	91.74[a]	85.29[b]
Kinetics of digestibility		
a	57.69	58.25
b	34.10[a]	29.33[b]
a+b	91.8[a]	87.79[b]
c	0.12	0.11
k	0.11[a]	0.14[b]
ED	75.86[a]	71.47[b]

[a,b]: Means in a row with different letter are different ($p \leq 0.05$).
T1=protein source with soybean paste; T2=protein source with sesame paste; SEM=Standard error of the mean; *a*=fast digestible soluble fraction; *b*=slow or potentially digestible fraction; *a+b*=maximum potential digestibility; *c*=rate at which *b* is digested; *k*=ruminal output rate; ED=effective digestibility.

Table 5. Body weight, dry matter intake and rumen fermentation characteristics of grazing calves supplemented with soybean meal paste (T1) or sesame paste (T2).

Variable	T1	T2	SEM
Initial weight (kg)	220.04	221.80	5.67
Final weight (kg)	257.52	254.24	6.57
Daily weight gain (kg d^{-1})	0.67	0.58	0.03
Supplement consumption (g d^{-1})	326.47	323.40	5.56
pH	6.66	6.82	0.11
Total bacteria (10^9 Cells mL^{-1})	2.07	1.58	0.76
Total protozoa (10^5 Cells mL^{-1})	2.63	2.19	0.82
Acetate (Mmol L^{-1})	28.31	23.63	2.36
Propionate (Mmol L^{-1})	9.69	7.24	1.01
Butirate (Mmol L^{-1})	6.45	4.27	0.71

CONCLUSIONS

Based on the *in vitro* and *in situ* evaluations, as well as on the productive behavior of the calves, sesame paste is a feasible option to replace soy paste as a source of proteins, in feed supplements for grazing calves, in the tropical region of Mexico.

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Colegio de Postgraduados

Scientific research on exotic and native fish farming in Mexico: a scientometric view

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ABSTRACT

Objective: To analyze through a scientometric study, the research effort in Mexico on the study of native and exotic fish species with aquaculture production technology, indexed in Scopus.

Design/methodology/approach: Fish species were selected based on a previous study and communications with researchers. Scientific articles from 1990 to 2023 were collected from the Scopus database, focusing on studies conducted by Mexican institutions. Data were transferred to Excel® sheets for analysis, including number of publications, topics, institutions, funding sources and open access documents.

Results: The species that shows the greatest number of articles published is *Oreochromis niloticus*, although there is a clear interest in addressing studies on native species. In general, scientific production is led by institutions such as UNAM, IPN, UABC, CIAD and CIBNOR. The dominant topics are aquaculture, ecology, biochemistry, immunology, and parasitology. CONAHCYT is the main source of funding. About half of the publications were open access.

Limitations on study/implications: The study is limited to the Scopus database, which is the one that includes the largest number of journals worldwide. However, by not including other databases the results could have a slight bias, although we do not consider that the trends changed significantly.

Findings/conclusions: Fish research in Mexico shows a positive outlook, with great interest in several species. However, it faces challenges such as the concentration of effort on exotic species, and the impact of the pandemic on research. A balanced approach is needed to encourage research on native species and facilitate open access to scientific information. This will strengthen fish research in Mexico and enhance its impact on the development of the country.

Keywords: Bibliometrics, native fish, open access, aquaculture, tilapia

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INTRODUCTION

Mexico stands out for its wide diversity of fishes, with a total of 2,763 species known in the country, representing approximately 9.8% of the total number of fish species known in the world. These species are comprised in 53 orders, 265 families and 967 genera (Espinosa-Pérez, 2014).

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Although the diversity of fish in Mexico is one of the highest in the world, only a few native species have been studied for the development of technologies to consider their cultivation. According to Chong-Carrillo *et al.* (2023), research directed to these purposes by the Public Research Centers (CIP) of the National Council of Science, Humanities and Technology (CONAHCYT), studies with native species have focused, for the most part, on marine fish with high added value, while freshwater species have been poorly attended. This is even though the country's major fish production is based on farmed fish in inland systems and waters, with introduced species such as tilapia, carp and catfish (SADER, 2022).

According to Dávila-Camacho (2019), success in the cultivation of native species is based on selecting those with commercial potential due to their rapid growth, high market demand and tolerance to environmental parameters. In mariculture in Mexico, the cultivation of totoaba (*Totoaba macdonaldi*) stands out with complete technological mastery. Other species with advances are the red snapper (*Lutjanus guttatus*), the red snapper (*Lutjanus peru*), and the bullseye puffer (*Sphoeroides annulatus*). In freshwater, the cultivation of the pejelagarto (*Atractosteus tropicus*) for restocking, ornamental and commercial purposes, with complete technology, stands out. Native mojarras such as the castarrica (*Cichlasoma uroptalmus*) and tenguayaca (*Petenia splendida*) are also cultivated.

Although, as mentioned above, research has been carried out with native fish species and there are successful cases of development of finished technologies for their cultivation, the largest production for consumption is based on carp of the *Cyprinus* genus, tilapia (*Oreochromis niloticus*, *O. aureus*, *O. mossambicus* and *Tilapia melanopleura*), commonly known as mojarras and channel catfish (*Ictalurus punctatus*) have also been recorded with important productions (Ortega-Mejía *et al.*, 2023). The first two species are exotic and introduced in most of the country's inland water bodies and both can be considered invasive species due to their high reproductive and adaptive capacity. Although, rainbow trout and channel catfish are native species to specific areas in northern Mexico, their cultivation has also spread to locations outside their natural range, so that, despite being native, they can also be considered invasive species (Mendoza-Alfaro *et al.*, 2021).

The objective of this article is to evaluate the research effort developed by Mexican institutions in relation to the study of native and exotic fish species with aquaculture production technology developed, under development or with potential, based on articles published in international and regional journals deposited in Scopus. This will make it possible to know the scenario in which this type of research is carried out and what its real social impacts are for the country.

MATERIALS AND METHODS

The fish species were selected based on Dávila-Camacho *et al.* (2019), and through personal communication with researchers related to the topic. All scientific papers published between 1990 and 2023 that included the species name in the title, abstract and keywords were selected from the Scopus® database (Elsevier, Netherlands). The database obtained was subjected to a filtering process that focused on studies carried out by Mexican institutions. Subsequently, we proceeded to eliminate those papers that did not comply with the parameters established for the genus and species of the fish under study. The complete records were transferred to datasheets of Excel® (Microsoft, USA) for later analysis. The database was organized to obtain, by species and in general, the number of publications, publication timelines, topics addressed, type of access to publications, institutions that have addressed the study, sponsors and type of access (open or paid). The results were graphed using Excel®.

RESULTS AND DISCUSION

The SCOPUS search yielded a total of 1,316 articles published by Mexican institutions on native and exotic fishes that are subject to study in Mexico for farming purposes. The study focused on 27 species, which are mentioned individually below.

Atractosteus spatula (catán)

The catán is a fish of the 'pejelagartos' group, it is the largest species within this group (Ríos-Espino, 2012), however, it barely reaches a total of seven published studies (Table 1). This species is in the status of foster aquaculture by the Mexican government, and its level of biotechnology mastery is reported as complete larval reproduction and culture and in fattening and experimental production (IMIPAS, 2018). The first study appears in 2002, with records ending in 2015. The Universidad Autónoma de Nuevo León is the institution that contributes to the totality of the studies published on this species (Figure 2), and the thematic axis cover areas of biochemistry, physiology and nutrition. The Consejo Nacional de Ciencia y Tecnología (now Consejo Nacional de Humanidades, Ciencias y Tecnologías-CONAHCYT) is the main source of funding mentioned and one document appears as open access (Figure 3).

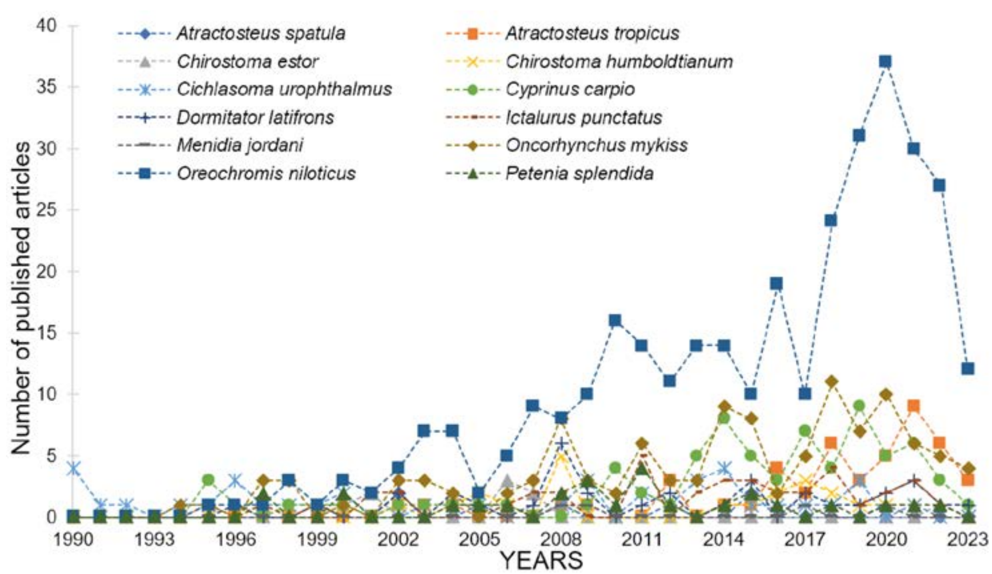


Figure 1. Timeline of studies of freshwater fish with developed, under development or potential production technology in Mexico according to Scopus.

Table 1. Fish species studied with developed, under development or potential production technology in Mexico. Scientific name, common name, status (exotic/native) and the number of publications found in Scopus are shown.

Scientific name	Common name	Common name (spanish)	Status	Number of published articles
Atractosteus spatula	Alligator gar	Catán	Native	7
Atractosteus tropicus	Tropical gar	Pejelagarto	Native	49
Centropomus undecimalis	Common snook	Róbalo común	Native	20
Chirostoma humboldtianum	Shortfin silverside	Charal plateado	Native	23
Chirostoma estor	Whitefish	Pescado blanco	Native	9
Cichlasoma urophthalmus	Mayan cichlid	Mojarra Castarrica	Native	47
Cynoscion othonopterus	Gulf corvina	Curvina golfina	Native	22
Cyprinus carpio	Common carp	Carpa común	Exotic	78
Dormitator latifrons	Pacific fat sleeper	Chame, chopopo, puyeque	Native	35
Ictalurus punctatus	Channel catfish	Bagre de canal	Native	40
Lutjanus guttatus	Spotted rose snapper	Pargo lunarejo	Native	106
Lutjanus peru	Pacific red snapper	Huachinango del Pacífico	Native	74
Menidia jordani	Silverside	Charal	Native	4
Mycteroperca rosacea	Leopard grouper	Mero leopardo	Native	32
Ocyurus chrysurus	Yellowtail snapper	Pargo canané rabirrubia	Exotic	19
Oncorhynchus mykiss	Rainbow trout	Trucha arcoíris	Native	114
Oreochromis niloticus	Tilapia	Tilapia del Nilo	Exotic	333
Paralabrax maculatofasciatus	Spotted sand bass	Lubina manchada	Native	31
Paralichthys californicus	California halibut	Lenguado de California	Native	22
Petenia splendida	Bay snook	Tenguayaca	Native	26
Rachycentron canadum	Cobia	Cobia o esmedregal	Native	9
Sciaenops ocellatus	Red drum	Corvina ocelada o roja	Native	22
Seriola lalandi	Yellowtail kingfish	Jurel, jurel de castilla	Native	22
Seriola rivoliana	Longfin yellowtail	Medregal limón	Native	28
Sphoeroides annulatus	Bullseye puffer	Botete diana	Native	46
Thunnus orientalis	Pacific bluefin tuna	Atún aleta azul	Native	13
Totoaba macdonaldi	Totoaba	Totoaba	Native	85

Atractosteus tropicus **(tropical gar)**

The tropical gar or pejelagarto (*A. tropicus*) is the species of this group of fishes (Lepisosteidae) best studied by Mexican institutions. This species is not only economically, but also socially and culturally important for the communities within its distribution area (Márquez-Couturier and Vázquez-Navarrete, 2015). A total of 49 documents were registered (Table 1), the first publication appeared in 1995, and it was not until 2002 that the number of publications began to increase (Figure 1). The leading research institution for this species is the Universidad Juárez Autónoma de Tabasco (UJAT), which registers 44 documents deposited in the Scopus database. The Centro

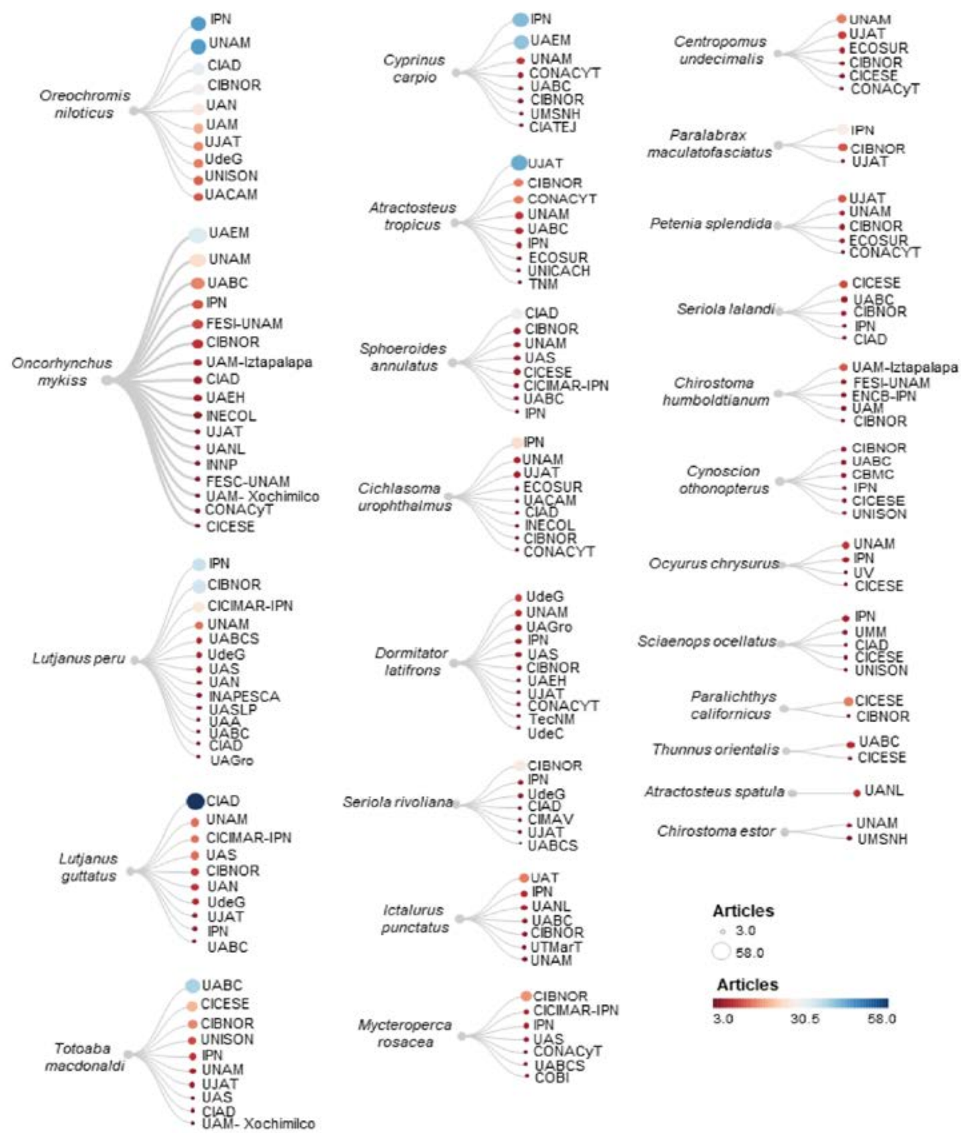


Figure 2. Mexican institutions that undertake studies of fish with developed, under development or potential production technology in Mexico, detected through Scopus. The size and color of the circle indicates the number of articles by institution. Nomenclature of institutions in alphabetical order: CBMC=Centro para la Biodiversidad Marina y la Conservación A.C., CIAD=Centro de Investigación en Alimentación y Desarrollo, CIATEJ=Centro de Investigación y Asistencia en Tecnología del Estado de Jalisco, CIBNOR =Centro de Investigaciones Biológicas del Noroeste, CICESE=Centro de Investigación Científica y de Educación Superior de Ensenada, CICIMAR/IPN=Centro Interdisciplinario de Ciencias del Mar del Instituto Politécnico Nacional, CIMAV=Centro de investigación en Materiales Avanzados SC., COBI=Comunidad y Biodiversidad AC., CONAHCYT=Consejo Nacional de Humanidades, Ciencia y Tecnología, ECOSUR=El Colegio de la Frontera Sur, ENCB-IPN=Escuela Nacional de Ciencias Biológicas-Instituto Politécnico Nacional, FESC-UNAM=Facultad de Estudios Superiores Iztacala, UNAM, FESI-UNAM=Facultad de Estudios Superiores Iztacala, UNAM, INAPESCA=Instituto Nacional de Pesca y Acuicultura, IPN=Instituto Politécnico Nacional, ITNM=Instituto Tecnológico Nacional de México, UAA=Universidad Autónoma de Aguascalientes, UABC=Universidad Autónoma de Baja California, UABCS=Universidad Autónoma de Baja California Sur, UACAM=Universidad Autónoma de Campeche, UAEH=Universidad Autónoma del Estado de Hidalgo, UAEM=Universidad Autónoma del Estado de México, UAGro=Universidad Autónoma de Guerrero, UAM=Universidad Autónoma Metropolitana, UAMIzt=Universidad Autónoma Metropolitana Iztapalapa, UAMXoch=Universidad Autónoma Metropolitana Xochimilco, UAN=Universidad Autónoma de Nayarit, UANL=Universidad Autónoma de Nuevo León, UAS=Universidad Autónoma de Sinaloa, UASLP=Universidad Autónoma de San Luis Potosí, UAT=Universidad Autónoma de Tamaulipas, UdeC=Universidad de Colima, UdeG=Universidad de Guadalajara, UJAT=Universidad Juárez Autónoma de Tabasco, UMM=Universidad Marista de Mérida, UMSNH=Universidad Michoacana de San Nicolás de Hidalgo, UNAM=Universidad Nacional Autónoma de México, UNICACH=Universidad de Ciencias y Artes de Chiapas, UNISON=Universidad de Sonora, UTMarT=Universidad Tecnológica del Mar Tamaulipas Bicentenario, UV=Universidad Veracruzana.

de Investigaciones Biológicas Del Noroeste (CIBNOR) is the second most productive institution and in third and fourth place are CONAHCYT and the Universidad Nacional Autónoma de México (Figure 2). The dominant thematic areas for this species are aquaculture, such as diet design, enzymatic biochemistry and expression of genes involved in metabolism.

CONAHCYT is the main funding source mentioned in documents and fifty-two percent of them are open access (Figure 3).

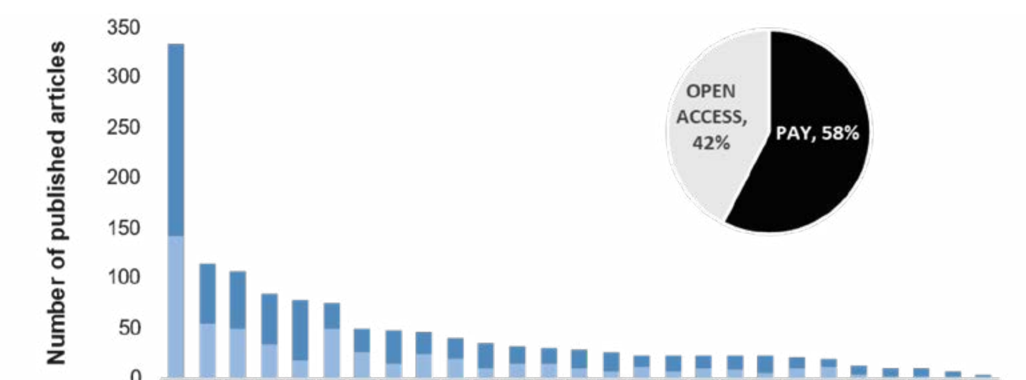
Centropomus undecimalis (common snook)

The common snook *Centropomus undecimalis* is distributed throughout the tropical and subtropical western Atlantic Ocean from the United States of America to Brazil (Arenas *et al.*, 2021). The scientific production for this species by Mexican institutions records a total of 20 papers deposited in the Scopus database (Table 1). The first document was registered in 2006, and its behavior does not show any trend over the years (Figure 4). The Universidad Nacional Autónoma de Mexico (UNAM) is the institution that devotes the most effort to this species followed by the UJAT and El Colegio de la Frontera Sur (Figure 2). The predominant themes for the study of *C. undecimalis* are ecology and aquaculture, with a wide variety of topics such as fisheries, enzymatic biochemistry and reproduction.

CONAHCYT is the main funding source followed by UNAM and El Colegio de la Frontera Sur. Nine documents are registered as open access (Figure 3).

Chirostoma humboldtianum (silver charal) and *Chirostoma estor* (white fish)

The silver charal is a native species found in shallow waters of the Lerma system of the Mexican highlands (Betancourt-Resendes, 2018). Despite being a species with fishery



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Pay	19	60	57	52	61	25	23	33	22	21	25	18	17	19	19	10	15	13	14	17	11	7	10	7	7	6	2
Open access	14	54	49	33	17	49	26	14	24	19	10	14	14	9	7	12	7	9	8	5	9	12	3	2	2	1	2

Figure 3. Number of publications by fish species with developed, under development or potential production technology in Mexico detected in the Scopus database, and divided by their access (open access and paid). Legends on the x axis: 1 *Oreochromis niloticus*. 2 *Oncorhynchus mykiss*. 3 *Lutjanus guttatus*. 4 *Totoaba macdonaldi*. 5 *Cyprinus carpio*. 6 *Lutjanus peru*. 7 *Atractosteus tropicus*. 8 *Cichlasoma urophthalmus*. 9 *Sphoeroides annulatus*. 10 *Ictalurus punctatus*. 11 *Dormitator latifrons*. 12 *Mycteroperca rosacea*. 13 *Paralabrax maculatofasciatus*. 14 *Seriola rivoliana*. 15 *Petenia splendida*. 16 *Paralichthys californicus*. 17 *Sciaenops ocellatus*. 18 *Cynoscion othonopterus*. 19 *Seriola lalandi*. 20 *Centropomus undecimalis*. 21 *Ocyurus chrysurus*. 22 *Thunnus orientalis*. 23 *Rachycentron canadum*. 24 *Chirostoma estor*. 25 *Atractosteus spatula*. 26 *Menidia jordani*. 27 *Chirostoma humboldtianum*.

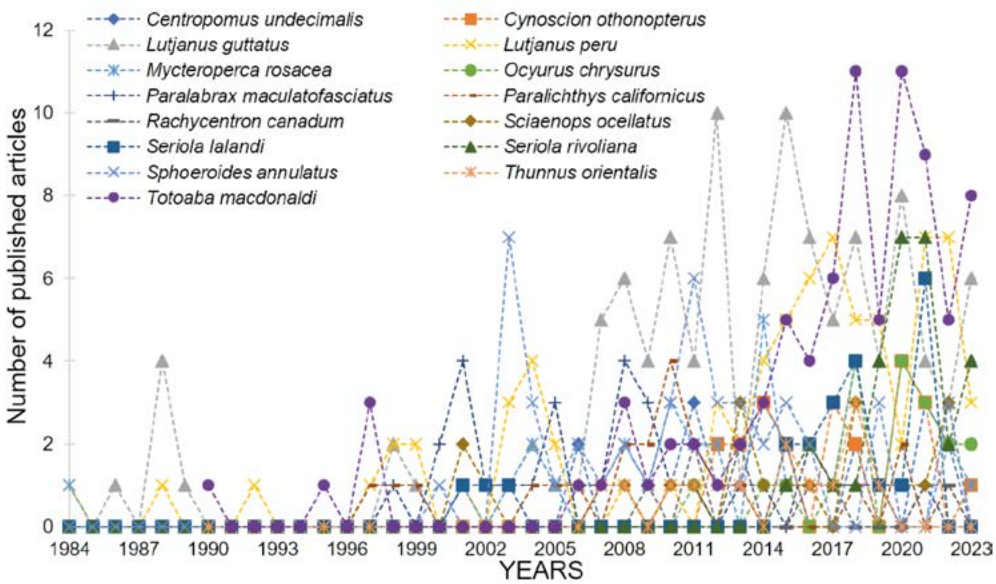


Figure 4. Timeline of studies of marine fish with developed, under development or potential production technology in Mexico according to Scopus.

importance for human food, it barely reaches a total of 23 papers registered in the Scopus database (Table 1). The first production records appear in 2006, and it has remained without any trend over the years (Figure 1). The Universidad Autónoma Metropolitana (UAM) is the main institution that dedicates research to this species followed by UNAM and in third place is the Instituto Politécnico Nacional (IPN) (Figure 2). The main topics treated for the species are ecology and fisheries, genetics and aquaculture, with works such as population genetics, karyotypes and aquaculture nutrition.

CONAHCYT is the main funding source mentioned in the documents on six occasions, followed by UAM with two, the rest of the funding sources barely appear with a single mention. Five documents were published with the open access category (Figure 3).

The whitefish, *C. estor*, is a species endemic to the lakes of Pátzcuaro and Zirahuén and shares many characteristics, both biological and commercially important, with *C. humboldtianum* (Rojas-Carrillo, 2005); however, its scientific productivity is only nine papers registered in the Scopus database (Table 1). UNAM and the Universidad Michoacana de San Nicolás de Hidalgo are the institutions that pay most attention to this species (Figure 2). The topics published are on ecology, parasitology and aquaculture. Only two papers were published under the open access category (Figure 3).

Cichlasoma urophthalmus (mojarra castarrica, mojarra colorada, mojarra criolla)

This native freshwater fish is in the top 5 of the most studied species, with 47 papers registered in the Scopus database (Table 1). The first record has been kept since 1986, and its production has been between 1 and 3 articles per year (Figure 1). The main institutions that dedicate research to this species is the IPN, which leads the research on this species, the second place is occupied by the UJAT and the third by El Colegio de la Frontera Sur

(Figure 2). There is interest in developing its production technology, with some pilot farming programs (Ramírez-Abarca *et al.*, 2011), although parasitology is the main thematic area, followed by aquaculture and ecology.

CONAHCYT dominates as the main funding source, followed the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO) and UNAM. Of the 47 documents, 14 appear in the open access category (Figure 3).

Cynoscion othonopterus **(Gulf corvina)**

The Gulf corvina is distributed throughout the tropical and subtropical western Atlantic Ocean from the United States of America to Brazil (Arenas *et al.*, 2021). It is a native marine species that only records a total of 22 papers deposited in the Scopus database (Table 1). 2020 was the year with the most records (four), and the behavior through the years is very variable, with highs and lows (Figure 4), with an average of approximately two articles per year, starting in 2012. The institutions that stand out are, in first place, CIBNOR and the Universidad Autónoma de Baja California (UABC), followed by the IPN, the Centro de Investigación Científica y Educación Superior (CICESE) and the Universidad de Sonora (Figure 2). The main line of research directed at this species is ecology with and aquaculture, population genetics and fisheries.

CONAHCYT is the main funding source, followed by a foreign foundations, the David and Lucile Packard Foundation, the Walton Family Foundation and the Universidad de Sonora. Nine published documents appear in the open access category (Figure 3).

Cyprinus carpio **(Common carp)**

The common carp is the second most studied exotic species by Mexican institutions, with 78 publications registered in the Scopus database (Table 1). The first study in Mexico for this species was recorded in 1984, while 2013 has the highest number of publications, with nine. There is no pattern in the number of publications over time, but there is an increase that falls during the pandemic (Figure 1). The IPN tops the list of institutions with the most studies for this species, the UNAM is positioned as the second, and UNAM as third (Figure 2). The studies with the highest proportion for *C. carpio* are those on toxicity, dealing with topics such as bioaccumulation, heavy metals, among others. The next most important thematic area is immunology, oxidative stress and hematology.

Among the main funding sources for studies focused on *C. carpio*, CONAHCYT obtained the most mentions, followed by the IPN. Of the 78 documents published, 17 appear in the open access category (Figure 3).

Dormitator latifrons **(chame, chopopo, Pacific fat sleeper, puyeque)**

D. latifrons (Richardson, 1844), commonly known as the Pacific fat sleeper, inhabits continental waters from California (USA) to Ecuador (Aréchiga-Palomera *et al.*, 2022). There is a record of 35 documents published in the Scopus database (Table 1). The first document appeared in 1996 and productivity has ranged from 1 to 3 documents per year (Figure 1). The Universidad de Guadalajara (UdeG) is the leader in research for this species, followed by UNAM and the third place is occupied by the Universidad Autónoma de Guerrero (UAG), with (Figure 2). The main areas addressed for this species are, in first place, parasitology, followed by ecology and aquaculture.

Only seven documents mention that funding is granted by CONAHCYT, one for the UdeG and another for the Universidad Autónoma de Sinaloa (UAS). Ten documents appear with the category of open access (Figure 3).

Ictalurus punctatus **(Channel catfish, American catfish)**

Channel catfish is a native freshwater species that is cultivated in various areas of the country (Lara-Rivera *et al.*, 2015). A total of 40 documents deposited in the Scopus database are registered (Table 1). The first record is from 1984, and its productivity ranges from one article to five over the years (Figure 1). The Universidad Autónoma de Tamaulipas (UAT) is the leader in research for this species, followed by the IPN and the Universidad Autónoma de Nuevo Léon (UANL) (Figure 2). The main thematic area is aquaculture, followed by genetics and biochemistry and parasitology.

CONAHCYT is the main funding source followed by IPN and the Secretaría de Educación Pública (SEP). There were 19 documents under the open access category, almost 50% of all the documents registered (Figure 3).

Lutjanus guttatus **(spotted rose snapper)**

The snapper, *L. gattatus*, is a marine native species of the tropical and subtropical eastern Pacific, its distribution extends from the southwestern coast of Baja California Sur and the Gulf of California, Mexico, to Peru. Low-scale, wild juvenile grow-out farms are reported in Baja California Sur, Sinaloa and Jalisco (IMIPAS, 2018). It is the most studied native species by Mexican institutions, with a total of 106 papers deposited in the Scopus database (Table 1). The Centro de Investigación en Alimentación y Desarrollo (CIAD) is the leader in research for this species followed by UNAM, the Centro Interdisciplinario de Ciencias Marinas del IPN, CIBNOR and the UAS (Figure 2). This species is one of those for which the greatest effort has been given to promote its cultivation, with some precedents of aquaculture activity led by CIAD and INAPESCA (IMIPAS, 2018). Aquaculture is the main thematic axis of study for this species with topics from larval stages to juveniles, search for diets according to their nutritional requirements, among others, and topics of reproduction. Ecology is the second thematic area for the species, and in third place is Parasitology.

CONAHCYT is the main source of funding for studies in this organization, followed by the Secretaría de Agricultura y Desarrollo Rural (SADER), and the Comisión Nacional del Pesca y Acuacultura (CONAPESCA). 46% of the documents were published under the open access category (Figure 3).

Lutjanus peru **(Pacific red snapper)**

The red snapper, *L. peru*, is a species with similar characteristics to *L. guttatus*; they are well accepted species in the regional market and their capture is persistent throughout the year (Chiappa-Carrara *et al.*, 2004). It is also a species that has generated a high scientific production by Mexican institutions, with a total of 74 papers deposited in the Scopus database (Table 1). The years 2017, 2021 and 2022 have the most published papers, with seven (Figure 4) with a dramatic decrease in 2023. The leading institutions in research for this species are led by the IPN, followed by CIBNOR and UNAM (Figure 2). The main thematic area is ecology, which include fisheries and deal with topics such as assemblages, connectivity and population characteristics. The next area is immunology, dealing with topics such as immune response, use of βglucans, use of probiotics, among others, and in third place appears aquaculture which is combined with immunology to deal with topics such as use of ingredients like probiotics or immunostimulators added to the diet of these organisms; the third most important topic is reproduction.

CONAHCYT is mentioned as the main source of project funding, followed by the IPN and SEP. A total of 27 documents were published under the open access category (Figure 3).

Menidia jordani **(river charal)**

The river charal, *M. jordani*, is distributed in the Mexico Basin, in the Lerma River Basin and its associated lake systems (Cuitzeo and Chapala), as well as in the Pánuco, Cazones and Tecolutla Rivers and their tributaries, with Lake Metztitlán as the eastern limit of its distribution in lentic environments (Ibáñez *et al*., 2008; Abeja-Cruz *et al*., 2013). The scientific information for this species, at least that reported for the Scopus database is very poor, with only four documents (Table 1). It is almost probable that the information generated on this species is available in gray literature (such as thesis, newspaper notes, and low print run books and manuals), and mostly on its ecology and fisheries.

Mycteroperca rosacea **(leopard grouper)**

This endemic, marine species is distributed from the southeastern coast of the Baja California peninsula, throughout the Gulf of California to the coasts of Jalisco, Mexico. It has strong importance in commercial fisheries and is a candidate for cultivation (Romo-Mendoza, 2019). A total of 32 papers deposited in the Scopus database were counted (Table 1). CIBNOR leads the research on this species followed by IPN with and UAS (Figure 2). The main thematic areas addressed for this species are, in first place, reproduction followed by Ecology and Immunology.

CONAHCYT dominates as the main source of project funding, followed by Sistema de Investigación del Mar de Cortés (SIMAC). There were 14 documents published under the open access category (Figure 3).

Ocyurus chrysurus **(canané snapper, yellowtail snapper)**

This organism has a wide distribution range that goes from Massachusetts, USA, to Brazil, including Bermuda, the Gulf of Mexico, the West Indies and the Antilles, and since it is considered a high-quality food, studies have been developed that involve its cultivation in controlled systems (Gutiérrez-Benítez, 2012). A total of 19 documents deposited in the Scopus database were recorded (Table 1), starting in 2011 (Figure 4). The institutions with the highest number of publications on this species are UNAM, IPN, Universidad

Veracruzana (UV) and CICESE (Figure 2). The main topic addressed in this species is Ecology, with nine documents, followed by Aquaculture with four.

CONAHCYT and UNAM are the main contributors of funding for the study of this species. Twelve documents were published under the open access category (Figure 3).

***Oncorhynchus mykiss* (rainbow trout)**

Rainbow trout, *O. mykiss,* (Walbaum, 1792) is a salmonid distributed from southern Alaska to northwestern Mexico (Hernández and Carrillo, 2018). According to the SADER (2021), trout production in 2020 was 3,898 tons. Although 18 states are reported as the producers, the main ones are (in order of production volume): Tamaulipas, Veracruz, Puebla, Michoacán and Chihuahua. The Scopus search yielded a total of 114 articles generated by Mexican institutions, the most important being the Universidad Autónoma del Estado de México (UAEM), UNAM, the Universidad Autónoma de Baja California (UABC), IPN, CIBNOR and the Universidad Autónoma Metropolitana (UAM) – Unidad Iztapalapa (Figure 2). With almost 50% of the articles detected, Agriculture and Biological Sciences is the most frequently addressed topic, followed by Veterinary Science, Environmental Science and Biochemistry, Genetics and Molecular Biology (the four topics account for almost 90% of the articles published). CONAHCYT is the institution that has provided the most funding for research on this species, followed by UNAM and the Universidad Autónoma del Estado de México, a total of 54 articles are available as open access (Figure 3).

***Oreochromis niloticus* (tilapia, Nile tilapia)**

These fish of African origin were introduced into many regions of the world during the second half of the 20th century for the purpose of providing a sustainable, inexpensive, high-quality source of protein. They have become widely popular because they tolerate high densities, are fast growing, disease resistant, easily adaptable to captivity, accept balanced feeding diets, the meat is of good quality and affordable price (Jácome *et al.*, 2019). In Mexico, the research directed to this species is the one that has made the greatest effort. A total of 333 documents deposited in Scopus are registered, from the year 1994 the first article is registered, while the year 2020 is positioned as the most productive, with 37 publications. Although there is no constant growth, there is an increase in the number of publications over the years (Figure 1). The institutions that direct most effort to the study of this species, in decreasing order, are the IPN and UNAM, CIAD and CIBNOR (Figure 2).

Despite being a species of commercial importance for human consumption, most of the studies are focused on the area of ecology, followed by genetic and immunological studies and microbiology and biochemistry. Although these studies are considered aquaculture topics, these areas are strongly related to aquaculture, as they address information on the metabolism and physiology of this species that is important for human consumption. CONAHCYT is the institution as the main source of funding, followed by IPN and UNAM. Of the 333 documents registered, 142 have open access status (Figure 3).

***Paralabrax maculatofasciatus* (spotted sand bass)**

The cabrilla arenera is a marine fish native to the Eastern Central Pacific. It is exploited throughout the year, mainly in local fisheries where it is marketed as one of the most important products in the northwestern region of Mexico (De la Re-Vega *et al.*, 2020). A total of 31 papers registered in the Scopus database were counted (Table 1). The IPN is the institution with the highest number of publications, followed by CIBNOR (Figure 2). Aquaculture and biochemistry were the main areas that dominate the study of this species.

CONAHCYT is the organization that provides the most funding to projects, followed by the IPN. A total of 14 documents were published under the open access category (Figure 3).

***Paralichthys californicus* (California halibut)**

The California sole (*Paralichthys californicus*) is a flatfish that inhabits the waters of the west coast of northern Mexico and the USA. It is considered a species with high aquaculture potential in both countries (Badillo-Zapata *et al.*, 2010). A total of 22 papers deposited in the Scopus database were registered (Table 1). CICESE is the institution with the most research on the species, followed by CIBNOR (Figure 2). Aquaculture is the main thematic axis for the study of this species, followed by Biochemistry, but this last area is related to aquaculture.

CONAHCYT is the source of funding for projects for the study of this species, followed by CICESE. Of the 22 published papers, 12 were published under the open access category (Figure 3).

***Petenia splendida* (tenguayaca, bay snook)**

Bay snook (*Petenia splendida* Günther, 1862), locally known as "tenguayaca" is a highly valued native cichlid for human consumption, inhabiting freshwater bodies from southeast of Mexico. It represents significant ecological importance and economic value (locally and regionally) for aquaculture (Rodríguez-Estrada *et al.*, 2020). A total of 26 papers were registered in the Scopus database (Table 1). UJAT leads in scientific production for this species, followed by UNAM, CIBNOR and El Colegio de la Frontera Sur (Figure 2). Ecology is the main dominant area, followed by aquaculture and parasitology.

CONAHCYT received only three mentions as a source of funding for projects aimed at the study of this species, and other institutions and foundations received only one mention. Seven documents were published with the category of open access (Figure 3).

***Rachycentron canadum* (cobia)**

This marine fish is a cosmopolitan organism from the Atlantic, has commercial importance and in Mexico is under the category of Aquaculture of promotion (IMIPAS, 2018). Studies in Mexico regarding this species are poor, with only a total of nine records deposited in the Scopus database (Table 1). UABC, CIAD and IPN are the institutions with the highest publication records, but with just two for each institution (Figure 2). Most of the topics related to this species are related to ecology and fisheries. There is no funding source that stands out, all with one mention, including CONAHCYT and the institutions mentioned above.

Sciaenops ocellatus **(Corvina ocelada or red drum)**

This native marine species is distributed in the northern Atlantic Ocean and the Gulf of Mexico. The level of mastery of its culture technology is complete and it already has a history of aquaculture production (IMIPAS 2018). Despite having the technological development for its cultivation, research directed in Mexico towards this species barely reaches 22 publications deposited in the Scopus database (Table 1). Institutions such as IPN, Universidad Marista de Mérida and CIAD stand out (Figure 2). Aquaculture is the main thematic focus for the study of this species, with ecology in second place.

CONAHCYT is the main source of funding for projects aimed at the study of this species, while the IPN and the Universidad Marista de Mérida occupy the second position. Seven papers were published under the open access category (Figure 3).

Seriola lalandi **(California horse mackerel, yellowtail jack mackerel)**

This native marine species has in Mexico a partial culture domain limited to fattening and there are antecedents of its culture based on the collection of juveniles (IMIPAS, 2018). Research directed at this species is concentrated in institutions in the north of the country, and its productivity accounts for 22 papers registered in the Scopus database (Table 1). The institutions that stand out are CICESE, UABC and CIBNOR (Figure 2). Aquaculture dominates as the thematic axis in the study of this species.

CONAHCYT is the main source of funding for projects aimed at the study of this species. Eight papers were published under the open access category (Figure 3).

Seriola rivoliana **(Bojala, lemon medregal)**

The lemon sole, *S. rivoliana*, is a native pelagic fish that is distributed circumtropically in the Pacific, Atlantic and Indian Oceans. It has been identified as having high aquaculture potential due to its rapid development, good meat quality and market value (Mendoza-Portillo, 2022). The scientific production of this species registers a total of 28 papers deposited in the Scopus database (Table 1). CIBNOR is the leader in research for this species, followed by IPN and the UdeG (Figure 2). Immunology, aquaculture and biochemistry are the main axes for the study of this species. CONAHCYT and CIBNOR are the main funding sources for projects. Nine published documents appear with the category of open access (Figure 3).

Sphoeroides annulatus **(bullseye puffer, botete)**

This native marine species, the botete or *S. annulatus*, is a species found in the Mexican Pacific with distribution from the Coast of San Diego, California (USA) to Peru (Abdo-De la Parra *et al.*, 2022). Forty-six papers are registered in the Scopus database (Table 1). CIAD leads the research for this species, followed by CIBNOR (Figure 2). The main thematic areas are reproduction, aquaculture and parasitology.

The topics addressed are hatchery, aquaculture nutrition and parasites associated with fisheries. CONAHCYT is the main source of funding for projects aimed at the study of this species, followed by SAGARPA. There were 24 documents published under the open access category (Figure 3).

***Thunnus orientalis* (bluefin tuna)**

The bluefin tuna *T. orientalis*, a marine fish distributed from Japan to the Baja California peninsula in Mexico. It is of undoubted importance in commercial and sport fishing. The domain of its biotechnology is partial and limited to fattening culture through the collection of wild organisms (IMIPAS, 2018). Despite its importance, in Mexico it barely reaches 13 publications registered in the Scopus database (Table 1). UABC and CICESE are the institutions with the highest numbers of publications with aquaculture as main topic.

CONAHCYT and UABC are the main entities that offer financial support for projects aimed to study of this species. Three papers were published under the open access category (Figure 3).

***Totoaba macdonaldi* (Totoaba)**

The totoaba is one of the most prized marine fish species in the upper gulf area. It is currently included in the list of endangered species and is permanently banned. The decline of its populations due to illegal fishing has forced institutions to redouble their efforts in the study to develop aquaculture technology (Cáceres-Martínez *et al.*, 2023). In fact, it is the second best studied native species by Mexican institutions, with a total of 85 published papers registered in the Scopus database (Table 1). An increase in the number of publications over the years can be observed (Figure 4). UABC is the leader in research, followed by CICESE and CIBNOR (Figure 2). Aquaculture is the main thematic axis in the study of this species, representing 35% of the published papers.

The topics covered include the use of additives, ingredient replacements to evaluate larval and juvenile growth, among others. Ecology and fisheries represent the second thematic axis treated in this species, fundamental for the evaluation and management of this species that is catalogued as vulnerable due to illegal fishing. CONAHCYT is the main entity with the highest number of mentions in funding, followed by UABC and CISESE. There were 33 documents published under the open access category (Figure 3).

General scenario

The results show that Mexican academic and research institutions have a clear interest in approaching the study of native species in a basic way and in the development of technologies for their cultivation. Applied research, based on basic studies of physiology, nutrition and reproduction, among others, have resulted, in some cases, in efficient cultivation technologies. However, the species that has shown the greatest growth, with the greatest number of articles published, is *O. niloticus*, a freshwater and exotic species. The common carp, *C. carpio*, also accumulates a good number of published articles, despite being another of the exotic species most widely distributed in dams and reservoirs in Mexico (Table 1). These two species alone account for 31% of all the articles published on fishes of commercial or social interest despite constituting only two of the 27 species covered in this work, or 7% of these. Most of the research has been directed towards marine species with high added value (Table 1). This has already been commented by Chong-Carrillo *et al.* (2023), who mention that the research conducted by the Public Research Centers (CIP) of the National Council for Science, Humanities and Technology (CONAHCYT) has focused, for the most part, on marine fish with high added value, while native freshwater species have received less attention.

The native fishes that show a growth in the number of publications are the rainbow trout *O. mykiss*, which after tilapia, is the fish with the most total published studies, followed by the tropical gar *A. tropicus*, the castarrica mojarra *C. urophthalmus*, and the channel catfish *I. punctatus*, species whose biological cycle has been closed and whose culture technology is very advanced. Of the total number of species included in this study, the 12 freshwater species, native or exotic, represent 44%, but in the total number of articles they have generated, they account for 58%.

Of the marine fishes recorded in the present study –15 in total– the lunar snapper *L. guttatus*, accumulated the most publications, followed by the totaba *T. macdonaldi*, the Pacific red snapper *L. peru*, and the botete *S. annulatus* (Table 1). These four species accumulate 311 published articles, which represents 24% of the total number of articles of the species covered in this study. Research with the remaining marine species (11) yielded 18% of the total publications. The cumulative number of articles published with marine species is 42%.

As it is possible to observe, and as mentioned before, native species have received great attention from the country's scientific community, and this complies with the concern expressed by FAO (2010, 2014, 2016, 2020, 2022) in relation to avoiding the introduction of exotic species with potential or known invasive capacity, and the need to research and develop technologies of regional, native or endemic species.

Figures 1 and 2 show the timeline of publications generated with freshwater species (Figure 1) and marine species (Figure 2). In both graphs it can be observed that there are species that show a profuse production of scientific articles, the least, and a majority that maintain a modest production over time. This may indicate that the knowledge generated about them is not yet sufficient to derive efficient cultivation technologies. This is more evident for native freshwater species. For the latter, the interest in tilapia in research development is clear, with an upward trend until 2020, for a dramatic decline to date. This decline is probably one related to the effects of the COIVD-19 pandemic that forced much of the country's scientific community into a work stoppage. This same phenomenon, although on a much smaller scale, can be observed in other freshwater species that were experiencing greater growth in research translated into published articles, such as rainbow trout, common carp and the common red snapper. This decrease does not show an increase in the last recorded year. In the case of marine species, the phenomenon is apparently repeated.

Species that showed a growth trend, despite significant variations in the timeline, are noted with significant declines from the year 2020 to increase again in the post-pandemic stage, which is not observed in freshwater species. This is the case of *L. guttatus*, *S. rivoliana* and *T. macdonaldi*. The consequences of the isolation forced by the pandemic suggest a negative effect on the continuation of research centers and universities. This phenomenon has been mentioned by Jamali *et al*. (2023), who comment that the closure of laboratories and the impossibility of carrying out field work had a severe impact on the development of young scientists in training and on their productivity, an important sector in the scientific population since it is the main work force of research groups.

Regarding the thematic areas, classified by Scopus, the articles published address aquaculture in more than 30% of the total, followed by Ecology and Immunology/Microbiology. These areas alone represent more than 50% of the publications (Figure 5). Most of the remaining publications deal, directly or indirectly, with aspects also related to aquaculture, such as Genetics, Biochemistry, Reproduction, Parasitology and Physiology (Figure 5).

The above shows that both basic and applied research have been directed to fill gaps in knowledge related to the capacity to carry out the culture of the species of interest and, in the case of Ecology, to determine population and environmental aspects (particularly of native species) that, although in *sensu stricto* do not follow the previous path, do allow contemplating a more complete scenario on the status of the species' populations, their interactions with the environment and the possibility of sustainable use.

Figure 3 shows the publications by species considering the access to them, either open access or paid access. Of the total number of articles published, 58% could only be accessed through the pay access system of the respective journals, while only 42% had open access. This may reflect the need for authors to use journals known as "mainstream", generally published by transnational publishers that only offer the opportunity for articles to be

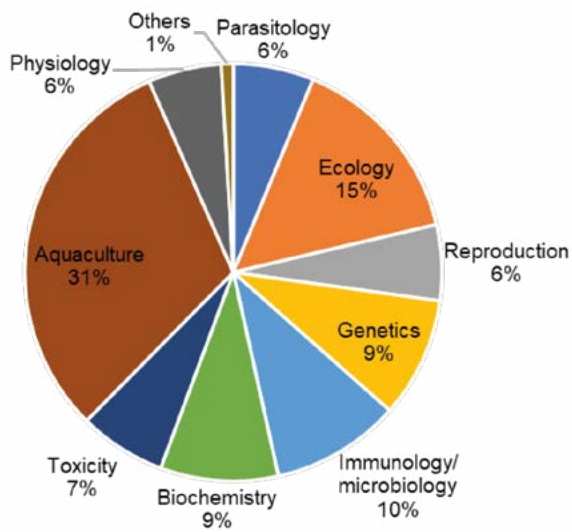


Figure 5. Thematic areas in studies of fish with developed, under development or potential production technology in Mexico and detected through Scopus.

freely read by charging very high fees. In addition, Mexican scientific policies, through CONAHCYT, promote publication in journals of "recognized prestige" included in indexes, also transnational, such as the Journal Citation Rank (JCR) and in databases such as Clarivate, Scimago Journal & Country Ranks and Scopus. In the case of open access journals, these are generally sponsored by academic institutions, public or private, whose publication costs are generally low. Many of these are also included in international indexes and databases. However, our study indicates that researchers prefer to publish in more prestigious journals with the idea that their work will be better located and cited (Figure 3).

With the popularization of the Internet and easy access to free databases, this justification has weakened as open access articles can be easily detected and consulted. Open access advocates argue that scholarly communities should organize against the current system in which scholars donate articles for free but then must buy them at high prices from commercial publishers (Wellen, 2004). Undoubtedly, the payment of high publication fees represents a deterrent for many researchers, especially those attached to public universities with limited economic resources. In addition, another negative factor is the loss of copyright, with the obligatory assignment to the journal. In such a way, the authors themselves do not have the legal right to disseminate their published work, although this is *de facto* done. Of the species addressed in this study, only four demonstrate open access publications superior to paid publications: *A. tropicus*, *S. annulatus*, *P. californicus* and *T. orientalis*. In the remaining, the vast majority, the ratio of open access: paid access is always higher than the latter. It is worth mentioning that in many cases this ratio is close, indicating an interest or need to publish in open access journals.

Reaching a scenario of only "open access" is not real; transnational commercial publishers are companies with great power, not only economic but even political, and they will continue to occupy a preponderant place in the dissemination of science. Promoting the development of open access publications, with rigorous editorial standards and mechanisms to deal with possible biases and inequities, emerges as a promising strategy to reform scholarly communication practices. The two models, paid and open access, should not be mutually exclusive; it is necessary to find ways for them to coexist and collaborate to broaden access and dissemination of scientific knowledge.

In relation to the institutions that have addressed the study of marine and freshwater fishes, Figure 2 shows their preponderance in the number of published articles on a scale from 3 (at least three published articles), deep red, to 58 (up to 58 published articles) deep blue. The identifying circle is directly proportional, in size, to the number of publications. The institutions that have studied the most species are (in order from highest to lowest) IPN>CIBNOR>UNAM>UABC>UJAT>CICESE>CIAD (Figure 6). This reflects the capacity of these institutions not only to have human resources but also financial resources, which allow them to demonstrate a clear superiority over the rest. The Public Research Centers (CPIs) of CONAHCYT are represented by CIBNOR, CICESE and CIAD, centers that have proverbially been spearheads in many of the studies carried out, mainly with marine species (Chong-Carrillo *et al.*, 2023). It is worth noting that four universities show a profuse publication, such is the case of the IPN (all its centers and

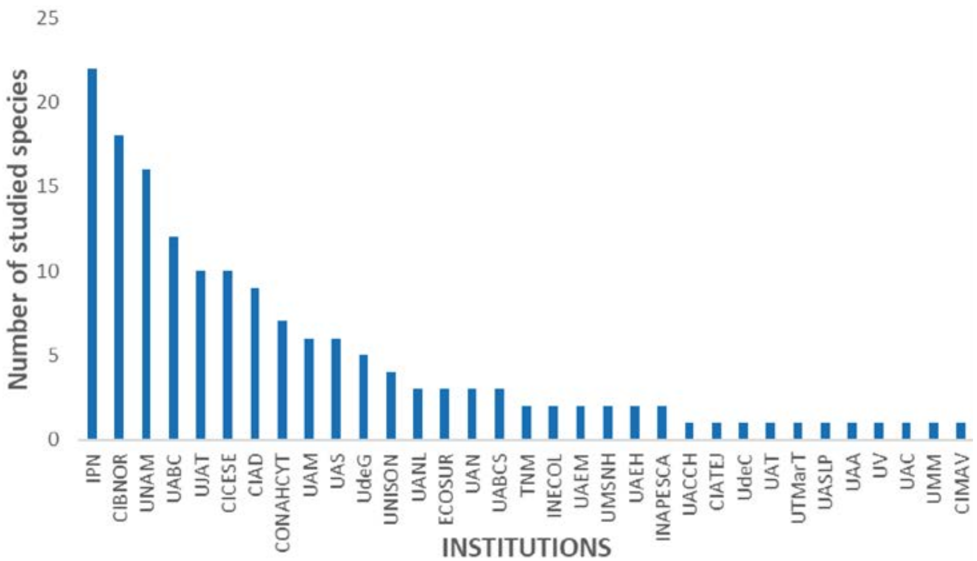


Figure 6. Number of fish species addressed by Mexican institutions detected in the Scopus database

units were included), which stands as the institution that has been involved in the study of the greatest number of species, followed by UNAM, UABC and UJAT.

Although tilapia is the species with the most articles published in the period analyzed, according to Scopus, the species that has received the most attention from the total number of institutions is the rainbow trout, *O. mykiss*, with 17 institutions, followed by red snapper, *L. peru* (14), puyeque, *D. latifrons* (11), tilapia, *O. niloticus* (10), snapper, *L. guttatus* (10) and totoaba, *T. macdonaldi* (10) (Figure 7).

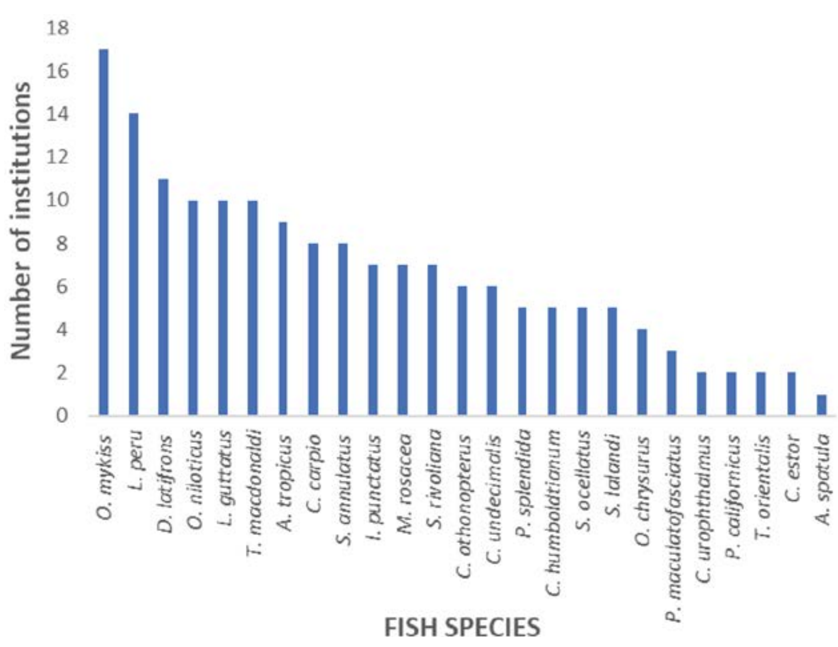


Figure 7. Number of Mexican Institutions that undertake fish studies with developed, under development or potential production technology in Mexico and detected through Scopus.

There does not seem to be a direct relationship between the number of institutions that study a fish and the number of articles published. The Pacific fat sleeper *D. latifrons*, stands out, despite being the third species that has received the most attention from various institutions, with only 35 registered publications (Table 1, Figure 1). A study should be carried out to determine the causes of this phenomenon, with this and other species.

In relation to the social impact that research could have on the species addressed (translated into technologies), the results suggest that although most species, both marine and freshwater, are native (and even some endemic), the institutions involved in their study, have not given them balanced and similar importance. Most of the species carried out for research are considered to have "high added value", rather than social, interest of Mexican institutions. This phenomenon was already addressed by Chong-Carrillo *et al.* (2023) who, based on projects developed by public research centers, demonstrate that few projects are aimed at solving priority needs (food, nutrition) of the population in general and focus on species considered of interest. for marketing in international markets. Native species with social and cultural value are little attended to, although there are relevant examples that suggest that research groups do dedicate efforts to them. Such is the case of the alligator gar, *A. tropicus*, which has been widely studied and has great social and cultural value in the southeastern Mexican region. Another example could be the castarrica mojarra, *C. urophtalmus*, which is already cultivated and is a fish that can be acquired by a large part of the population, also in the Mexican southeast. Even so, the appearance of tilapia (*O. niloticus*) as the most studied species, with the greatest number of publications, is evidence of the need for institutions to provide new information that allows its more efficient cultivation based on a spirit of social benefit. The above has also been mentioned by Chong-Carrillo *et al.* (2023) who show that some public research centers allocate significant funding to the study of this exotic species. This study showed that CONAHCYT is the main entity that allocates the most funding to fish research. However, it is evident that, although there are efforts for the study of certain native species with a view to their technological development, most Mexican institutions do not seem to have sufficient interest in directing their studies to regional needs, and food security of vulnerable groups. However, CONAHCYT itself has redirected its objectives in relation to the social impact of research as a fundamental aspect in the development of the country.

CONCLUSIONS

There is a clear interest of Mexican institutions in addressing the study of native fishes with publications in national and international journals, however, the publication trends suggest a significant focus on exotic species of commercial or social interest. *Oreochromis niloticus* and *Ciprinus carpio* are the species with the highest number of published articles, representing 31% of the total. On the native fishes in Mexico, *Oncorhynchus mykiss* is the species that has received the most attention, followed by *Lutjanus guttatus*, *Totoaba macdonaldi* and *Atractosteus tropicus*.

A decline in publications is observed from 2020 onwards, possibly related to the effects of the COVID-19 pandemic. Laboratory closures and difficulties in carrying out field work have had a negative impact on the productivity of the scientific community.

The research addresses aspects related to physiology, nutrition, reproduction, genetics, biochemistry, parasitology and physiology, evidencing an integrated approach to the development of culture technologies. More than 50% of published articles focus on aquaculture, ecology and immunology/microbiology. Approximately 58% of the articles are only available through paid systems, reflecting the researchers' preference for "mainstream" journals. Open access represents 42% and highlights the need to find a balance between both models to broaden access and dissemination of knowledge. Institutions such as IPN, CIBNOR, UNAM, UABC, UJAT, CICESE and CIAD lead in number of articles published. There is no direct relationship between the number of institutions and the number of articles published for each species. Research aimed at solving priority needs related to food is few, and most are focused on the study of tilapia, even though it is an exotic species and considered invasive.

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Colegio de Postgraduados

Current status of the global production chain of giant river prawn (*Macrobrachium rosenbergii*)

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ABSTRACT

Objective: To determine the current state of the global production chain of *Macrobrachium rosenbergii*, identifying its links and the characteristics of the techniques used.

Design/Methodology/Approach: Studies over a period of 20 years (2001-2022) about the production chain of *M. rosenbergii* were reviewed and analyzed. The information was synthesized in tables and the most relevant production and farming data are offered as an output.

Results: The following links of the production chain were identified: production, processing, and commercialization. The *Macrobrachium rosenbergii* farming and the main technologies used to improve its production are described.

Study Limitations/Implications: There is limited systemic information about the *M. rosenbergii* production chain. The topics addressed the specifics of production, processing, and marketing.

Findings/Conclusions: To improve the *M. rosenbergii* production chain, the following aspects must be guaranteed: the supply of post-larvae (PL) specimens, the development of better monoculture practices, and its integration with other species of commercial importance. The organization of the production chain must also be reviewed, considering its direct and indirect participants.

Keywords: farming, crustaceans, production.

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INTRODUCTION

Macrobrachium rosenbergii is a species of freshwater crustacean, native to the Indo-Pacific region. It is commercially important and is farmed in various countries around the world (New and Valenti, 2000). According to the Food and Agriculture Organization (FAO) of the United Nations, it has been introduced as an aquaculture product in at least 40 countries (FAO, 2022a). The global production of *M. rosenbergii* in 2020 was 294,000 tons, representing 2.6% of the total crustacean production (FAO, 2022b). Modern farming of this species started around 1960. In 1978, the first major FAO project designed to expand the farming of this species in Thailand began (New, 2009). Since then, it has been developed on all continents, particularly in Asia and the Americas (FAO, 2022a). In view

Image by Kreingkrai Luangchaipreeda at Pixabay of the increase in global prawn demand, guaranteeing a responsible production process throughout the entire production chain is an essential measure; this chain is composed of a set of linked elements that constitute a complex network with various multidisciplinary characteristics (Valenti and Tidwell, 2006). In particular, the farming of *M. rosenbergii* has a low environmental impact and offers opportunities for the socioeconomic development of developing countries, becoming a market alternative that contributes to job generation. Therefore, the objective of this work was to determine the current state of the global production chain of *Macrobrachium rosenbergii*, identifying its links and the characteristics of the techniques used.

MATERIALS AND METHODS

The Web of Science, EBSCO, Scopus, Scielo, Redalyc, Dimensions, and Google Scholar databases were consulted, using the keywords "*Macrobrachium rosenbergii*," "productive chain," and "freshwater prawn," in the 2001-2022 period. The resulting information was analyzed according to the production chain links (production, processing, and commercialization) proposed by Valenti and Tidwell (2006). Relevant information is provided in the description of each of them.

RESULTS AND DISCUSSION

Production

Production is divided into three stages: post-larvae (PL) production, nursery, and grow out. Obtaining PL in commercial hatcheries —whether they are private or governmental— depends on access to economic resources, brackish water, technology, and infrastructure. Its success is mainly based on location, design, and management, as well as on its proximity to the PL market of nursery and grow-out producers (Valenti *et al.*, 2010).

Post-larva production

The production of post-larvae meets the appropriate conditions of the reproducer management, embryonic process, and larval process.

Commercial hatcheries usually obtain prawn breeders from grow-out ponds, although they also frequently acquire them from the natural environment in the countries of

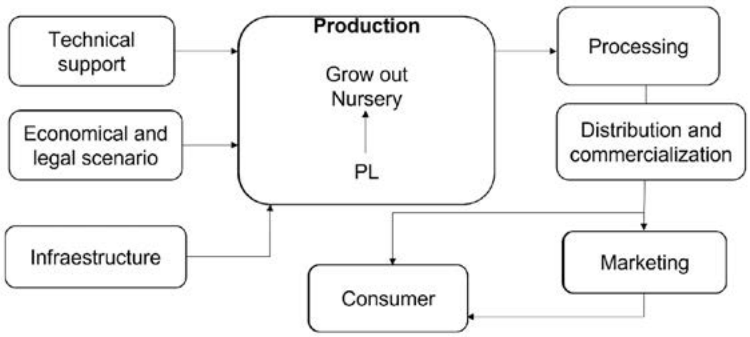

Figure 1. Production chain of *M. rosenbergii*, modified from the original model proposed by Valenti and Tidwell (2006).

origin (Daniels *et al.*, 2000). After the reproduction process, female prawns (commonly known as ovigerous females) carry the eggs attached to their abdomen (Valenti Daniels *et al.*, 2010). The embryonic process begins with the selection of ovigerous females and their introduction to aquaculture systems (tubs with aeration), using brackish water at 5 ppt, keeping the temperature within a 25-30 °C range, and preferably with a pH of 7.0 to 7.2, until the eggs hatch (New, 2002). According to Wei *et al.* (2021), *M. rosenbergii* larvae record a better growth and survival rate at a salinity level of 13 ppt. The larval development of *M. rosenbergii* has eleven stages that occur in brackish water, before their metamorphosis into PL, and eventual migration to freshwater in the wild (Brown *et al.*, 2010). Overall, the reproduction and incubation period of the larvae of this species lasts from 20 to 25 days (Wei *et al.*, 2021).

The larval process of *M. rosenbergii* is carried out in raceways systems and recirculating aquaculture systems (RAS), using clear water (Valenti *et al.*, 2010). The first system is based on the regular exchange of water, which reduces the accumulation of the toxic substances that originate from the metabolism of the larvae, from the *Artemia* nauplius offered as live food, and from the bacterial decomposition of food waste, feces, and dead organisms (New and Valenti, 2000). The second system uses the continuous or semi-continuous processing of water to reduce nitrogen and solid waste, as well as disinfection to control diseases (Valenti *et al.*, 2010). These systems enable high densities of larvae in ponds; the maximization of the use of space, water, supplies, and natural resources generates commercial and environmental benefits (Piedrahita, 2003).

The diet of growing *Macrobrachium rosenbergii* larvae basically consists of newly hatched *Artemia* nauplius, supplemented with inert food (Valenti and Daniels, 2000).

Table 1 shows suitable farming conditions for larval production.

The survival rate in PL production in experimental and commercial hatcheries varies between 40% and 50% in continuous flow systems and 60% to 80% in RAS (Valenti *et al.*, 2010).

In addition to salinity, another major environmental factor is the photoperiod, which has a positive impact with continuous light conditions, improving the survival of *M. rosenbergii* larvae (Wei *et al.*, 2021).

The production of both female and male monosex post-larvae has likewise been evaluated. Although the farming of males is in demand, some authors favor monosex female

Table 1. Optimal conditions for farming *M. rosenbergii* larvae.

Conditions for the larval rearing	Contribution	Reference
Salinity	Salinity level of 13 ppt.	(Wei *et al.*, 2021)
Density	100 larvae L^{-1}.	(David *et al.*, 2016, 2018)
Photoperiod and light spectrum	24 hours continues of light, green or white.	(Wei *et al.*, 2021)
Feed	They concluded to feed during daylight with *Artemia* nauplii up to 5 not exceeding 10 mL^{-1}.	(Aviz *et al.*, 2018)
	They evaluated of semi-moist product with squid	(Santos-Gutiérrez *et al.*, 2011)

production (Levy *et al.*, 2017; Malecha, 2012), given the feasibility with which they can be managed at high densities and homogeneous sizes. Some commercial hatcheries (such as the prawn biotechnology company Enzootic) have developed high-density monosex female technology for selective breeding. This RAS vertical hatchery in Thailand has been designed to produce 450 million PL per year (Shechter and Sagi, 2021).

Nursery

The nursery phase of *M. rosenbergii* is an intermediate stage between the recent metamorphosis to PL and grow-out, when it is grown at high densities, until they reach advanced sizes (Coyle *et al.*, 2009). PL (with a <0.01 g average weight) achieve an average weight ≥ 0.3 g in 30-60 days (Tidwell *et al.*, 2005). Once the nursery phase is over, PL are generally called "juveniles." Juveniles are more resistant than PL to predation, cannibalism, and environmental fluctuations (New, 2002). The duration of the nursery phase and the optimal size of juveniles shows geographical differences, depending on the climatic conditions of temperate and tropical zones (Coyle *et al.*, 2009).

Table 2 shows the nursery phase conditions: the stocking density is the number of post-larvae that can be stocked in the pond to guarantee a greater survival.

The use of artificial substrate or shelters in ponds —such as bamboo sheets, polypropylene nets, fine nylon material (Apud *et al.*, 1983), or waste PVC pipes (Asiain-Hoyos *et al.*, 2014)— provides prawns with a three-dimensional contact surface (D'Abramo *et al.*, 2006), increasing survival and production (D'Abramo *et al.*, 2000).

The intensification of *M. rosenbergii* farming implies a greater use of resources (*e.g.*, water and feed) than in traditional farming systems. Therefore, other alternative systems (Table 3), such as Biofloc technology (BFT) and synbiotics have been studied (Santos *et al.*, 2022). BFT allows the recycling of nitrogenous waste as microbial proteins, which subsequently are fed to the prawns (Ballester *et al.*, 2010).

Symbiotic systems use a holistic approach to balance the relationship between phytoplankton and other microorganisms (such as bacteria and zooplankton), resulting in a "mature" environment and stable water quality parameters (Kawahigashi, 2018).

Grow-out

The *Macrobrachium rosenbergii* species has been farmed under monoculture (Table 4) and polyculture systems, until the specimens reach commercial size in both in commercial

Table 2. Nursery phase conditions of *M. rosenbergii* post-larvae.

Nursery	Contribution	Reference
Stocking density	860 PL/m^2 produced a greater number of nursed juveniles (5.5/L and 527/m^2) in 56 days.	(Coyle *et al.*, 2003)
Photoperiod	24 hours continues of light.	(Tidwell *et al.*, 2001)
Artificial substrate	Preference for dark shelters.	(Kawamura *et al.*, 2017)
	They increased the substrate surface area in cages by 75%, improving growth and survival rate.	(Thapa *et al.*, 2021)

Table 3. Alternative farming systems for *M. rosenbergii*.

Systems	Contribution	Reference
Biofloc technology (BFT)	BFT can be used for the nursery phase.	(Ballester *et al.*, 2017)
	They used BFT with a salinity of 15 ppt for the nursery phase.	(Hosain *et al.*, 2021)
	Higher protein and lipid content due to the nutritional contribution of the biofloc material.	(Pérez-Fuentes *et al.*, 2013)
Symbiotic	A symbiotic diet was evaluated in postlarvae.	(Chen *et al.*, 2017)
	Longer time of symbiotic preparation may promote better performance.	(Santos *et al.*, 2022)

hatcheries and in research centers. These types of systems are classified according to their stocking density: extensive (1-4/m^2), semi-intensive (4-20/m^2), and intensive (>20/m^2). They have also been grown in brackish water, cages, and pens; however, this species is usually farmed in earthen ponds fed with fresh water, at a wide range of temperatures (Valenti *et al.*, 2010).

Prawns can play an important role in the success of commercial polyculture and integrated aquaculture, which are already carried out in some countries (particularly in Bangladesh, Brazil, China, India, and Vietnam) and are the subject of experiments in many others (Zimmermann *et al.*, 2009). According to Marques *et al.* (2016) integrated aquaculture (Table 5) of *M. rosenbergii* includes various farming systems, such as fish-prawns (New and Valenti, 2017), rice-prawns (Ahmed *et al.*, 2008; Ahmed and Garnett, 2010), aquaponics (Ma *et al.*, 2020; Tong *et al.*, 2021), and the integration with terrestrial animals and plant crops.

Processing

Market sells frozen and peeled prawns, frozen spots, and headless prawns. Meanwhile, the gourmet market particularly prefers whole prawns, given the distinctive characteristics of the chelae and the head.

In addition to these presentations, Freeman *et al.* (2016) examined consumer willingness to pay for prawns with and without added value, using sensory analysis and experimental

Table 4. Grow-out conditions of *M. rosenbergii* in monoculture.

Monoculture	Contribution	Reference
Phased cultivation	Separating prawns several times by size showed compensatory growth.	(Valverde, 2021)
Density	They evaluated two densities (2.5 and 6 /m^2, was more rentable the lower density.	(Valverde y Varela, 2020)
Probióticos	The daily addition of probiotics (*Bacillus subtilis* and *Bacillus licheniformis*), even at low concentrations, influences greater survival.	(Frozza *et al.*, 2021)
Artificial substrate	Growth and survival of juvenile prawns improved in the presence of artificial substrate.	(Tuly *et al.*, 2014)

Table 5. Grow-out conditions of *M. rosenbergii* in integrated aquaculture.

Integrate aquaculture	Contribution	Reference
Biofloc technology (BFT) and polyculture	They evaluated the BFT and clear water in an aquaponic system with *M. rosenbergii* and *O. niloticus*, BFT increased content of fat in the fillets of Nile tilapia and the control of the nitrates production.	(Barbosa *et al.*, 2022)
	They evaluate BFT and RAS in polyculture y monoculture with *M. rosenbergii* and *O. niloticus*. The BFT provides better growth responses in monoculture for *O. niloticus* and in polyculture with *M. rosenbergii* compared to RAS.	(Hisano *et al.*, 2019)
Aquaponic	They evaluated the feasibility of producing romaine lettuce (*Lactuca sativa* L. var. Longifolia) in a semi-intensive aquaponic system of *O. niloticus* and *M. rosenbergii*.	(Calderón-García *et al.*, 2019)
Polyculture	They evaluated the feasibility of polyculture with *Litopenaeus vannamei* and *M. rosenbergii*.	(Ni *et al.*, 2021)

auction. They confirmed that the willingness to pay per consumer increased significantly with two alternative salting processes: salt acclimatization (live prawns acclimatized in salt before harvest) and marinating (peeled prawns marinated in salt for 18 hours).

Dasgupta and Williams (2008) evaluated the operations of two representative processing facilities: a site-built plant and a processing trailer. The latter option provided mobile and convenient services for multiple producers.

Karim (2008) noted that prawn farming, processing, and exportation are an important economic activity in Bangladesh. However, importing countries and economic zones (including the United States and the European Union) are imposing harsher food safety measures; consequently, exporting countries (such as Bangladesh) must guarantee that all the levels of their aquaculture activities meet the conditions of international trade, if they are to survive the tough global competition (Karim 2008). According to Ahmed (2008), around 35 plants were engaged in the processing and exportation of prawns in southwestern Bangladesh, between November 2007 and February 2008.

Freshwater prawn is different from marine shrimp and its favorable culinary characteristics should be promoted. Ongoing research and a more efficient promotion of better breeding and processing technology is necessary to exploit the opportunities for expansion of the aquaculture production sector (Banu and Christianus, 2016).

Commercialization

Commercialization is carried out in domestic and international markets, with the main exporters being China, Thailand, India, and Bangladesh. The latter exports to the United States, the United Kingdom, Belgium, Germany, and Japan (Rabiul Islam *et al.*, 2017). Commercialization is also carried out through gastronomic festivals in Brazil and the United States; these strategies promote local consumption (Dasgupta *et al.*, 2007; Marques and Moraes-Valenti, 2012). In other countries, PL, live prawn, and processed products are marketed through internet pages or social networks.

Dasgupta *et al.* (2007) described the current situation of prawn commercialization in Kentucky, which is largely based on niche marketing, in which fresh prawn is supplied to a specific sector of consumers who are willing to pay a premium for a quality product.

For their part, Ahmed *et al.* (2007) evaluated prawn commercialization in Mymensingh, Bangladesh, and found that several intermediaries are part of the commercialization chain that connects producers and consumers. They also identified that 60% of prawn production is exported, particularly to the United States, Japan, and Europe, while the rest (40%) is sold in local markets.

Ahmed *et al.* (2016) also evaluated the prawn commercialization chain in the southeastern region of Bangladesh, before and after the implementation of a project funded by Denmark. Before the project was implemented, several intermediaries were part of a more extensive commercialization chain and they used to share a considerable amount of the marketing margin. However, after the implementation of the project, both the commercialization chain and the intermediaries were reduced, which facilitated the direct sale of prawn to processing plants.

CONCLUSIONS

The review of the current state of the *M. rosenbergii* production chain detected relevant information about the species and the technologies used to farm it. There is plenty data about farming and production issues, unlike the other links (*e.g.*, processing and commercialization). This situation opens an opportunity area to develop new research and generate more information.

In order to improve the production chain of *M. rosenbergii*, the supply of PL must be guaranteed, better monoculture practices must be developed, farming must be integrated with other species of commercial importance, and the organization of the production chain should be restructured —according to the direct and indirect participants. The trend towards innovative and alternative farming systems is undeniable; however, its application will depend on the characteristics of the region and its resources.

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Colegio de Postgraduados

Socio-agronomic characterization of agricultural farmers that survive in the community of San Diego, Texcoco

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ABSTRACT

Objective: to characterize farmers who still grow food in the San Diego community through knowledge of their agricultural production systems.

Design/Methodology/Approach: field visits and tours were carried out to the farmers' plots to obtain information that would allow the design of a questionnaire for the agronomic and social classification of farmers in San Diego. The questionnaire was applied to 26 of them; who narrated some historical facts about their survival in food production.

Results: most of the farmers interviewed are on average 55 years old, have a level of education equal to or higher than middle school, have three economic dependents, use the yoke for tilling their soils and hybrid seed. Likewise, they apply organic fertilizers and chemical fertilizers to their plots for the growth of crops, for the control of pests and diseases they use agrochemicals; and the control of weeds is done manually, supplying water for the crops through gravity irrigation.

Study limitations/Implications: this research was conducted during the CovID-19 pandemic. For this reason, only 26 farmers could be located. Most of them were at home.

Findings/Conclusions: in the community of San Diego, two types of farmers can be identified, those who carry out practices related to conventional agriculture, and others who maintain traditional practices, but combine them with conventional agriculture.

Keywords: urban agriculture, socio-agronomic characterization, land use change, agricultural survival.

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INTRODUCTION

The expansion of agricultural production systems based on the establishment of monoculture and the application of agrochemicals has generated a socio-environmental crisis worldwide, making it necessary to revalue rural production systems in a social and scientific way. Because those production systems are conditioned by the principles proposed by the Food and Agriculture Organization to achieve sustainable food and agriculture in the world (FAO, 2018). Such agricultural production systems provide elements for resilience to the environmental, health and food crises that affect us today. In addition, considering the conservation of natural resources such as soil and water during agricultural production and access to food for the population (Cuadras-Berrelleza *et al.*, 2021).

In Mexico, rural areas developed by traditional methods of self-sufficiency, as well as by modern agricultural methods, are affected by processes of deforestation, land use change, agricultural modernization and rural urbanization associated with industrialization; which in turn, devaluate and alter their environmental, sociocultural and economic environments (López-Valentín *et al.*, 2020). Land use change is the impact suffered by natural landscapes through changes in ecosystems, which has manifested itself in recent decades in the reduction of agricultural area to increase the spaces for the construction of urban infrastructures. This, in turn, generates various effects that are usually permanent such as the loss of biodiversity, climate variation, or degradation of natural resources (Amorim-Homem *et al.*, 2023).

In the municipality of Texcoco de Mora, the effects of the intensification of land use change and growth of the urban sprawl are manifested, such as accelerated population growth, overexploitation of natural resources. For example, it is highlighted that since 2020, only 1.89% of the total population carries out agricultural activities (Data Mexico, 2023). According to Tejeda-Sartorius *et al.* (2015) the community of San Diego is a particular area of Texcoco, because it has good quality irrigation water from wells.

In recent years, the abandonment of the Mexican rural areas has intensified in rural farming communities, due to the migration of their inhabitants to urban areas, seeking to improve their economic conditions (Sandoval-Genovez *et al.*, 2022). In communities like San Diego (Texcoco, State of Mexico) these environmental, cultural and socioeconomic processes affect the rural population, which has survived since past decades following the arrival of the green revolution, finding a way to continue producing food (Ascencio-López *et al.*, 2018).

MATERIALS AND METHODS

This study was established in the community of San Diego, located southeast of the municipality of Texcoco de Mora (State of Mexico) from February to April, 2022. The geographical coordinates are located between 19° 29' 51.04"-19° 30' 3.13" N and 98° 51' 20.04"-98° 52' 22.22" W and it has an area of approximately 1.81 km^2. The research integrated quantitative and qualitative methods. The qualitative aspect included visits and field trips in the plots of eight farmers, representatives of the community's irrigation organization. Through non-participant observation, the form of production of the main crops, historical facts, ways of thinking, main motivations and productive logic were known.

For the quantitative aspect, we worked with 26 farmers, selected through the snowball technique, to whom a questionnaire was applied as a data collection instrument. The questionnaire integrated two axes: 1) socioeconomic characteristics of the farmers and 2) management characteristics of the main crops. Based on the data of agricultural and socioeconomic variables, a hierarchical cluster analysis was carried out with the Statistical Package for the Social Sciences® (SPSS, 2020).

RESULTS AND DISCUSSION

Socioeconomic characteristics

According to the data obtained from the interviewed farmers, their average age is 55 years, with the youngest being 35 years old and the oldest being 76 years old. The younger farmers are in the range 30 to 50 years old, representing 69.24% of the total number of farmers, while the remaining percentage correspond to older farmers in the range 50 to 80 years old. In 2020 the population over an age of 50 years was 10.29% in Texcoco de Mora, (State of Mexico, Mexico). It is worth highlighting that in 2022, 2.72% of total inhabitants in the State of Mexico worked in agricultural activities (Data Mexico, 2023).

Farmers over 50 years of age are vaults of traditional knowledge and awareness of local agroecosystems; all this knowledge was acquired in the experience of agricultural production. These farmers can provide important elements to improve food sovereignty and security at the regional scale (Pai-Natacuas *et al.*, 2022). In terms of marital status, more than half (65.38%) of farmers are married. In the aspect of education, 15.38% of farmers have elementary education, 26.92% have middle school, and 57.70% have a level of education equal to or greater than high school, coinciding with the results presented by Data Mexico (2023).

According to the farmers interviewed, complementing traditional knowledge acquired over generations with academic knowledge influences food production through traditional practices in combination with those of modern agriculture (Rodríguez-Fernández *et al.*, 2010); 70% of the farmers have three economic dependents, while 4% of farmers have six dependents. According to Alcázar-Sánchez and Gómez-Martínez (2022), in communities such as San Diego, agriculture is an activity of great economic importance for the maintenance of families, since they grow food both for small sales and self-supply.

Resources available for agricultural production

The total agricultural area of the farmers is 37.58 ha, of which 22% corresponds to greenhouses and 78% to the open field. According to the SIAP (Mexico's Agri-Food and Fisheries Information Service), during the last few years, greenhouse area has increased exponentially. So much that, in 2021 the area cultivated in open fields was less than 1% in the State of Mexico (SIAP, 2022). Montenegro-Gómez *et al.* (2022) pointed out that, in open-field production systems, soils have higher organic matter content and lower salinity, sodicity, and phytopathological disease indices.

In terms of soil tillage, 53.85% of farmers use the yoke, either totally or in combination with agricultural machinery. The use of the yoke increases both the efficiency of nutrients and water use in plants, and their retention in the soil, in addition to reducing production costs by avoiding the use of fossil fuels (Domínguez-Vento *et al.*, 2011).

Regarding the use of seeds, 73% of farmers use hybrid seed, either individually or in combination with native seed. While 46.15% of farmers plant native seed, either individually or in combination with hybrid seed. Farmers who sow native seeds commented that those are more resistant to lodging, pests, diseases and soil salinity problems compared to hybrid seeds. In addition to the fact that native seeds are more economically accessible, since farmers exchanged those seeds among themselves (Montaño *et al.*, 2021).

When it comes to crop fertilization, 84% of farmers apply inorganic and organic fertilizers to reduce production costs; to improve crop yields and the quality of their soils. The 57.69% of the farmers apply fertilizers through fertigation (46.15% in drip system and 11.54% in gravity fed irrigation); and 42.31% incorporate fertilizers directly into the soil (19.23% with tractor and 23.08% manually). Farmers who have a drip irrigation system commented that they implemented it to save water and improve water use efficiency, compared to gravity fed irrigation. This statement agrees with the research of Montemayor-Trejo *et al.* (2012), where maize was irrigated with a drip system, thus using 75% of the volume of water used in gravity fed irrigation. In addition, crop yield was 30% higher when compared to gravity fed irrigation.

In the phytosanitary aspect, the pests that cause the most damage to crops in San Diego, are whitefly (*Bemisia tabaci*), fruitworm (*Heliothis virescens*) and green aphid (*Myzus persicae*). While the main diseases are powdery mildew (*Leveillula taurica*), late blight (*Phytophthora infestans*) and root rot (*Fusarium oxysporum*). The most used control for pests and diseases is the use of agrochemicals (76.92%), to a lesser extent is the use of biological control agents (11.53%). Also, there are some farmers who do not control, as they do not have considerable populations of insect pests or disease incidence. It should be noted that the constant applications of agrochemicals result in environmental pollution, genotoxicity, carcinogenic effects and various poisonings that cause damage to the nervous and reproductive systems in the surrounding communities where those products are applied (Rodríguez-López *et al.*, 2020; Zúñiga-Venegas *et al.*, 2022).

Regarding weed control, it is noteworthy that 73.06% of farmers use ecological techniques (42.30% manually and 30.76% carry out cultural control through plastic mulches). Farmers who opt for manual control collect wild plants for sale, mainly for their own food and medicinal use. According to González-Ortega and Fuentes-Ponce (2022), those farmers who choose alternative controls to chemical products reduce input costs and environmental impact derived from herbicides.

Water management in the community is widespread, 70.07% of the farmers indicated that they use gravity fed irrigation, while 29.93% have a drip irrigation system. It should be noted that 42.30% of the irrigation water used by farmers comes from wells, while 57.7% used water from wells and rainfall. The latter are strategies for water and food security at the local community scale (Suástegui, 2021).

Regarding the need for technical advice for crop management, about 38% of farmers are interested in being trained on organic agriculture, followed by pest and disease control (26%), and crop nutrition (11.5%). They also expressed the need to have access to government financing programs (23%). According to farmers, these issues are necessary to increase the sustainability of their agri-food systems in such a way that they are more environmentally and economically sensitive, and their operation subsidized by ecological processes of the soil (Mello-Fagundes and Pérez-Cassarino, 2020).

Production systems

Through hierarchical cluster analysis, two groups of agricultural farmers were obtained. The first group is composed of eight farmers characterized by an age range 40 to 45 years; they are married, have university studies and four economic dependents, who produce their crops mainly in greenhouses with their own machinery and hybrid seed. Also, they use inputs of chemical origin as a source of nutrition and as control agent of pests and diseases of their crops. They control wild plants through the passage of machinery, have a drip irrigation system, and have complications in crops production due to high input prices and low harvest costs.

The second group is composed of 18 farmers described as being over 60 years of age, have middle school education, have three economic dependents. They produce their crops in open field with the help of rented machinery, use hybrid and native seeds, apply inputs of chemical and organic origin as a source of nutrition and control agent of pests and diseases of their crops. They control the weed plants in their plots manually, have a gravity fed irrigation system and have complications to produce their crops due to the damage caused by pests and diseases and the lack of knowledge regarding organic agriculture.

According to the classification of production systems established by Chávez-Caiza and Burbano-Rodríguez (2021), two production systems prevail in the community of San Diego: conventional (group 1) and semi-conventional (group 2). So, highlighting that in both systems, the type of soil tillage is with machinery, in the nutrition and control of crop pests and diseases, chemical synthesis products are used and the seeds used are hybrids. It is worth mentioning that, in the semi-conventional system, the aforementioned aspects are complemented by using organic products for the nutrition and control of pests and crop diseases.

In both systems, agroecological practices are implemented, such as the use of organic fertilizers, local seeds, living barriers, use of wild plants, manufacture of agricultural tools and implements, intercropping and crop rotation, creation of contour lines, incorporation of *Brassica* sp. remains, integration of the animal component, and the maintenance of pollinators; and conventional agriculture practices such as the use of chemical fertilizers for crop development, pest and disease control through the use of agrochemicals, soil tillage with agricultural machinery, use of hybrid seeds and monoculture.

According to the information from farmers, the two production systems are conditioned by the agricultural tradition inherited from their parents and grandparents. This is the main reason why farmers continue to work their plots despite the impact generated by the change in land use and local market conditions, where there are times when the price of their crops is too low and even the investment cost is not returned (López-Vazquez *et al.*, 2015; Ascencio-López *et al.*, 2018).

CONCLUSIONS

In the community of San Diego (State of Mexico, Mexico) there are two types of agricultural production systems. In the first one, farmers work with practices related to the conventional system; while in the second, they work jointly with the conventional and agroecological (semi-conventional) production schemes. The wholesale of crops and the combination of agroecological and conventional practices are strategies that allow these farmers to survive through their own food production within the community.

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Colegio de Postgraduados

Fruits and seed characteristics of chihua squash (*Cucurbita argyrosperma* Huber.) accessions from Yucatan, Mexico

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ABSTRACT
Objective: To evaluate some fruit and seed characteristics of six chihua squash genotypes obtained in the Yucatan Peninsula.
Materials and methods: Six collections of *Cucurbita argyrosperma* Huber, obtained in the Yucatan Peninsula, were evaluated in a randomized complete block design for two years. The number of fruits per plot, fruit weight and size, seed weight and number of total, empty and full-filled seeds per fruit were quantified, and degrees Brix of the pulp.
Results: Differences in fruit production were found between collections. The dominant fruit size was medium, fruits between 500 and 999 g, with the Chetumal collection standing out, while the variables of seed weight and number of seeds were affected between collections and between years, because of low rainfall during the production cycle of the second year. All collections recorded a reduction in weight and an increase in the number of empty seeds. Slight variations were identified in the concentration of degrees Brix of the pulp, with the Chetumal collection standing out above the rest.
Conclusions: The evaluated collections present variability within them, which can be useful to select materials for different purposes and to obtain more benefits from this crop.

Keywords: Fruit size, number of seeds, degree Brix, seed weight.

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INTRODUCTION

Mexico is the center of origin of the genus Cucurbita and there is evidence that shows that the species *Cucurbita argyrosperma* was domesticated in southern Mexico (Rio-Santos *et al.*, 2018; Khoury *et al.*, 2019). Historically, squash has been part of the diet of many American peoples (McCreight, 2016). The genus Cucurbita has 27 species of which five are domesticated: *C. pepo* L.; *C. fisifolia* Bouché; *C. moschata* (Duchesne ex Lam) Duchesne ex Poiret; *C. máxima* Duchesne ex Lam; *C. agyrosperma* Huber (Eguiarte *et al.*, 2018). According to several authors (González *et al.*, 2010; Martínez-González *et al.*, 2021), most of the genetic diversity of Cucurbita is found in Mexico, which manifests in the wide range

of sizes, shapes, colors, and yields in the field. Sánchez-de la Vega *et al.* (2018) indicated that *C. agyrosperma* is the one of lowest diversity, as is the case of *C. fisifolia* (Lebeda *et al.*, 2006) in comparison to the rest of the domesticated species. In the Yucatan Peninsula, squash is an important part of the complex milpa production system, where three of the 15 species of the American genus of Cucurbita are cultivated: *C. argyrosperma*, *C. moschata* and *C. pepo*, and where the main objective is obtaining seed for food. The diversity in the native populations of squash is maintained by traditional farmers, who are capable of recognizing variants and selecting the fruits from which they will obtain their seed in each sowing cycle, based on the preferred characteristics of color and shape (Canul *et al.*, 2005; Basurto-Peña, 2014; Hernández-Galeno *et al.*, 2015). The sizes of the fruit of the squash species that are cultivated are the result of evolutionary changes; multiple studies on morphological, genetic and agronomic diversity have shown a great variability within and between specimens of the species (Lira *et al.*, 2002; Basurto-Peña *et al.*, 2015). *C. agyrosperma* has been one of the least studied; Sánchez-Hernández *et al.* (2004) started a program for genetic improvement of this species with the aim of obtaining outstanding materials for seed production. Although it can be consumed in various ways, the seed is the main product, so in the last 10 years its production has been promoted in different regions of Mexico as monocrop system (Serna *et al.*, 2004), in response to economic studies that show that *C. agyrosperma* has economic potential that can contribute in benefit of the producers (Ireta-Paredes *et al.*, 2018). The objective of this study was to evaluate some characteristics of the fruit and seed of six genotypes of *Cucurbita agyrosperma* Huber squash cultivated in the Yucatan Peninsula, with the aim of identifying materials with potential for commercial production.

MATERIALS AND METHODS

The evaluation of six chihua squash accessions was established in the Uxmal Experimental Site (INIFAP), located on km. 72 of the Mérida-Campeche Highway en route to the Muna ruins, Yucatan (20° 24’ 37.23” latitude North and 89° 45’ 24.36” longitude West). The genotypes were obtained from different regions of the Yucatan Peninsula and given the name of the collection site, two from Chetumal (Chetumal and Cacao), three from Campeche (Edzna, Pixoyal and Becal), and one from Yucatán. Sowing was done under rainfed regime (June 13, 2017, and June 20, 2018) with the climate information presented in Figure 1.

The evaluation management was carried out in a randomized blocks design. Each plot consisted in four furrows with separation of 2.40 m and 9 m long with an area of 64 m^2 per experimental unit (Hernández *et al.*, 2006). The agronomic management recommended by Ruiz García *et al.* (2020) was conducted. The fruits were harvested when they reached physiological maturity and were evaluated in the facilities of the Mococha Experimental Field located on km 25 of the former Mérida-Motul Highway. The data obtained were total number of fruits per plot and average weight of fruits, and then they were classified according to their weight into large, of 1000 g or more, medium of 500 to 999 g, and small of less than 500 g, in addition to eliminating the fruits damaged by rotting. From each fruit, seeds were extracted and dried until constant weight; the total seeds per fruit were counted,

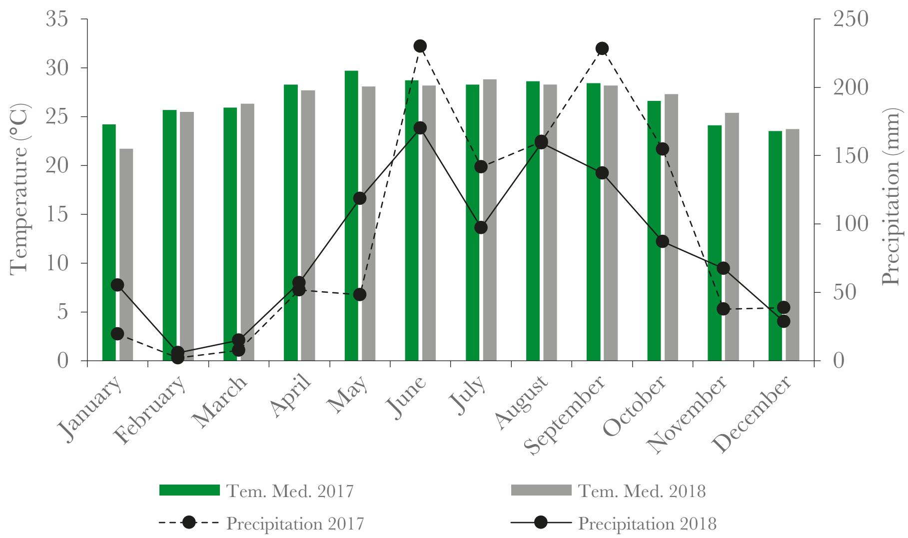

Figure 1. Mean temperature and precipitation in Yucatan during 2018 and 2017. Graph elaborated with data from: https://smn.conagua.gob.mx/es/climatologia/temperaturas-y-lluvias/resumenes-mensuales-de-temperaturas-y-lluvias.

and the hollow seeds and the seeds with complete filling were separated and counted. A sample of five fruits was obtained from the total fruits, and degrees Brix of the pulp were determined with the help of a refractometer Atago PR-32β brand while the equatorial diameter of the fruits was obtained through the most prominent part of the middle part and the polar diameter was taken from the base passing through the fruit stem. These data were reported in centimeters. All the variables were subjected to analysis of variance and Tukey's means comparison ($P \leq 0.05$) with the help of SAS 9.1®.

RESULTS AND DISCUSSION

Fruit production during the two years of evaluation of the collections showed variations. Differences were found ($p \leq 0.05$) between the collections; the collection from Chetumal surpassed all the collections in fruit production, while the collections Yucatán and Pixoyal were lower, with a difference of 50% compared to the collection with the highest number of fruits (Figure 2a). When it comes to the fruit size, the production was concentrated in the medium size and was higher in 53.72 and 54.49 % than the large and small sizes (Figure 2b); differences were found ($p \leq 0.05$) between the accessions, with Chetumal standing out, which produced the highest amount of fruits that were concentrated in the medium size, followed by Becal, while the collections Yucatán and Pixoyal were lower in all the fruit sizes and this production trend of medium fruits prevailed in the two years of evaluation (Figure 2c). When it comes to fruit weight during 2017, the collections Pixoyal, Edzna and Becal resulted in the highest averages with weights of 2234, 2191 and 1844 grams, respectively, while for 2018 these collections had averages of 976, 918 and 1005 g, respectively, reducing their weight in up to 50 % (Figure 2d).

The seed production in weight and number of seeds per collection was affected in the two years of production. Differences are observed ($p \leq 0.05$) when comparing seed

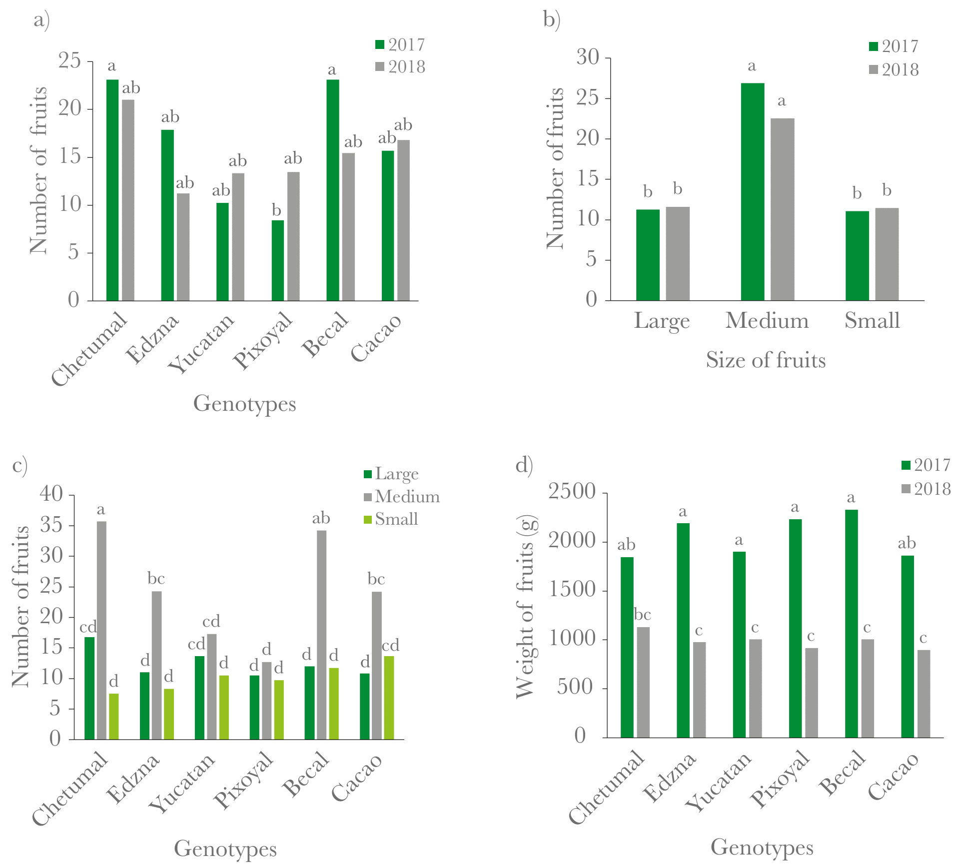

Figure 2. Characteristics of the chihua squash fruit production during 2017 and 2018 in the Uxmal Experimental Site. a) Number of fruits produced by collection per year; b) Number of fruits produced per year and classified by sizes; c) Number of fruits produced by collection per size; d) Average weight of the fruits obtained during the evaluation.

production from each collection in each year: for 2017, Pixoyal and Becal stand out from the rest in number of seeds with 366 and 343, respectively, while Pixoya is superior for total seed weight with 76 g; for the year 2018, all the collections showed low seed production, as well as low seed weight per fruit, and in that year Chetumal and Becal stand out regarding seed weight with averages of 38 and 34 g/fruit, respectively; however, Edzna and Yucatán showed the highest number of seeds per fruit with 167 and 171 (Figure 3a).

An important characteristic is the amount of complete or full-filled seeds. When the behavior of each collection along the years is observed, it is possible to highlight that Pixoyal produced the largest number of complete seeds per fruit (315) and Chetumal the smallest number of hollow seeds (29), while in the year 2018 the number of complete seeds per fruit per collection remained between 79 and 105 seeds; however, the amount of hollow seeds increased with regards to what was obtained in the year 2017, Chetumal showed 50% more hollow seeds than in 2017, while Edzna increased on average to 42 hollow seeds in 2018, affecting the weight of the seed produced. Although the collections from Becal and Cacao reduced the amount of complete seeds by 2018, they also reduced the hollow seeds in 2018 in comparison to 2017 (Figure 3 b).

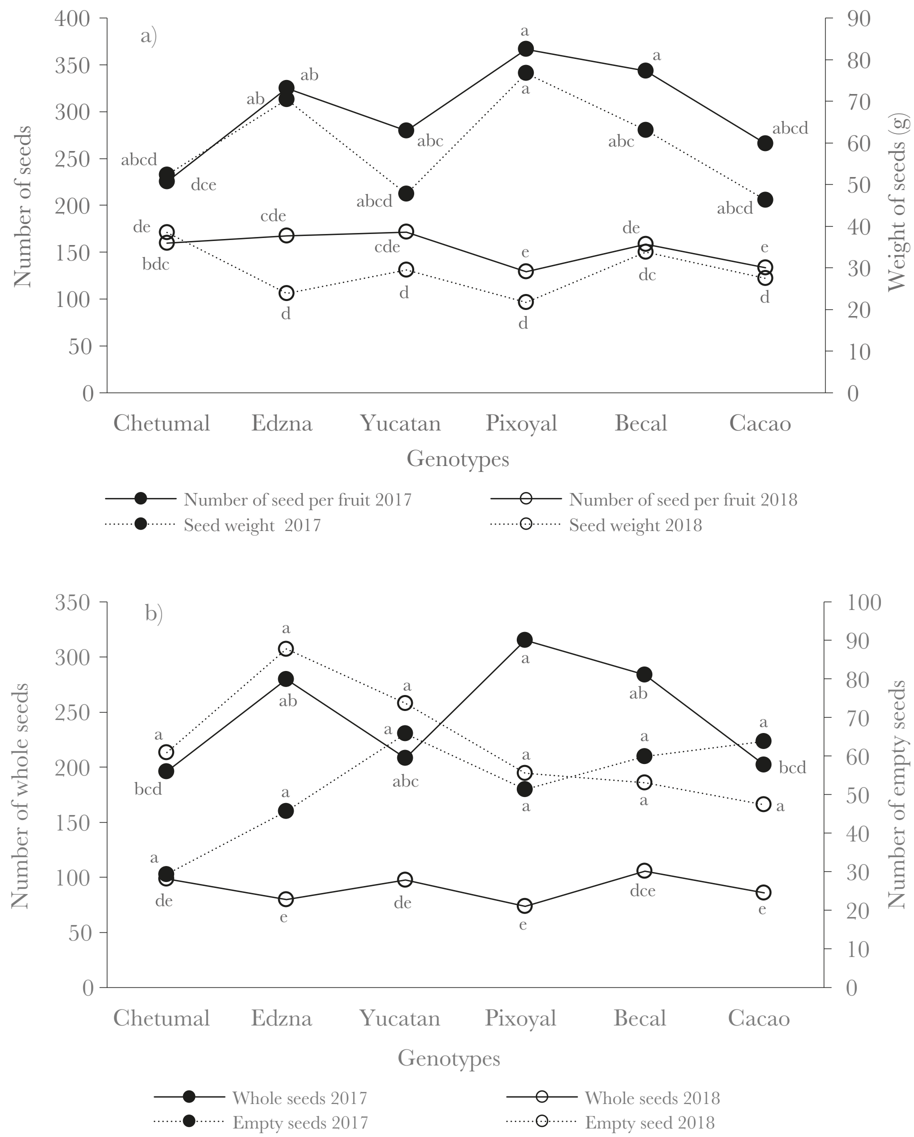

Figure 3. Characteristics of chihua squash seed production during 2017 and 2018 in the Uxmal Experimental Site. a) Number of seeds produced and seed weight; b) number of full-filled and hollow seeds during the evaluation.

On the other hand, the variables of polar and equatorial diameter had differences between the collections ($p \leq 0.05$); the collection from Edzna and Becal exceeded in both variables, while in Pixoyal only the polar diameter and the cacao collection reported the lowest values in both variables; in terms of the years evaluated, 2017 presents the highest values, and the behavior of the Edzna and Becal collections in the two years of evaluation remain as outstanding in both variables during the two years (Figure 4).

The amount of degrees Brix in each of the collections has discordances ($p \leq 0.05$); in 2017, Becal recorded on average 4 °Bx, although for 2018 Chetumal resulted in an increase of 1.19 °Bx in comparison to 2017, the same as Cacao (0.14 °Bx) and Becal decreased 1.19 °Bx and the collections Ezdna (0.68 °Bx), Yucatán (0.34 °Bx) and Pixoyal (0.26 °Bx) (Figure 5).

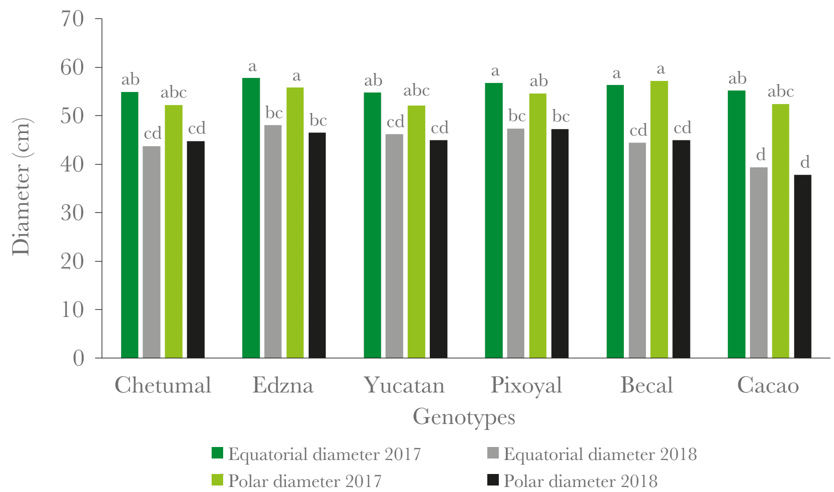

Figure 4. Equatorial and polar diameter of fruits from six chihua squash collections produced in the Uxmal Experimental Site, Yucatán.

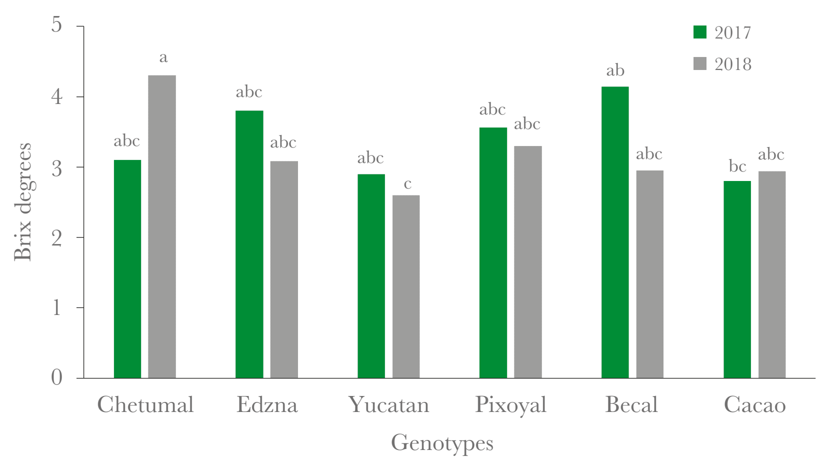

Figure 5. Degrees Brix of six chihua squash collections produced during 2017 and 2018 in the Uxmal Experimental Site, Yucatán.

A wide variety of squashes can be found in the Yucatan Peninsula, particularly *C. moschata* and to a lesser degree *C. agyrosperma*, according to Sánchez-de la Vega *et al.* (2018). This species presents low levels of genetic variation in the Yucatan Peninsula and high levels of kinship with local wild species, which indicates that the populations of *C. agyrosperma* are genetically isolated. The crop is particularly associated to the milpa system, where corn, bean and chili pepper are produced (Canul *et al.*, 2005). There are characteristics to which farmers pay special attention to select the seeds for the next production cycle; for example, amount and size of the fruit, amount and size of the seed, fruit color (Barrera-Redondo *et al.*, 2020). The variables of fruit weight, seed weight, and number of seeds agree with what was reported by Garza-García *et al.* (2020) in hybridization assays on collections of *C. agyrosperma* obtained in the Huasteca region. When it comes to the fruit size, Hernández-Galeno *et al.* (2015) report some characteristics of fruit size from *C. agyrosperma* collections obtained in the state of Guerrero, which they describe as elliptical fruits with diameters of 70.6±7 cm; rounder fruits with smaller diameter were found in the results obtained (Figure 5), probably due to the number of medium fruits (Figure 2). The characteristics evaluated were variable between them and between the years evaluated and respond to the conditions of moisture and temperature present during cultivation. The characteristics of fruit weight and fruit size were affected in the two years evaluated, and this behavior was identified by Sánchez-Hernández *et al.* (2014) in fruits obtained in two localities; for the particular case of this study, differences in fruit weight were obtained between the years. Although there was sowing in the same site but in different year, this affected the production both of number of fruits and the fruit size, which is why it is believed that the accessions evaluated are affected by the conditions of rainfall between both years, during 2017 there was higher rainfall than in the year 2018 (Figure 1). According to Aguirre *et al.* (2020), the climatic conditions where *C. agyrosperma* is distributed range from 15 to 30 °C and average annual rainfall of less than 1000 mm to 2000 mm, which is a very broad interval, so it would be necessary to understand the optimal conditions for the production of this species.

Urbanek Krajnc *et al.* (2017) indicate that the climate changes lead to considerable losses in the production in squash production as well as anatomical modifications. This condition of water stress could affect in the same way the variables of seed weight, total number of seeds, number of complete seeds and hollow seeds, since the lowest values were obtained during 2018. These results also agree with what was found by Garza-Ortega *et al.* (2010), who reported differences in the variables of fruit weight, seed and concentration of soluble solids during two cycles of evaluation of lines, hybrids and landraces of *C. agyrosperma*; these differences are related to the climatic conditions present during the production cycles. Despite this reduction in yield, all the collections showed production indicating that there can be certain tolerance to water stress (Pedroza-Sandoval *et al.*, 2018).

On the other hand, options have been sought for exploitation of the fruit, since this species was used to obtain the seed, wasting the pulp; in this sense, Dorantes-Jiménez *et al.* (2016) report *C. agyrosperma* with potential for the diets of farm animals. However, Ruiz García *et al.* (2020) have reported variations in the amount of protein and oils in different collections of *C. agyrosperma*. In this sense, the amount of sugars can play an important role in the palatability of the pulp and in the acceptance by animals of the feeds; the collections found variations in the concentrations of sugars of the fruits evaluated, primarily when conditions of water stress were present. Sánchez-Hernández *et al.* (2000) did not show degrees Brix in the evaluation and they report an improvement in taste, from insipid-bitter to sweet, for the two years of selection; however, the concentration of sugars in the pulp will depend on the amount of water it can contain.

CONCLUSIONS

The characteristics of *C. agyrosperma* evaluated help to identify materials that can be used with different objectives, such as animal feed or exploitation of seeds for oils or direct consumption. The collections evaluated behaved differently depending on the conditions of temperature and moisture during the years evaluated; however, these responses contribute to identify characteristics that can be taken advantage of, such as the increase of sugars in the pulp, or else to generate information that contributes to the elaboration of a technological package for management of the species.

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Colegio de Postgraduados

Agrochemicals and crop productivity losses

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ABSTRACT

Objective: to reflect briefly on the importance of the use of agrochemicals in the productivity of some crops according to their contribution to yield, and some implications of their agricultural consumption.

Design/methodology/approach: based on related literature and some experiences in the field on the use and consumption of pesticides.

Results: pesticides are synthetic, microbial, or derived organic compounds used in plant growth programs to prevent or control pests, diseases, and weeds. Also, mineral nutrition (with macro and micronutrients) is considered as agrochemicals. However, the effects on the contamination of soils, groundwater, lakes, seas, and oceans due to its use is increasing. Mexico is a large consumer of fungicides, insecticides, fertilizers, and herbicides for agricultural uses.

Limitations on study/implications: unmeasured use of any type of pesticide can produce tons of pesticide-trash. Some traces of active or inert ingredients can be detected in bodies of water.

Findings/conclusions: the constant risk of agronomic yield losses can be substantial without the use of agrochemicals. Without the application of pesticides, yield losses can reach 100%.

Keywords: contamination, pesticides, production, yield losses.

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INTRODUCTION

In general, pesticides or agrochemicals are organic and inorganic synthetic molecules, biorational, microbiological or derived from them, for the management of pests, diseases, weeds, and nutrition, with the aim of sustaining or improving agronomic performance.

Regarding world consumption, for 2021 it was 4.1 million metric tons and is expected to grow to 4.4 by 2026. China is the largest consumer of pesticides, with 1.8 million metric tons in 2021, followed by United States, Brazil, and Argentina as the second, third and fourth main consumer, respectively. Despite its benefits in agricultural productivity, throughout the use process (from pesticide storage warehouses to its use in crop fields) a large amount of garbage is generated that ends up in groundwater deposits and surface bodies, as documented by [1] and [2]. Moo-Muñoz [3] reported that more than 106

thousand tons of agrochemicals were produced in Mexico in 2017. In addition, during 2000-2017, of the total of pesticides produced, fungicides represented 45%, followed by insecticides (28%) and herbicides (27%).

Toxicity and nobel prize related to pesticides

In the world of food production, highly carcinogenic compounds have paraded, such as the herbicidal compound 2,4-D (chlorophenoxy) and its analogue 2,4,5-T. The reason for the Nobel Prize in Physiology or Medicine in 1948 to Prof. Paul Hermann Müller was the description of the properties of the insecticide DDT, an organochlorine that was introduced since 1942, banned in 1972 in the USA and in Mexico until 1999. Its high stability and magnification caused conditions in humans.

There are too many stories for and against the use of agrochemicals in the scientific literature.

Notwithstanding the effects on the contamination of soils, groundwater, lakes, seas and oceans, the dependence on its use is increasing [4].

Dr. Michael Herrman, a United Nations specialist, stated that the world population at the end of 2022 would be about 8 billion. This leads us to ask ourselves: How could we feed a growing population without the intensive use of agrochemicals? Could we reduce its use in agriculture? As an example, in wheat if it were possible to reduce at least 50% of agrochemicals, yield losses would be of the order of 5 to 13% compared to traditional intensive methods [5]. Therefore, it has been shown that agrochemicals will continue to be used in agriculture despite scientific documentation of adverse effects.

Modes of action insecticides and fungicides

According to Crop Life International, there are many modes of action that allow counterattacking or preventing various pests and diseases in the fields. The information from IRAC (Insecticide Resistance Action Committee) and FRAC (Fungicide Resistance Action Committee) are useful tools to teach our students in a summarized way (Tables 1 and 2). It is important to note that IRAC information is also reported for microbial organisms. For detailed information, visit www.irac.info and www.frac.info/.

Yield losses

Yield losses in agriculture is something that has been sought to be prevented since ancient times. Due to this, production environments, genetics of the cultivated materials, as well as the incidence of pests and diseases, among other factors, have led to the coexistence of the use of agrochemicals and productivity. Some time ago many crops were completely devastated despite the use of pesticides due to poor technology and little understanding of damaging factors. Today, yield losses range from 5 to 100%, the latter in extreme conditions.

Total crop losses from pest feeding or plant damage can be as high as 100 percent. In summary, Table 3 is presented, showing examples of losses in some crops caused by pests, diseases, and weeds of anthropocentric importance.

Table 1. Classification of modes of action of insecticides (including acaricides and nematicides). Based on https://www.frac.info/

Group 1: Acetylcholinesterase (AChE) inhibitors	**Group 7: Juvenile hormone Mimics**	**Group 13: Uncouplers of oxidative phosphorylation via disruption of proton gradient**	**Group 19: Octopamine receptor agonists**	**Group 25: Mitochondrial complex II electron transport inhibitors**	**Group 33: Calciumactivated potassium channel (KCa2) modulators**
Group 2: GABA-gated chloride channel antagonists	Group 8: Miscellaneous non-specific (multi-site) Inhibitors	Group 14: Nicotinic acetylcholine receptor (nAChR) channel blockers	Group 20: Mitochondrial complex III electron transport inhibitors – Qo site	Group 28: Ryanodine receptor modulators	Group 34: Mitochondrial complex III electron transport inhibitors – Qi site
Group 3: Sodium channel modulators	Group 9: Chordotonal organ TRPV channel modulators	Group 15: Inhibitors of chitin biosynthesis affecting CHS1	Group 21: Mitochondrial complex I electron transport inhibito	Group 29: Chordotonal organ nicotinamidase inhibitors	
Group 4: Nicotinic acetylcholine receptor (nAChR) competitive modulators	Group 10: Mite growth inhibitors affecting CHS1	Group 16: Inhibitors of chitin biosynthesis, type 1	Group 22: Voltage-dependent sodium channel blockers	Group 30: GABA-gated chloride channel allosteric modulators	
Group 5: Nicotinic acetylcholine receptor (nAChR) allosteric modulators site I	Group 11: Microbial disruptors of insect midgut membranes	Group 17: Moulting disruptors, Dipteran	Group 23: Inhibitors of acetyl CoA carboxylase	Group 31: Baculoviruses	
Group 6: Glutamate-gated chloride channel (GluCl) allosteric modulators	Group 12: Inhibitors of mitochondrial ATP synthase	Group 18: Ecdysone receptor Agonists	Group 24: Mitochondrial complex IV electron transport inhibitors	Group 32: Nicotinic Acetylcholine receptor (nAChR) allosteric modulators site II	

Table 2. Classification of modes of action of fungicides *. Based on https://www.frac.info/

A: Nucleic Acids Metabolism	G: Sterol Biosynthesis in Membranes
B: Cytoskeleton and Motor Proteins	H: Cell Wall Biosynthesis
C: Respiration	I: Melanin Synthesis in Cell Wall
D: Amino Acid and Protein Synthesis	P: Host Plant Defense Induction
E: Signal Transduction	M: Chemicals with Multi-Site Activity
F: Lipid Synthesis or Transport / Membrane Integrity or Function	

* Groups were designed by the fungal control agents by cross resistance pattern and mode of action 2022.

Table 3. Relevance of the use of agrochemicals and potential losses in agronomic yield by crop.

Crop	**Yield losses (%) by avoiding the use of agrochemicals**	**Reference**
Corn	20 a 41	[6], [7]
Bean	37 a 100	[8]
Wheat	10 a 28	[7]
Tomato	15 a 95	[9]
Rice	25 a 41	[7]
Potato	8 a 21	[7]
Soybean	11 a 32	[7]

CONCLUSIONS

The ongoing risk of agronomic yield losses can be substantial without the use of agrochemicals. However, the integration of complementary techniques to synthetic pesticides (for example, biorational management, ethological control strategies or microbial control) should be considered in the cases or environments where they are applicable. The measured use of pesticides is mandatory to protect human health and ecosystems in general.

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Diagnosis and distribution of *Citrus tristeza virus* in northern Veracruz, Mexico

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ABSTRACT
Objective: of the research was to know the incidence of CTV to try to associate it with the yellowing and death of citrus trees.
Design/Methodology/Approach: the presence of the virus was diagnosed in seven citrus-producing municipalities in northern Veracruz. A total of 804 samples from citrus trees were collected in 90 locations belonging to the municipalities of Álamo, Castillo de Teayo, Cazones, Chicontepec, Ixhuatlán, Papantla and Tihuatlán.
Results: out of all the samples, 380 were positive for CTV; 68% corresponded to attenuated variants and 40% to severe variants. The following symptoms were observed in all the municipalities: death of branches (68%), yellowing of shoots (41%), trees with small leaves (38%), and debarking of the trunk (32%); the incidence of small fruits was 31%, and finally, generalized yellowing (19%).
Limitations/Implications of the study: to manage the disease there are various alternatives, the most frequent is the use of tolerant rootstocks, however, with the existence of severe variants there may be tree deaths even with tolerant rootstocks, so it is necessary to search for and implement other far-reaching options.
Findings/Conclusions: the results show that even with the regulations for the production and mobilization of plants, the virus is widely distributed in the seven municipalities of northern Veracruz.

Keywords: citrus, incidence, variants, virus.

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INTRODUCTION

Citrus trees can be affected by different pathogens such as bacteria, fungi, viruses, and viroids which limit the production of citrus plantations (Bar-Joseph *et al.*, 2010). The disease known as citrus decline virus is caused by the *Citrus tristeza virus* (CTV), which has been present in Mexico since 1983 when the first positive plants were detected in Tamaulipas, Veracruz (1986 and 1992), and then in Yucatán, Quintana Roo, Campeche, Morelos, Michoacán (2000), Colima and Nuevo León (2005), with severe variants in some states (Loeza-Kuk *et al.*, 2008; Herrera-Isidrón *et al.*, 2009; Rivas-Valencia *et al.*, 2017). In 2001, the NOM-031-FITO-2000 norm was published, which established "the campaign against the citrus decline virus", although the dispersion of the disease still continues (Rivas-Valencia *et al.*, 2010; Contreras-Maya and Villegas-Monter, 2020).

Citrus decline has been one of the most devastating diseases in the citrus producing zones of the world; in the 1930s and 1940s it affected practically the entire citrus production in Argentina, Brazil and Uruguay, and by 1950 in Colombia and Peru, which had rootstock of sour orange (*Citrus aurantium*), and as a result 20 000 000 trees were eliminated (Müller and Rezende, 2004). In 1980, another similar disaster happened in Venezuela and Jamaica (Mendt, 1992; Roistacher, 1999). However, it is unknown why such an epidemic had not happened in Mexico, considering that since 2003 there are reports of severe isolates in *C. sinensis* in the states of Baja California, Nuevo León, Tamaulipas and Veracruz (Herrera-Isidrón *et al.*, 2009; Mendoza *et al.*, 2003; Loeza-Kuk *et al.*, 2005; Contreras-Maya and Villegas-Monter, 2020; Contreras-Maya *et al.*, 2022). There are several isolates of CTV that differ biologically and primarily in the symptoms that they cause in citruses (Dawson *et al.*, 2013, 2015). The variants responsible for the sudden decline have been designated as severe, while the ones that do not cause symptoms are known as mild. The CTV is transmitted through infected shoots and in a semi-persistent way by many species of aphids (Harper *et al.*, 2016).

In northern Veracruz, in recent years, symptoms of yellowing, dry branches, small leaves and fruits, and even tree deaths have been seen, which lead to the deterioration and low yield of plantations. There is lack of knowledge of the pathogens associated to yellowing. Therefore, the aim is to evaluate the presence and distribution of CTV and its variants in seven orange producing municipalities in northern Veracruz, Mexico, with the aim of updating the phytosanitary status of CTV and to give it the importance it has as a threat to Mexican citrus production, which could be devastating as it has happened in other countries, since the sour orange rootstock predominates.

MATERIALS AND METHODS

Sample collection

The study was conducted in citrus plantations located in the municipalities of Álamo (153 samples), Castillo de Teayo (76), Cazones (117), Chicontepec (100), Ixhuatán (79), Papantla (183), and Tihuatlán (96), which are the leading orange producers in northern Veracruz, where 804 citrus samples were collected in 90 localities. The sampling was directed at trees with symptoms of yellow shoots, death of branches, small fruits, trunk debarking, and small leaves. Four vegetative shoots were collected in each tree in active growth (a shoot per cardinal orientation), which were labeled and placed in polyethylene bags and stored in cold, to transport to the Biotechnology and Plant Physiology Laboratory, Montecillo Campus, Colegio de Postgraduados. In addition, a questionnaire was carried out with the producers to understand the origin of the plants, as well as the age, variety, rootstock and management.

RNA extraction and molecular analysis

Once the samples arrived to the laboratory, they were stored at −4°C for their processing, which consisted in cleaning the leaves with interfolded paper towels (Sanitas®, Mexico) and alcohol at 75%, then the leaf midribs were extracted with single-edge razors (CORTY®, Mexico) and finely chopped for storage in microtubes of 2 ml (AXYGEN®, Mexico) at −20 °C. Tissue maceration was done in a disruptor (Retsch®, Mexico) at 30 frequencies per 20 min, with 0.25 g of leaf midrib plus 1 ml of saline buffer (Tris-HCL 10mM, EDTA 1mM, NaCl 2M, Bovine albumin 0.05%; Sigma-Aldrich, USA) and 3 stainless steel pellets (3/16”) in microtubes of 2 ml. Once the tissue is macerated, the microtubes were centrifuged for 5 minutes at 13500 rpm at 4 °C; the pellets were retrieved and the supernatant discarded; again, 800 μL of saline buffer were added, there was vortex and then it was centrifuged for 5 minutes at 13500 rpm at 4 °C and the supernatant discarded.

For the extraction of nucleic acids, the Minas *et al.* (2011) protocol was followed with some modifications. In the phase of cell lysis, CTAB 2% was used (Tris-HCL 1M (pH 8; Sigma-Aldrich, USA), EDTA 20 mM (pH 8; Sigma-Aldrich, USA), NaCl 1.4 M (pH 8; Sigma-Aldrich, USA), and CTB 2% (w/v; Sigma-Aldrich, USA)), vortex happened. All the samples were incubated at 65 °C for 40 minutes, centrifuged at 13500 rpm 4 °C for 5 min, 800 μL of the supernatant was transferred to microtubes of 2 ml adding 400 μL of phenol: chloroform: isoamyl alcohol (25:24:1; invitrogen, USA) to each sample, it was mixed by inversion during 3 min at room temperature. During this phase of enzyme inhibition and precipitation of nucleic acids, the microtubes were centrifuged at 14000 rpm for 10 min at 4 °C to recover 500 μL of the supernatant in a microtube of 1.5 ml, adding 500 μL of isopropyl alcohol (−20 °C) and 50 μL of ammonium acetate 7.5 M (CH_3COONH_4; Sigma-Aldrich, USA), and it is left to incubate all night at −20 °C. The samples were centrifuged at 14000 rpm for 10 min at 4 °C, and the supernatant was discarded. Finally, the pellets or pills were washed with ethanol (Sigma-Aldrich, USA) at 70% (v/v) 1 ml at 14000 rpm for 5 min at 4 °C; the pellets were left to dry at room temperature and were re-suspended in 50 μL of water free of nucleases (IDT, USA), stored at 4 °C for 24 h.

Then, the qualities (260/280 nm; 260/230 nm) and RNA concentrations were verified through spectrophotometry using the Nano Drop 2000 (Thermo Fisher Scientific, USA) with 1 μL of total RNA sample.

In the reverse transcription reaction (RT; Reverse Transcription), each microtube of 0.2 ml was added with 0.75 μL of each primer (P25-R, VT-R, T30-R at 10 pmol, Table 1) in 6.87 μL of water + 3 μL of RNA from each sample and incubated at 72 °C for 5 min in a thermocycler (MAXIGENE AXIGEN, USA), unique cycle. Then, each sample was added with 7.87 μL of the mixture that contained 5 μL Buffer 5X of M-MLV + 1.5 μL of dNTP’s Mix + 0.625 μL of RNAsin + 0.75 μL of M-MLV Reverse Transcriptase (Promega, USA), placing the tubes in the thermocycler at 42 °C for 60 min followed by 72 °C for 10 min, unique cycle.

The samples were analyzed through qPCR for the detection of CTV in a thermocycler C1000 (Bio-Rad, USA). The final reaction volume was 20 μL: 10 μL of Ssoadvance universal for sybr green mix (Bio-Rad, USA), 0.5 μL of each primer P25F-23 and P25R-20 (125 nM) (Saponari *et al.*, 2008), 7 μL of water free of nucleases and 2 μL of cDNA. Each sheet contained two Not target and NTC control which validated the absence of contaminants or dimers in the reaction; they were fit with the adhesive cap manipulating it only by the edges. The thermocycling program was 55 °C for 2 min with initial denaturalization followed by 95 °C for 5 min, then 40 cycles at 95 °C for 15 s, and aligning at 57 °C for 40 s. Finally, a melting curve of 65 to 95 °C was used with increase of 0.5 °C/cycle.

Considering the viral concentration, samples were selected for the final point PCR reaction, with the objective of identifying existing severe and attenuated variants, using for this purpose a thermocycler (MAXIGENE AXIGEN, USA). It was done by adding to each tube 9 μL of the mixture that contained 2 μL Green buffer GoTaq DNA Polymerase + 0.4 μL of $MgCl_2$ (10 Mm) + 0.2 μL of dNTP's (10 Mm) + 0.6 μL of each primer (VT-F, VT-R, T30-F and T30-R) + 0.1 μL of GoTaq DNA Polymerase (Promega, USA) + 2 μL of cDNA + 5.1 μL of water. The PCR conditions for the five variants were: one cycle of 3 min 94 °C, 30 cycles of 30 s at 94 °C, 30 s at 56 °C, 45 s at 72 °C and a final extension of 10 min at 72 °C (Saponari *et al.*, 2008; Contreras-Maya and Villegas-Monter, 2020; Contreras-Maya *et al.*, 2022). The products were visualized in non-denaturalizing agarose gel at 2%, dyed with ethidium bromide. The gel was observed and photographed in a QUANTUM ST5® (Vilver Lourmat, USA) transilluminator, using positive, negative and 100bp DNA Ladder controls (Promega, USA). Some of the samples that were positive were sent to Macrogen Corp. (South Korea) for sequencing and thus corroborate the presence of CTV.

Impact of symptoms and CTV

According to the producers and the field visits that were carried out, the symptoms were grouped into: generalized yellowing, yellow shoots, death of branches, small fruits, trunk debarking, and small leaves. Therefore, the sampling was directed at trees that presented one or more of the symptoms mentioned before; 804 samples were collected, among which 722 were orange trees (*Citrus sinensis* L. Osbeck), species that predominates in northern Veracruz, 33 Persian lime (*Citrus latifolia* Tanaka), 31 mandarin (*Citrus reticulata* Blanco), and 18 grapefruit (*Citrus paradisi* Macf.). To determine the impact of each of these symptoms, the number of plants with symptoms was divided by the total number of trees collected by municipality and multiplied by 100. In the case of the impact of CTV, the number of samples that were positive in the qPCR reaction was also taken into account, as well as the total number of samples by municipality.

Table 1. Sequences of primers for RTqPCR and RT-PCR.

Name	Polarity	Sequence 5´-3´	Size (pb)	Product size	Reference
P25-F	Sense	AGCAGTTAAGAGTTCATCATTAC	23	101	Saponari *et al.*, 2008
P25-R	Antisense	TCAGTCCAAAGTTTGTCAGA	20		
VT-F	Sense	TTTGAAAATGGTGATGATTTCGCCGTCA	28	302	Roy *et al.*, 2010
VT-R	Antisense	GACACCGGAACTGCYTGAACAGAAT	25		
T30-F	Sense	TGTTGCGAAACTAGTTGACCCTACTG	26	206	
T30-R	Antisense	TAGTGGGCAGAGTGCCAAAAGAGAT	25		

RESULTS AND DISCUSSION

The RT-qPCR technique allowed detecting the pathogen in 380 positive samples to the citrus decline virus. The primers P25F-23 and P25R-20 amplified for the protein layer of the virus. The samples that amplified with Cq (Cycle quantification) $<$35 were considered positive and presented temperature or melting curve (fusion curve) Tm=80.5 °C (±0.5 °C) that corresponded to the product obtained by the primers, and in addition, the NTC did not present amplifications, which confirmed the absence of primer dimers or of any type of non-specificity (Figure 1).

Distribution of the virus

Mexico is considered one of the leading citruses producing countries; however, in recent years the yield has decreased due to different biotic and abiotic factors (Orozco-Santos *et al.*, 2014). Among these, we can highlight problems with diseases such as the one caused by the citrus decline citrus, whose impact varied from 27% in Chicontepec to 63%

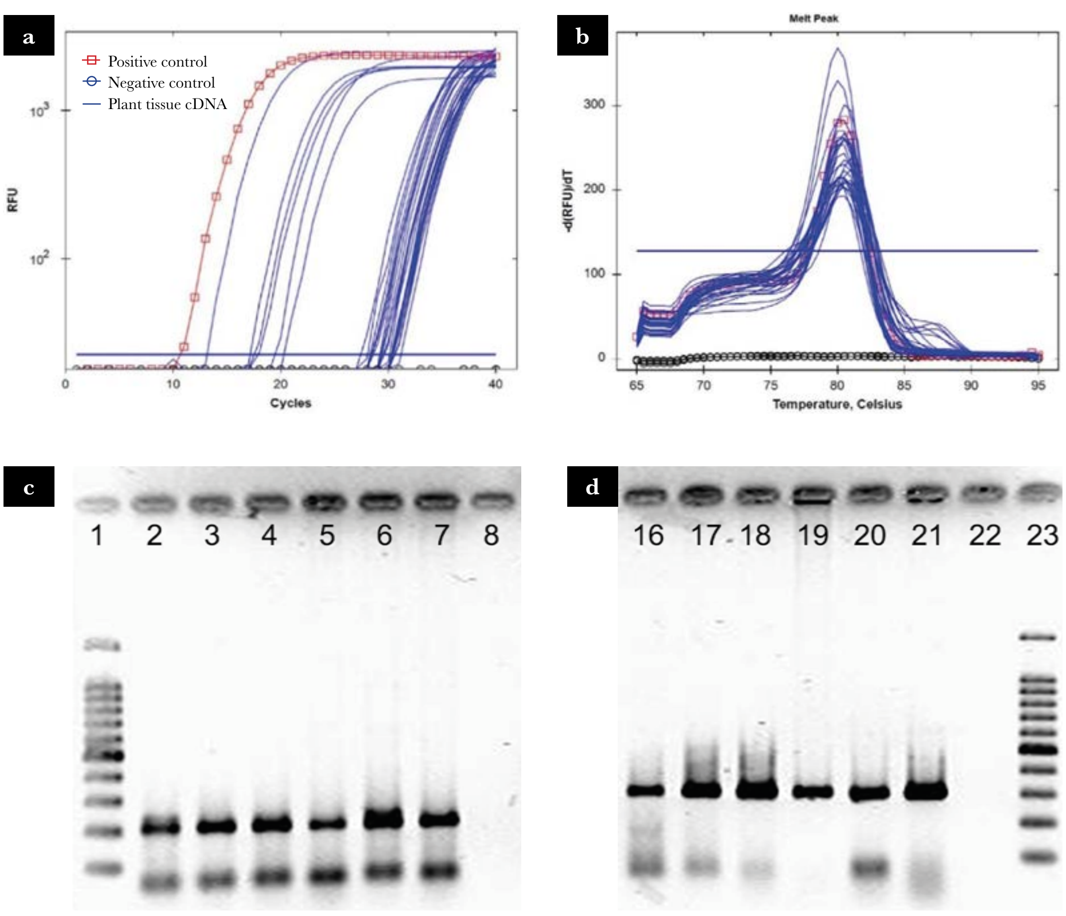

Figure 1. Amplification by qPCR of *Citrus tristeza virus* in samples of symptomatic plant tissue. a) Amplification curves obtained from cDNA; b) Fusion curves for CTV amplification, the temperature (°C) is presented on the (x) axis, and the fluorescence units (RFU) on the (y) axis; c) Amplifications obtained by final point RT-PCR for attenuated variants (T30); and d) Amplifications for severe variants (VT) of CTV, where lanes 1 and 23 correspond to the molecular weight marker of 100 pb; 2 and 16 positive controls; lanes 8 and 22 negative controls; lastly, 3-7 and 17-21 samples of citruses that were positive.

in Álamo. In Álamo and Papantla, more than 50% of the samples were positive, situation that is worrying because of the distribution of the disease since more than 50% of the surface planted with citruses in northern Veracruz is concentrated in these municipalities. It should be indicated that in Chicontepec and Ixhuatlán there were lower percentages; however, this does not exempt them from reaching risk zones because at any time the impact could increase because most of the producers use non-certified plants purchased in nurseries of the municipality of Álamo with the highest percentage of CTV according to the study's results.

Propagation of the virus and its variants

The CTV is transmitted in a semi-persistent way by *Aphis gossypii* and *Toxoptera citricida*, among other aphids (Cambra *et al.*, 2000). However, the high impact allows indicating that the virus has also been disseminated by the mobility of the infected plant material (purchase of non-certified plants), in addition to recent reports that CTV can be transmitted by diaphorina citri (Wu *et al.*, 2021; Zhang *et al.*, 2023). Taking into account that more than 95% of the plants used come from non-certified nurseries, where the plants are produced outdoor and the shoots are collected from trees of unknown origin and that the federal campaign against CTV was eliminated since 2014, it is easy to understand why there is massive death of citrus trees in northern Veracruz and there is no doubt that the same can happen in the rest of the country in a short amount of time.

The detection of severe variants of CTV in mandarin plantations in California in the year 2000 originated from the illegal imports of shoots (Matos *et al.*, 2013), the discovery of VT type variants in commercial citruses in Florida (Hilf *et al.*, 2005), which represent clear examples that severe variants can emerge due to oversight in the introduction of plant materials, which are a continuous threat for the citrus industries in many countries. The eradication of infected trees, as well as the development of means to protect the plantations against severe variants, become critical to coexist with the virus.

It should be mentioned that in Mexico, the first detections of CTV were carried out in Tamaulipas (1983) and Veracruz (1986); however, despite the eradication of the initial focal points, the dispersion of the disease has continued (Silva-Vara *et al.*, 2001; Rivas-Valencia *et al.*, 2010; Contreras-Maya *et al.*, 2022). Currently, there are several aphid species that can transmit CTV, the main ones are *Aphis gossypii* (Glover), *A. spiraecola* (Patch), *Toxoptera aurantii* (B de F) and *T. citricida* (Kirkaldy). *T. citricida* is found in all the citrus producing states of the country, with the risk of causing severe outbreaks of citrus decline because severe variants have been detected in Nuevo León, Colima, Baja California and Tamaulipas (Mendoza *et al.*, 2003; Loeza-Kuk *et al.*, 2005; Herrera-Isidrón *et al.*, 2009), and recently in several municipalities of Veracruz (Contreras-Maya *et al.*, 2022). Taking this into account, the situation is more worrying because the disease is widely distributed and there are increasingly more vectors that favor the dispersion. A factor that is not considered is the number of non-certified nurseries, which in northern Veracruz exceed 400 selling points, because they have never been controlled and are probably the most important vector.

Variants of CTV of the attenuated and severe type were found in every municipality sampled, and more than two variants were even found in the same tree. It should be pointed out that in Álamo, 60% of the CTV-positive samples were VT type while in Castillo only 3% (Figure 2). The presence of severe variants in all the municipalities and the use of sour orange as rootstock explain the accelerated death of plants in northern Veracruz, but it is important to consider that psorosis, exocortis, and cachexia are also present based on the symptoms observed in the plant trunk and branches. These diseases have been reported previously by Almeyda *et al.* (2007) and Contreras-Maya *et al.* (2018).

Symptoms associated to citrus death

Concerning the symptoms, trees were observed with death of branches (68%) in every municipality, with higher impact in the municipalities of Tihuatlán, Papantla, Cazones, Castillo de Teayo and Álamo. Another symptom that presented high impact was yellowing of shoots (41%), principally in Tihuatlán, Papantla, Álamo and Cazones. The symptoms of small leaves and trunk debarking had values of 38 and 32%, respectively; the trees with small fruits had impact of 31%, and lastly generalized yellowing (19%) (Figure 3). However, it is difficult to attribute the death of plants to a causal agent, because the problem presents in citrus producing zones is multifactorial. Nevertheless, there are plants that die because of the presence of CTV and it can be verified in the field because the leaves and the fruits remain adhered to the plant (Figure 3e), where the classical syndrome is present, or else the rootstock dies from the base and this causes the death of the variety (Figure 3d). The producers manage to distinguish a sudden death of the trees and colloquially called it "died out of nowhere", term that is frequently heard in the community of Santa Emilia, Álamo, Veracruz.

Depending on the virus variant and the citrus graft/rootstock combination, CTV causes two primary symptoms, which have had an impact on the global production of citrus. The first is the sudden decline of trees grafted in sour orange (*Citrus aurantium*). During the past century, the severe outbreaks of CTV caused a fast decline that developed in the citrus producing regions and destroyed nearly 100 million trees (Moreno *et al.*, 2008). The solution to the outbreak was to use disease-tolerant rootstock. Although this allowed an effective control of CTV, these rootstocks require a different management than sour orange (Folimonova *et al.*, 2013), including fertilization, which is scarcely used in the study

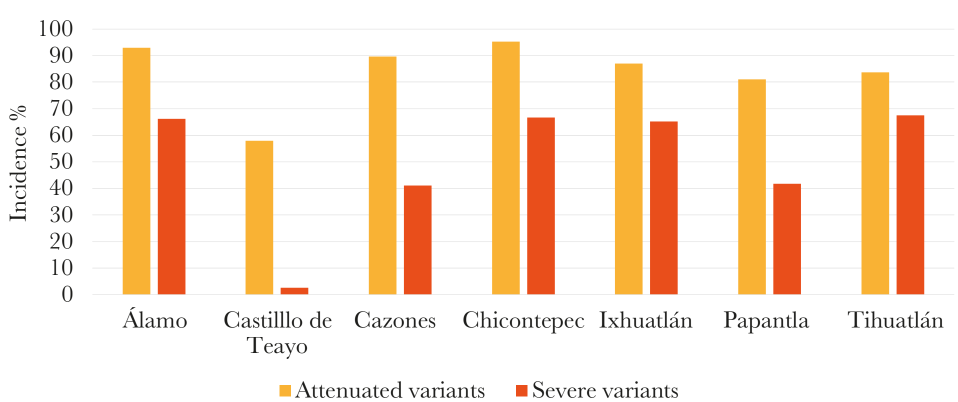

Figure 2. Impact of *Citrus tristeza virus* variants in seven municipalities of Veracruz, based on the samples collected by municipality and which were positive in the qPCR reaction.

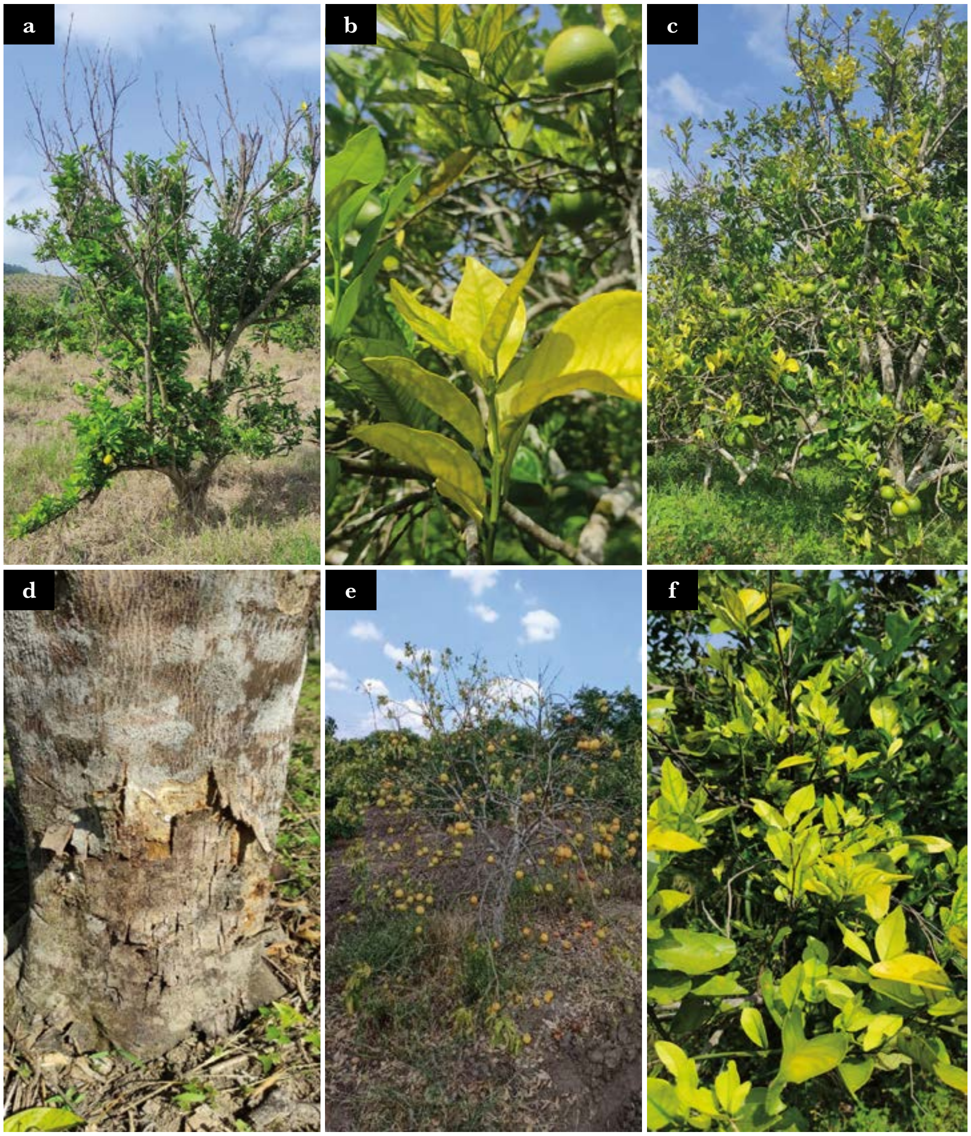

Figure 3. Symptoms observed and associated to *Citrus tristeza virus* in seven municipalities of northern Veracruz. a) Dry branches (68%), b) yellow shoots (41%), c) small leaves (38%), d) trunk debarking (32%), small fruits (31%), and generalized yellowing (19%).

zone, tree pruning, considered until now as unnecessary in citrus trees, although essential for the "new citrus production" and irrigation; due to climate change, it is necessary to apply four to six irrigation events in the months of April-May, July-August, and this if we want to continue producing citrus trees.

Another symptom caused by some variants of CTV is stem pitting, which affects grapefruit (*C. paradisi*), orange (*C. sinensis*), and Mexican key lime (*C. aurantifolia*), regardless of the rootstock used. The stem pitting is the result of the areas of virus multiplication (Tatineni *et al.*, 2011). However, during the sampling carried out, stem pitting was not observed, although rootstock death was, starting from the thinnest roots to those of first order, and then the stem base which climbs progressively; this is what we see in the field, death from the tips of the terminal branches and the continuing descending death, which confuses technicians and producers because they do not observe the base of the trunk, in plants with these symptoms that are consistently found in VTC.

From the symptoms evaluated, death of branches, leaves with yellow midribs, small leaves and fruits are related to *Citrus tristeza virus*, which is why the cause of death can be due to this virus; however, we cannot forget that the plants also have psorosis, exocortis, cachexia, HLB, leprosis, in addition to the attack from fungi (*Colletotrichum* spp. and *Lasiodiplodia* spp.) oomycetes, lethal fungus (*Usteulinea deusta*); these pathogens weaken the plant and as consequence it is more susceptible to diseases. We should also take into account that in northern Veracruz most of the producers do not fertilize and pruning is incipient; these two factors also impact the "presence, or make the plants more sensitive to attack from diseases". With the results obtained, it is possible to point out that: there is NO control in the mobility of plants (certified material is generally not used), and there is no control of vector insects. There is lack of knowledge of the main diseases of economic importance, which evidence the lack of training of technicians and producers in general.

CONCLUSIONS

The high impact of CTV and severe type VT variants explain in part the death of plants in northern Veracruz, although we must not forget that there are other quarantine diseases that are also contributing and aggravating the problem.

Some of the samples collected were young plants, which were positive to *Citrus tristeza virus* and this shows the risk that viability and productivity present in the orange plantations in northern Veracruz.

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Colegio de Postgraduados

Current challenges and forecasts in maize grain production and consumption in Mexico

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ABSTRACT

Objective: to analyze production and consumption of maize grain in Mexico, with time series and recurrent neural networks, to describe the present and future situation of maize cultivation.

Design/Methodology/Approach: key variables were analyzed in graphs and maps created in Excel® and SCImago Graphica®, respectively. Forecasts for the year 2050 were obtained in Python© with Recurrent Neural Network (RNN) of the Long Short-Term Memory (LSTM) type, and were compared with the years 1980 and 2020.

Results: the largest production of white and yellow maize grain was obtained by the United States and China. Mexico ranks seventh, is not competitive in exports, and relies on imports of yellow maize grain from the United States to supply demand. The Mexican states that implemented technology packages showed higher yields and production. By 2050, maize grain production in Mexico will increase due to the technological advances of Agriculture 5.0 Although it would not be enough to supply the apparent consumption of the growing population, for this reason imports will increase.

Limitations/Implications of the study: analysis of the possible future, created from time series through RNN-LSTM, helps to guide decision-making in the present.

Findings/Conclusions: new agricultural public policies are needed to guide, in the long term, the challenges of maize grain production and consumption in Mexico to guarantee food sovereignty.

Keywords: yields, imports, exports, time series, deep neural networks.

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INTRODUCTION

Maize (*Zea mays* L.) has its center of origin and domestication in Mexico. It is one of the most economically important crops in the world, as it is necessary for human food, animal feed, and to obtain industrial products. Maize grain, and green maize fodder are harvested (López-Torres *et al.*, 2016; Cadet Díaz and Guerrero Escobar, 2018). There are several varieties; their use depends on agricultural goals (Sánchez-Ramírez *et al.*, 2023).

In 2021, maize grain was the second most produced crop in the world, with 1210 million tons [Megagrams] (M Mg), and with the second largest harvested area, about 206 million hectares. For this reason, it ranked only after sugarcane (1859 M Mg) and wheat (221 million ha), respectively (FAO, 2023). However, it is necessary to increase yields with the least use of resources to meet global demand in the future (FAO, 2022).

Maize yields in Mexico increased due to the management of improved varieties, agrochemicals, machinery, credits, and irrigation (Cadet-Díaz and Guerrero-Escobar, 2018). In self-consumption production, farmers conserve native maize varieties (Sánchez-Ramírez *et al.*, 2023) that will be necessary in the genetic improvement of Agriculture 5.0. In addition to biotechnology, in the long term, artificial intelligence, Big Data, blockchain and automation will increase productivity and the supply of maize grain (Ahmad and Nabi, 2021; Martos *et al.*, 2021).

Time series are used to characterize the supply and demand of maize grain in Mexico. In addition, they make it possible to calculate forecasts necessary to guide decision-making in the present (Aladag, 2017; Mills, 2019). One of the most widely used methods is recurrent neural networks – RNNs, especially those of the deep LSTM type, because they give more accurate results to complex linear and nonlinear problems (Abbasimehr *et al.*, 2020).

The objective of this research was to analyze production and consumption of maize grain in Mexico, with time series through recurrent neural networks, to describe the present and future situation of maize cultivation.

MATERIALS AND METHODS

Data set

Data were downloaded from the Mexican Agri-Food and Fisheries Information Service (SIAP, 2023b) and the United Nations Food and Agriculture Organization (FAO, 2023). The key variables analyzed for maize grain in Mexico were production, apparent consumption, exports, imports, yields, and harvested area.

Time series analysis

Discrete time series were implemented with annual data (x_t), from 1961 to 2021, x_{t-1}, x_{t-2},... x_{t-n} (Aladag, 2017; Mills, 2019). A descriptive analysis was performed in the time series with graphs created in Excel®, maps in SCImago Graphica® and forecasts towards 2050 in Python© version 3.12, with the use of recurrent neural networks – RNN, for the variables production, apparent consumption, import and export to compare the present (2020) with the data from 30 years ago (1990) and up to 30 years towards the future (2050).

Recurrent Neural Networks

A Deep Neural Network (DNN) model of the recurrent type, known as recurrent neural networks for short- and long-term memory analysis (RNN-LSTM), was selected to obtain the forecasts to 2050. They were designed under the theory of Greff *et al.* (2017) and Abbasimehr *et al.* (2020), with the following architecture: an input layer, six hidden layers with 32 neurons each, and an output layer (Figure 1). The input layer of the RNN-LSTM received the time series data, and the output layer of the network generated the forecast up to 2050. An RNN-LSTM was trained for each of the key variables to be predicted.

For the RNN-LSTM performance evaluation, the root mean squared error (RMSE) was used $\sqrt{\frac{1}{n}\sum_{t=1}^{n}\left(x_t-\hat{x}_t\right)^2}$, where x_t and $\hat{x}_t$ are the actual and predicted values of the series, respectively; n is the length of the test set (Abbasimehr *et al.*, 2020).

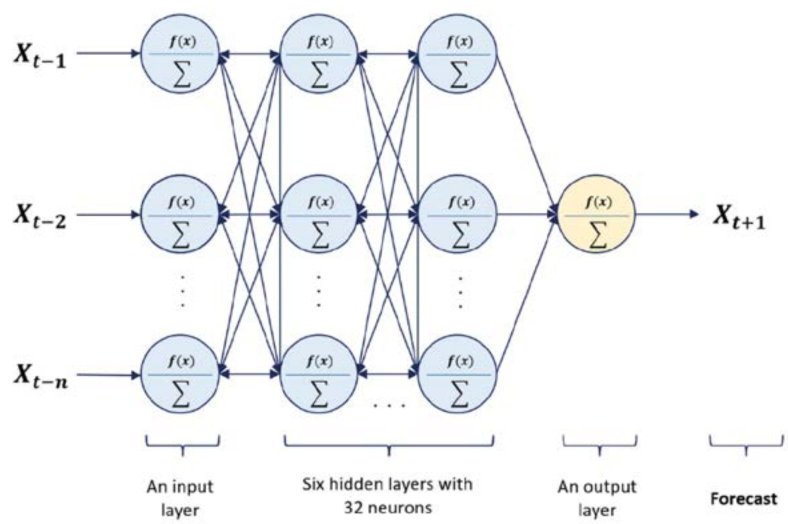

Figure 1. Architecture of a recurrent neural network for short- and long-term memory analysis (RNN-LSTM). Source: elaborated by the authors with information from Greff *et al.* (2017) and Abbasimehr *et al.* (2020).

RESULTS AND DISCUSSION

World scope of maize grain production

In 2021, the countries with the highest corn grain production (M Mg) were the United States, 384; China, 273; Brazil, 89; Argentina, 61; Ukraine, 42; India, 32; and Mexico, 28 (Figure 2). The United States and China contributed 54% (657 M Mg), with respective 320 and 1413% rates of change. This unprecedented increase led to a 490% increase in global maize production (FAO, 2022; 2023).

In order to maintain this pace further, production systems need to be oriented to solve the following challenges: climate change; pandemics; war and conflicts; genetically modified organisms; lack of credit; high prices; scarce and underage skilled labor (Cadet-Díaz and Guerrero-Escobar, 2018; Kato-Maldonado and Huerta-Moreno, 2022; Shahini *et al.*, 2022).

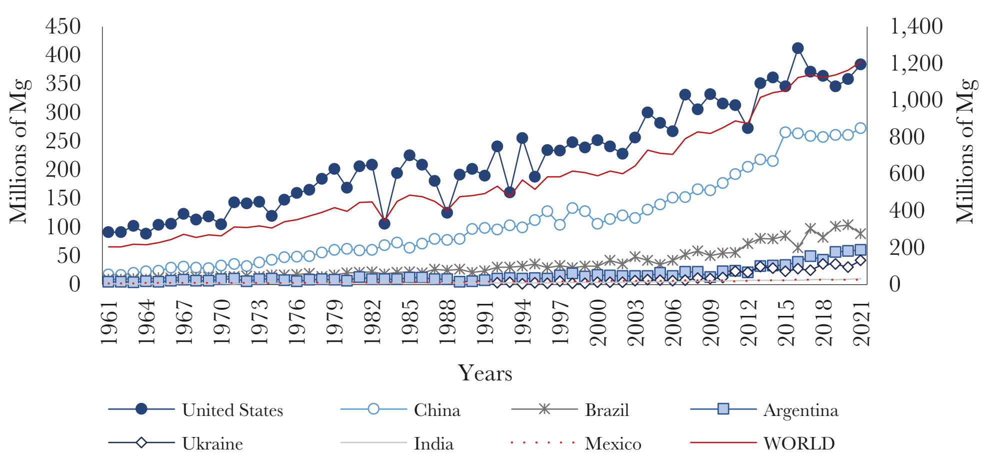

Figure 2. Behavior of maize grain production in the main producing countries and world total, 1961-2021. Source: elaborated by the authors using data from FAO (2023).

Production and consumption perspectives of maize in Mexico

The problems described are aggravated in developing countries, such as Mexico, because it disrupts free trade, raises prices for supplies (Shahini *et al.*, 2022) and poverty levels. In 2022, 46.8 million Mexicans (36.3%) were in poverty and 9.1 million (7.1%) in extreme poverty (CONEVAL, 2023). In addition, these are maize producers for self-consumption who are located in rural and peri-urban areas (López-Torres *et al.*, 2016; FAO, 2022).

Another challenge faced by Mexico is to supply the apparent domestic consumption (44.80 M Mg, Figure 3) (FAO, 2023) for 127 million Mexicans (ONU, 2023), with the domestic production of white (24.29 M Mg) and yellow (2.80 M Mg) maize grain. The latter was not enough and 15.81 M Mg of yellow maize grain was imported from the United States in 2021 (SIAP, 2023a).

Yellow maize was destined for livestock feed (15.50 M Mg) and the starch industry (3.02 M Mg; SIAP, 2023a). This makes Mexico dependent on production from the United States (Espinosa Cortés, 2022) and uncompetitive in exports [0.097 M Mg] (FAO, 2023).

On the other hand, in 2021 the main producer states of this staple in Mexico were Sinaloa, Jalisco, State of Mexico, Guanajuato, and Michoacán, with 5.54, 3.95, 1.94, 1.93, and 1.91 M Mg, respectively (Figure 4). Sinaloa, Guanajuato, Chihuahua, Michoacán, and Sonora produced 5.47, 1.48, 1.43, 0.82 and 0.79 M Mg, under irrigation systems. The states with the highest rainfed production were Jalisco, State of Mexico, Guerrero, Veracruz, Chiapas, and Michoacán, with 3.60, 1.57, 1.29, 1.25, 1.24, and 1.08 M Mg, respectively (SIAP, 2023b).

The production of rainfed maize in these states was significant due to the greater amount of harvested area (Figure 5) not to yields. Jalisco only obtained a yield of 6.66 Mg ha^{-1}; State of Mexico 3.74; Guerrero, 2.80; Veracruz, 2.26; Chiapas, 1.83; and Michoacan, 3.16 Mg ha^{-1} (SIAP, 2023b). The importance of rainfed maize production is based on the diversity and conservation of native maize varieties, grown by small-scale producers in traditional production systems, under extreme climatic and topographic conditions (López-Torres *et al.*, 2016).

In contrast, the highest yields (Mg ha^{-1}) of cultivated maize obtained with irrigation systems were Sinaloa, 11.89; Sonora, 11.56; Baja California, 11.47; Chihuahua, 11.13; and

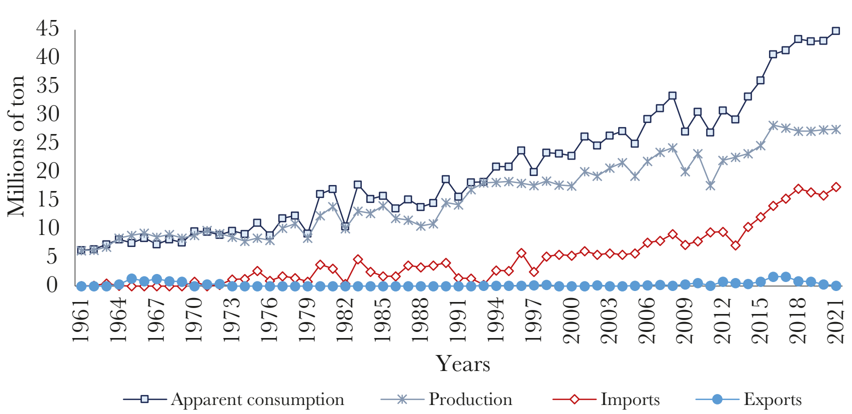

Figure 3. Historical behavior of apparent consumption, production, imports, and exports of maize grain in Mexico, 1961-2021. Source: elaborated by the authors using data from FAO (2023).

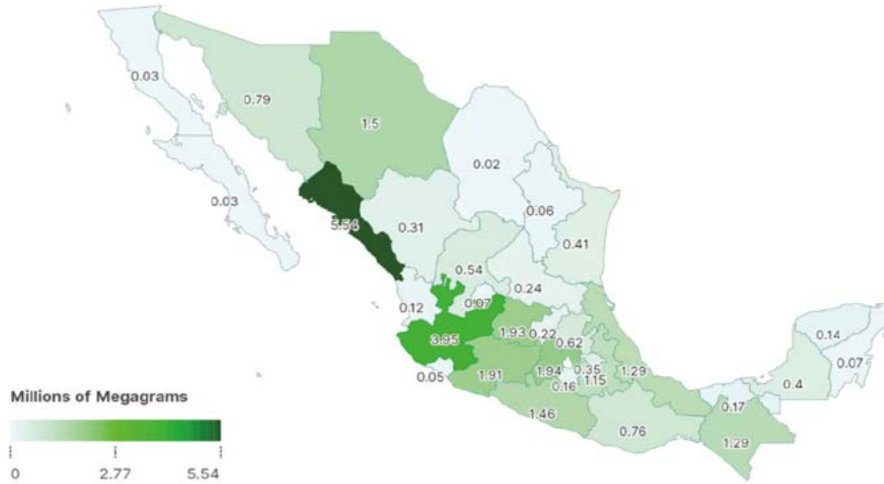

Figure 4. The states in Mexico with the highest production of maize grain in 2021. Source: elaborated by the authors using data from SIAP (2023b).

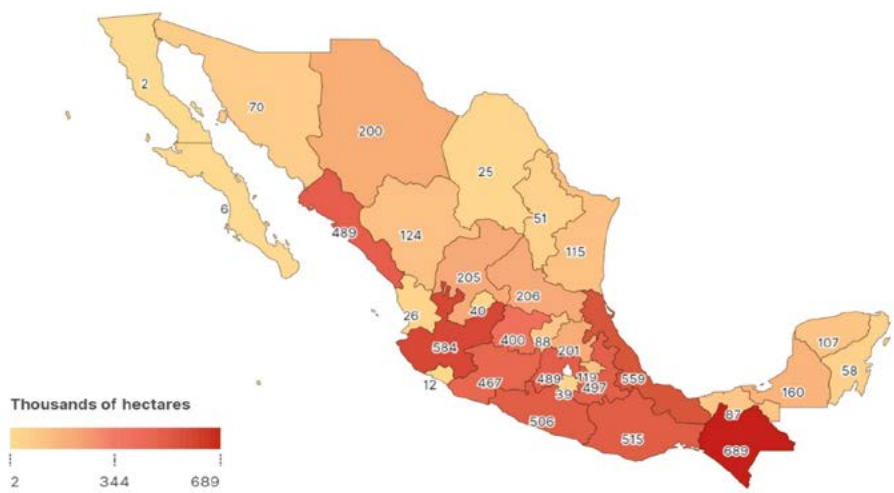

Figure 5. The states in Mexico with the largest harvested area of maize grain in 2021. Source: elaborated by the authors using data from SIAP (2023b).

Guanajuato, 10.20 Mg ha^{-1} (SIAP, 2023b). In order to be more competitive in these states, big-scale producers use, in addition to irrigation, genetically improved maize varieties, machinery, credits, fertilizers, and more efficient controls for pest and diseases (Cadet-Díaz and Guerrero-Escobar, 2018). Therefore, generating a 288% rate of variation in domestic yields (Figure 6; FAO, 2023).

In this scenario, maize grain production is expected to increase 37% by 2050, compared to 27.42 M Mg in 2020, in turn the equivalent of 2.57 times what was produced in 1990 (Figure 7; FAO, 2023). However, it will depend on digital education and the effective technological adoption of Agriculture 5.0 in Mexico, which emphasizes artificial intelligence, Deep Learning models; internet of things; Big Data; cloud computing; Blockchain, wireless sensors; drones, self-operated tractors, and robots; 3D printing; vertical farming, and urban

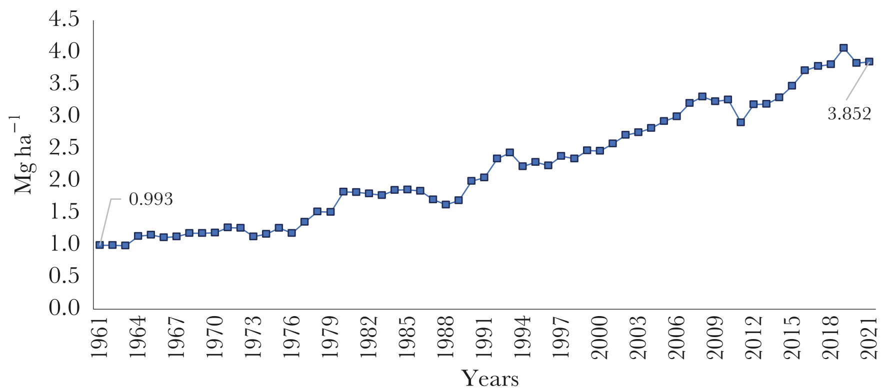

Figure 6. Behavior of the domestic yield of maize grain in Mexico, 1961-2021. Source: elaborated by the authors using data from FAO (2023).

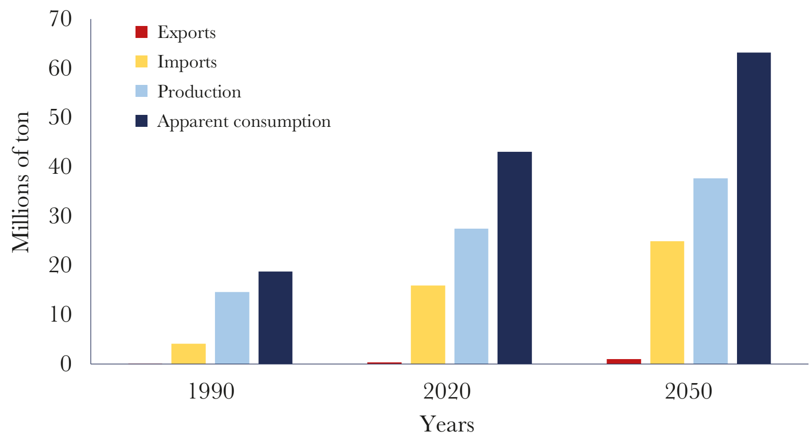

Figure 7. Forecasts for the year 2050 of the export, import, production and apparent consumption of grain corn in Mexico. Source: elaborated by the authors using data [1990 and 2020] from FAO (2023).

production of organic crops (Cadet-Díaz and Guerrero-Escobar, 2018; Ahmad and Nabi, 2021; Martos *et al.*, 2021; Buka *et al.*, 2022).

Agriculture 5.0 will also bring new challenges: reduction of repetitive jobs; skilled labor; modernization of educational programs; unprecedented polarization of producer types; exclusion, complexity, scarcity and the high costs of big Deep Learning models and Artificial intelligence; vulnerability of the privacy and security of data; technological dependence; environmental impact resulting from digitalization and robotics; reduction of traditional agriculture; oligopolistic markets for a global food production order; and loss of sovereignty in food production (Ahmad and Nabi, 2021; Martos *et al.*, 2021; Buka *et al.*, 2022; Espinosa Cortés, 2022; FAO, 2022; Kato-Maldonado and Huerta-Moreno, 2022).

These and other challenges that may arise in the future and have not been identified at present, shall reduce maize supply to satisfy the apparent consumption of this staple in Mexico (63.16 M Mg; FAO, 2023) due to the demand of the estimated 144 million people that would be here in 2050 (ONU, 2023). Consequently, for 2050 an increase of 8.99 M

Mg in imports will be required, compared to 2020; this value will be equivalent to a 6-fold increase on what was demanded in 1990. Further domestic demand will cause exports to a 0.682 M Mg increase in maize grain production, compared to values today (FAO, 2023).

CONCLUSIONS

Maize is one of the most important grains in world agriculture production. The United States, China and Brazil focus their resources on yield and production increases in the future. In Mexico, maize grain production was deficient to meet current demand, so there is dependence on the imports of yellow maize grain from the United States.

According to the 2050 forecasting scenario, maize grain production in Mexico may increase due to education, development, and adoption of advanced Agriculture 5.0 technologies. But it will not be enough to meet domestic demands, for it is predicted that imports of yellow maize grain will still be needed. Agricultural public policies adapted to each region and type of producer are required to go beyond the short term, in order to design sustainable and resilient production systems that guarantee food sovereignty, restoration of natural resources and conservation of native maize varieties.

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Biofortification of forages through the application of selenium nanoparticles

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ABSTRACT

Objective: This review paper examines the application of nanoparticles in agriculture, through biofortification with Se NPs in plants.

Methodology: A comprehensive review of the literature on the use of Se NPs in plant biofortification was made.

Results: According to documented studies, the foliar application of NPs significantly improves the morphological and physicochemical characteristics of plants. They promote root development, plant growth and increase the content of raw protein and ether extract in forages. An increase in the production of amino acids and essential fatty acids is also observed.

Limitations: The findings are promising, but there is still slight research on how NPs affect the environment and the safety of their use in the food chain. Further studies are needed to fully understand the long-term effects.

Conclusions: The application of Se NPs for biofortification can improve the nutritional quality of forages, contributing to more efficient and sustainable livestock production. However, to ensure the safety of its implementation it is necessary to continue investigating.

Keywords: sustainable agriculture, animal nutrition, nanotechnology.

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INTRODUCTION

Livestock farming faces the challenge of increasing its production in the face of expectations of world population growth due to the products and by-products that come from these animal sources and are required to feed the population, with forecasts of reaching close to 10 billion people in 2050 [1]. The expectations to meet this challenge are high, but this requires more efficient livestock production with fewer polluting emissions, both greenhouse gases and substances that contaminate soils and waters.

Forages are the primary support of sustainable agriculture and constitute one of the leading resources for the success of the animal production system [2]. Selenium (Se) is an essential micronutrient for animals; soil typically contains low levels of Se. Plants can benefit their growth and development by increasing their tolerance to different types of stress and synthesizing phytochemicals with antioxidant properties [3, 4]. Therefore, Se may be present, but in chemical forms, it is not available to organisms; as a result, Se content is deficient in plants, animals, and other organisms in the food chain [5, 6]. Biofortification increases the nutritional value of food crops by increasing the mineral and vitamin content in the plants [7]. Biofortification has been carried out in agricultural products (wheat, sorghum, peas, rice, and vegetables) and some forage varieties, such as alfalfa, due to the lack of Se in the soil [8]. Several biofortification methods are used for plants, but the foliar application method is more effective than soil fertilization [9]. In recent years, the benefits that Se provides for the health of farm animals have been demonstrated, such as improving reproductive performance and immunological and homeostatic function [10]. Worldwide, several studies relate the absence of consumption of this element to decreased development, growth, and reproductive and metabolic problems, especially in ruminants [11, 12].

Nanotechnology and agriculture

Nanotechnology is a multidisciplinary scientific research area that works on the design, characterization, manufacturing, and application of structures created by controlled size and shape manipulation at the nanometric scale (less than 100 nm) that produce complex systems in which they modify some specific characteristics [13]. Due to the above, many applications have been developed, including medicine, genetics, pharmaceuticals, and agriculture. In 2013, the FAO published information on the state of the art of nanotechnology in food and agriculture. Its report documented ten-year research focused on sustainability and solutions to environmental problems. These investigations focus on releasing active ingredients (disease management and crop protection), minimizing fertilization loss and increasing yield, and producing bionano compounds from traditional crops [14]. Reports of such research on possible applications in agriculture have been both positive and negative. However, there are reports in which favorable results have been found, for example, in the case of fertilization, germination, growth promoters, sensors of contaminants and other substances, and recovery and treatment of soil and water [15, 16]a. Our knowledge about the interactions of nanoparticles for agricultural use with the environment still needs to be improved. Due to its complexity, there is still a long way to go to understand it completely. The UN 2013 raised the need to increase food production since by 2050, there is an estimated population of 9.1 billion people. There is currently a trend towards producing energy crops derived from efforts to mitigate climate change and due to the imminent reduction of hydrocarbon reserves. At the same time, in the expert forum organized in 2009 by FAO, "How to feed the world in 2050," it was proposed that it will be necessary to increase cereal production by 70% by 2050 [17]. Nanotechnology could provide tools for modern agriculture and even solve future problems related to food production and energy demand, focusing on sustainability. However, some NPs have been shown to affect the development and yields of crops.

Nanotechnology and caring for the environment

The current food system worldwide is not sustainable and, for decades, has caused environmental damage, including greenhouse gas emissions, the irrational use of drinking water, the contamination of soils and aquifers by nitrogen and phosphorus, the use of pesticides, etc. However, recent research on environmental nanotechnology shows it can be a valuable tool for reducing environmental pollution. [18] developed nano encapsulates and nanocomposites for food and feed additives, biocides, pesticides, and contact materials. [19] applied nanomaterials as catalysts in phytoremediation processes and used stabilizers to improve their performance.

Applications of nanoparticles in agriculture

Applied science in agricultural systems presents a series of adversities and a wide range of challenges, such as a decrease in crop yields, low efficiency in the use of macro and micronutrients, a decrease in soil organic matter, deficiencies in multi-nutritional foods, climate change, the reduction in the availability of arable land, as well as the scarcity of water and labor for the countryside [20]. Various experiments have been carried out regarding the agricultural sector to know the optimal size, shape, and concentration of NPs to be applied to plants trying to improve their penetration and vascular translocation through the vascular bundles of the xylem and phloem.

The literature mentions that concentrations or doses less than 5 ppm of NPs can promote more significant plant growth. The size of the NPs is a relevant factor that significantly intervenes in the action of their penetration and translocation inside the plant tissues; therefore, the larger the size of the NPs, the less penetration will be into the vascular system in the plants when foliar spray techniques are used [21]. When applied to plant leaves, NPs can penetrate plant tissues as an aerosol or spray.

Evidence shows that 14.7% of the nanomaterial applied to plants is lost when using the spray or aerosol technique, compared to 32.5% or more with conventional agricultural products applied traditionally. An alternative is the application of foliar spray for the manipulation of nanoscale materials. When fertilizers are applied in a foliar way, they can easily penetrate the interior of the plants through the opening of the stomata, which have micrometric sizes fluctuating from 10 μm to 60 μm [22, 23].

Pastures and forages

Mexico has the eighth-largest cattle population in the world, with approximately 31 million heads, representing 2.31% of the world herd. However, Mexico has a greater diversity of ruminants, such as sheep and goats [24].

In the tropics of Mexico, the exploitation of livestock, specifically cattle, is carried out mainly by grazing native grasses and introducing grasses for livestock feeding. Pastures are grass and legume plants that grow in the pasture and serve to feed livestock. At the same time, forage is defined as any grass or legume harvested to be supplied as food to animals, whether green, dry, or processed. They can have different nutritional characteristics (hay, silage, stubble, saccharin, ammonification) [25, 26].

Selenium and Selenium nanoparticles

Selenium (Se) is a trace element that is scarce in the Earth's crust and is distributed in all parts of the Earth, being an element of volcanic origin. It accompanies sulfur and is found in clay soils (Figure 1). It is a by-product of the industrial manufacture of sulfur and sulfuric acid. Chemically, it forms with hydrogen and oxygen, the same compounds as sulfur H_2SeO_4, H_2SeO_3, H_2Se, and SeO_2. It can also replace sulfur in certain amino acids (cysteine, methionine) [27]. Selenium is an element found constantly but in small quantities in animal tissues. Selenium is one of the essential micronutrients for animals and is very important for animal nutrition [28]. Selenium is critical because it is necessary to form proteins and be a cofactor of antioxidant enzymes such as glutathione peroxidase (GPX), which reduces reactive oxygen species (ROS). Se deficiency in mammals causes physiological and nutritional deficiencies and pathologies since Se forms at least 25 selenoproteins that fulfill antioxidant, antiviral, and antitumor functions [29-31]. The synthesis and application of selenium nanoparticles (SeNPs) present several advantages, including chemical stability, biocompatibility, low toxicity, increased bioactivity, improved targeting, and versatile means to control the release profile.

Biofortification of plants with selenium nanoparticles

Biofortification increases the nutritional value of food crops by increasing the mineral, vitamin, and mineral content of conventional plant crops. This action is called agronomic biofortification. Biofortification involves the application of different minerals or nutrients in a foliar or edaphic manner, taking advantage of plant management, soil factors, and plant characteristics to obtain a higher content of critical micronutrients in the edible portion of the plant. The importance of Se in plant nutrition has been known for a long time. There are increasing ways to use Se NPs in agriculture, such as edaphic form (addition of Se to soil), hydroponic, and plant foliar cultivation. Plants can benefit from the use of Se NPs in a variety of applications, including (1) as care and management of pests and diseases caused by infectious microorganisms such as bacteria and fungi, (2) as near-essential trace elements, through the promoting plant biochemical pathways and thereby refining the

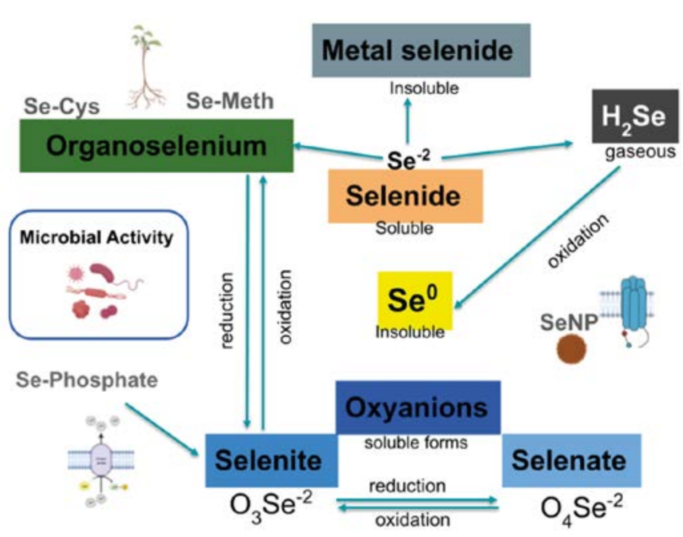

Figure 1. Changes in Selenium and selenium nanoparticles (SeNP) in the soil environment. Selenium (Se), a natural soil element, is found in the environment and organisms in two forms: inorganic (selenate, selenite, elemental Se and selenide) and organic (mainly Se-Meth selenomethionine and Se-Cys selenocysteine).

progress, yield and nutritional value of crops, (3) biofortifying crops using Se to increase their content, (4) alleviating abiotic stress and (5) increase the nutraceutical quality of consumable foods [32].

Uptake of Se NPs in plants

Concerning the phyto-uptake of SeNPs and their translocation, determining the safety and toxicity of NPs provokes a deep understanding of their uptake in plants (Figure 2). There are different ways for the plant to capture this trace element in the form of NP. An absorption mechanism of SeNPs occurs through the cell wall and penetration of the plasma membrane. Only NPs with sizes smaller than 100 nm can enter through the different pores (stomata) and pass through the cell wall successfully. The plant cell walls act as a barrier, preventing the entry of external substances or materials, including NPs, into the plant cell walls [33-35]. It has been shown that NPs can adhere to surfaces such as plant roots and could influence plant chemical and physical absorption [33]. One of the explanations that demonstrates the most outstanding scientific solidity is the translocation of nanomaterials, which can move intracellularly and extracellularly between plant tissues until they reach the xylem [36]. Once the NPs are within the plant's vascular system, the designed NPs could be transported to the aerial parts along with plant water transpiration and nutritional flux in nutrient transmission.[37] applied selenium nanoparticles in grass plants, *Festuca arundinacea*, reporting significant differences in biomass parameters, root length, and leaf length ($p<0.05$) between the treatments (1.5, 3.0, and 4.5 ppm NPsSe) and the control. The increase in root length, leaf length, and biomass production of se-treated plants could be attributed to the application of selenium nanoparticles; Se stimulates the growth and development of plants because it improves the synthesis of photosynthetic pigments and organogenesis [3, 38]. Some authors [39] observed that foliar application of different amounts of SeNPs affected morphological characteristics such as shoot length, root size, and fresh weight of the bean plant. In other study [40] reported that by applying SeNPs foliar, plant height, fresh weight of the shoots, and root size were increased when concentrations of 10 mg SeNPs were used in wheat plants, attributing that the SeNPs stimulated the organogenesis and growth of the plant roots.

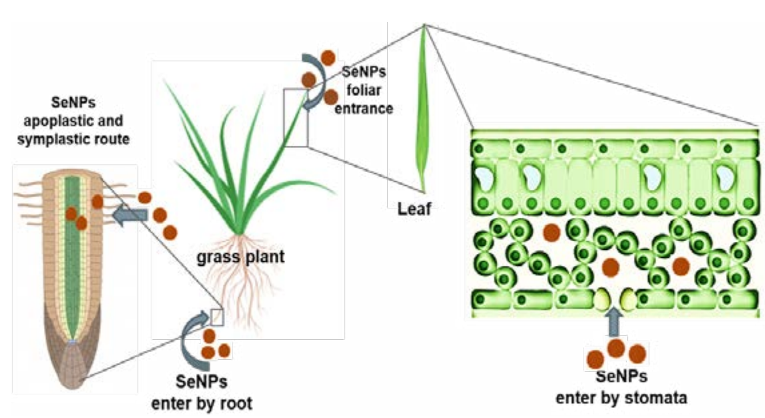

Figure 2. Uptake of Selenium NPs in plants, foliar, and soil route of adsorption.

Proximal composition of fortified forages

This research [41] found significant differences ($p<0.05$) between the treatments in the variables of crude protein (CP), ether extract (EE), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), and Nitrogen Free Extract (NFE). The increase in PC content as a plant response to foliar biofortification of NPsSe could be manifested because Se promotes the increase in the synthesis of sulfur amino acids (Cys and Met) and selenium-aminoacids such as SeCys and Semet, which are incorporated into proteins. However, there is also evidence that other non-protein amino acids are synthesized, such as γ-glutamyl methyl selenium-cysteine (γ-gluMetSeCys), methyl-SeCys and methyl-Semet ([32, 42], where Se was used as a fertilizer, and increased protein was obtained in the root and leaf of alfalfa (*Medicago sativa* L.). Similarly, [43] found that foliar spraying of selenate produced an increase in the levels of protein content and total root nitrogen in radish (*Raphanus sativus*). Other research [44] found that foliar supplementation of sodium selenate in wheat plants (*Triticum aestivum* variety BRS 264) increased total nitrogen (up to 20%) compared to the control. In addition[45] reported that fertilization with Se promotes the increase in fatty acids (oleic, linoleic, and linoleic), increasing the concentration of lipids; however, the metabolic pathway is unclear. Furthermore [46] found that foliar application of sodium selenite and selenate in four rice genotypes (Ariete, Albatros, OP1105, and OP1109) increased the content of total lipids, as well as increased the concentration of oleic acid (C18:1), linoleic (C18:2), and palmitic acid (C16:0). Also, [47] mention that the synthesis of compounds such as the content of starch, total soluble sugars, and reducing sugars are enhanced, caused by the possible increase in the activity of carbohydrate metabolism enzymes. The results obtained in this study are similar to those reported by [42], where Se was used to improve carbohydrate metabolism, finding an increase in these compounds in alfalfa root and leaf (*Medicago sativa* L). In the same way, [44] found that the carbohydrate content in wheat plants increased when biofortified with selenium, showing this trend in each crop.

Use of nanoelements in Animal Nutrition

Nanotechnology and its applications can revolutionize agricultural production. This technology has had various applications in animal production, including the use of new molecular and cellular tools for animal reproduction, preservation of the animal's identity from birth to the consumer's table ("traceability"), biosecurity of food from animal origin, a better understanding of the phenomena that govern animal nutrition from dietary ingestion to the uptake and use of nutrients, and others [48].

In animal nutrition, as in other areas, it is possible to apply nanotechnology to obtain information about a nutrient or bioactive component and its release at specific sites of action, greater availability and maintenance of adequate levels for more extended periods, greater use of food [49]. Minerals are one of the most widely used supplements in animal nutrition; However, the form in which these minerals are found influences their bioavailability, so if they have low bioavailability, the animal will not use them correctly. An example is Selenium (Se), an essential trace element with a narrow range of beneficial and toxic effects. Recently, nano selenium has attracted the attention of many researchers due to its high bioavailability and low toxicity. Nonmetric particles exhibit novel characteristics, such as a larger contact surface, high surface activity, high absorption capacity, and low toxicity.

CONCLUSIONS

Biofortification with Se NPs benefits forage grass's morphological and physicochemical characteristics. Se NPs improve root development and synthesis, growth, and grass yield. Applying Se NPs increases the content of crude protein and ether extract.

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Sampling unit and optimal sample size for the detection of *Aeneolamia albofasciata* (Lallemand) eggs in sugarcane

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ABSTRACT

Objective: To compare the efficiency of a 30×30×5 cm iron sampling frame (Frame30) with a smaller 15×15×5 cm one (Frame15), as sampling method for spittlebug eggs [(*Aenolamia albofasciata* (Lallemand)] that reduces the amount of soil removed, optimizes time used, and reduces the sampling effort.

Design/methodology/approach: Mean, variance, coefficient of variation, sampling effort, spatial arrangement, and sample size were determined with both sampling frames. Forty systematic soil samples were obtained using each frame in two plots planted with the variety MEX 69-290, and two others with MEX 91-662. Each soil sample was mixed and homogenized to obtain a subsample of 250 g, from which eggs were extracted by decantation in saline solution.

Results: Both frames estimated different numbers of eggs in the four plots ($\bar{x} \pm s$) (Frame15: 2.71±1.71; 3.49±1.81; 2.74±2.08; 4.44±2.22; Frame30: 4.42±3.58; 6.65±3.92; 4.40±3.45; 7.84±4.54). Significant differences were found between sampling frames (P<0.0001) and between plots (P<0.0001), but not in the plot-sampling frame interaction (P=0.1509). The optimal sample size (accuracy 0.1) was smaller with Frame15 (40, 27, 57 and 25), compared to Frame30 (65, 34, 61 and 34). Both frames estimated a conglomerated spatial arrangement of eggs using three methods.

Limitations on study/implications: This study suggests changing the sampling frame used in Veracruz, Mexico, for a smaller, more efficient one.

Findings/conclusions: Frame15 reduced by 75% the soil removed, provided more accurate population estimates, and simplified field and laboratory management, compared with Frame30.

Keywords: spotted spittlebug, eggs, sampling metal frame, systematic sampling.

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INTRODUCTION

The spotted spittlebug or salivazo [*Aeneolamia albofasciata* (Lallemand)] is one of the pests that most affects sugarcane production (*Saccharum officinarum*) in the Gulf of Mexico region (García-García *et al.*, 2006) and the coastal zone of the Pacific Ocean (Parada-

Domínguez *et al.*, 2019), within an altitudinal strip comprising 10 to 1700 masl (Alatorre-Rosas and Hernández-Rosas, 2015). The nymphs, which secrete a protecting foamy substance (Obando *et al.*, 2013), feed from adventitious roots, main roots, stem and basal bracts (Hernández-Rosas and Figueroa-Rodríguez, 2011), while winged adults suck sage from the underside of the leaves, at the same time that they emit enzymes that block the vascular conducts and provoke linear chlorotic spots, as well as longitudinal necrosis (Badilla, 2002).

The preferred substrate for oviposition of the spotted spittlebug eggs is the soil around the variant of sugarcane, between the roots, at few centimeters of depth (Thompson and León, 2005). The eggs can enter or exit diapause depending on the moisture (Hernández-Rosas and Figueroa-Rodríguez, 2011; Badilla, 2002). This biological stage does not cause harm, but its quantification (León-Hernández *et al.*, 2014; Ramos-Hernández *et al.*, 2018) allows estimating the population density of nymphs and adults (Auad *et al.*, 2011), for which the application of methods for biological control (Bautista-Galvéz and González-Cortés, 2005; Parada-Domínguez *et al.*, 2019), mechanical, trapping, and chemical control (Badilla, 2002; García-García *et al.*, 2006; Ortiz-Laurel *et al.*, 2014) are necessary.

In sugar mills of the central zone of the state of Veracruz, iron frames measuring 30×30×5 cm are used (Frame30) for soil extraction, necessary to count the spittlebug eggs; however, their use requires removing a large volume of soil (4500 cm^3 or 6 kg), which represents greater sampling effort for the operator.

This study compares the number of spotted spittlebug eggs, the optimal sample size, the indicators of spatial distribution, and the sampling effort between soil samples collected with the Frame30 (30×30×5 cm), versus a smaller frame (Frame15, 15×15×5 cm), in sugarcane plots of the varieties MEX 69-290 and MEX 91-662, during three sampling events.

MATERIALS AND METHODS

The soil samples used to count spotted spittlebug eggs were taken from four sugarcane commercial plots located in two municipalities of the state of Veracruz, Mexico: plots 1 (Lat. 19.332350, Long. −96.369933) and 2 (19.338927, −96.353470) in El Salmoral, municipality of La Antigua, grown with the variety MEX 69-290, and plots 3 (19.314149, −96.355744) and 4 (19.305975, −96.368308) in La Víbora, municipality of Paso de Ovejas, grown with the variety MEX 91-662. The physical and chemical characteristics of the soil from the four plots were obtained through the analysis methods indicated in the NOM-021-RECNAT (2001) during the three sampling dates: July 23, August 11, and August 30 in 2012.

A surface of 1.0 ha was selected in each plot, surrounded by an edge of 6 m of crop. To achieve high accuracy (0.1), 40 systematic sampling events were conducted per plot (Villanueva-Jimenez *et al.*, 1993; Southwood and Henderson, 2000). In each sampling point, two sampling frames were used (15×15×5 and 30×30×5 cm), made from laminated iron beams with 3.2×50.8 mm thickness. The frames were buried to soil level, opposite to the variant and on the same furrow.

The analysis was performed in the Plant Health Laboratory of Colegio de Postgraduados, *Campus* Veracruz, where each sample was weighed and homogenized to obtain a subsample of 250 g of soil (± 208.33 cm^3). Next, each subsample was mixed with a saturated NaCl solution (70%) and was left resting for 30 min; then, it was sifted (30, 40 and 60 nets) and washed with running water. The sieve content from 60 nets was mixed inside a separation funnel of 500 mL with saline solution (NaCl, 30%) and left resting for 10 min. The largest particles and the saline solution were kept in the bottom, while the spittlebug eggs floated in the supernatant. The eggs and the content from washes of the decantation funnel performed with a pipette with water were retained in a 7×10 cm organza fabric. The fabric was placed on a paper filter circle inside a Petri dish; 10 mL of distilled water was added to moisten the soil particles dragged with the eggs. The total number of eggs from each subsample was counted, with the help of a Stemi DV4 Carl Zeiss® stereoscopic microscope.

The optimal sample size was calculated with: $\hat{n}=\left(\frac{s}{\bar{x}C}\right)^2$, where $\hat{n}$ is the optimal sample size, s is the standard deviation, $\bar{x}$ is the mean, and C is the accuracy (Karandinos 1976; Southwood and Henderson, 2000).

To determine the spatial disposition, the variance/mean ratio (Ledo *et al.*, 2012), the Morisita index (Ledo *et al.*, 2012), and Lloyd's agglomeration mean index (Lloyd, 1967; Ledo *et al.*, 2012) were used. The variance/mean ratio $=\frac{s^2}{\bar{x}}$; with variance (s^2) and mean ($\bar{x}$), indicates that when the calculated value is >1, the population is aggregated; if = 1, it is random; if < 1, it is ordered. The Morisita index, $I_\delta = n\left[\frac{\sum x_i^2-\sum x_i}{\left(\sum x_i\right)^2-\sum x_i}\right]=\frac{n\bar{x}I_A}{n\bar{x}-1}$, with $\sum x_i$, the sum of the sampling observations, $\sum x_i^2$ sum of the square of the sampling observations, and $\left(\sum x_i\right)^2$ the square of the sum of the sampling observations. If $I_\delta > 0$ indicates aggregation, if = 0 it indicates randomness, and if < 0 it presents ordered dispersion. Lloyd's mean agglomeration index (I_A) was calculated through: $I_A=\frac{\dot{x}}{\bar{x}}$ y $\dot{x}=\bar{x}+\left(\frac{s^2}{\bar{x}}-1\right)$, where $\bar{x}$ is the mean, s^2 is the variance, and $\dot{x}$ is the average of individuals in relation to other individuals. For the relationship between the mean agglomeration index (I_A) and the mean ($\bar{x}$) to be easier to compare, the aggregation percentage was estimated, as the quotient between the index I_A and the mean: $\frac{I_A}{\bar{x}}*100\%$. To compare the efficiency of both sampling frames, the accuracy was estimated through the mean standard error ($S_{\bar{x}}$) and the efficiency with the sampling effort (E_M), defined as: $E_M=S_{\bar{x}}(t)$, where t, is the time invested and $S_{\bar{x}}=\frac{s}{\sqrt{n}}$.

A factorial analysis was carried out through PROC GLM from SAS, to identify the effect of the factors: plot (1, 2, 3 and 4), sampling frame (Frame30 and Frame15), and their interaction. The effect of the sampling dates (July-23, August-11, and August-30, 2012) was analyzed as means repeated in time (PROC MIXED, SAS).

RESULTS AND DISCUSSION

Soil fertility

The soil pH of the plots was alkaline, and differences were found in the clay content, which allows classifying the sampled soil as loamy clay or clay. The content of organic matter (OM) in the soil was rich and it did not present salinity. The macro and micronutrients (N, P, K, Ca, Mg, Fe, Cu, Zn and Mn) were found in adequate levels ($>$1.0 mg kg^{-1}) (Table 1), except for the zinc content (Zn) of 0.90 mg kg^{-1} in plot 1 MEX 69-290, considered as marginal. The soil from the four plots presented good fertility (Salgado-García *et al.*, 2013).

Sample of the optimal sample

The means and variances are lower in Frame15 than in Frame30 (Table 2). Approximately half the number of eggs was collected with Frame15 than with Frame30, but the variance was significantly lower. The samples obtained with Frame15 were more homogeneous. In addition, the sample sizes estimated with a high level of accuracy (0.1) (Villanueva-Jiménez *et al.*, 1993; Southwood and Henderson, 2000) with Frame15 were lower than those calculated with Frame30.

Spatial disposition of spotted spittlebug eggs

The variance/mean ratio and the Morisita index (I_δ) indicate that all the plots present aggregated distribution, except for plot 2, Frame15, variety MEX 69-290, where the variance/mean ratio (0.938$<$1) indicates random distribution. With the Morisita index (0.989$<$1) all the plots presented aggregated distribution. In every case, higher aggregation percentages (I_A) were obtained with Frame15 than those estimated with Frame30, which can indicate that the aggregation of eggs is higher in the 15 cm closer to the stems of the variant than the 30 cm closer to the variant (Table 3).

Effectiveness of the sampling unit in different plots

To compare the accuracy between Frame15 and Frame 30, the standard error ($S_{\bar{x}}$) was estimated, and the unit of effort (E_M) was calculated for efficiency (Table 4).

Table 1. Chemical and physical characteristics of the sol in the sugarcane plots sampled in the trial.

Plot	Variety	pH	Organic matter (%)	Electric conductivity (dS m^{-1})	Total Nitrogen (%)	Phosphorus (mg kg^{-1})	Potassium	Calcium	Magnesium	Iron	Copper	Zinc	Manganese	Texture
							cmol (+) kg^{-1}			mg kg^{-1}				
1	MEX 69-290	8.1	3.9	0.12	0.20	12	0.4	35.2	4.2	16	0.9	0.9	20	Clay loam
2	MEX 69-290	7.6	4.3	0.12	0.22	36	0.5	28.3	5.2	27	2.2	2.2	32	Clay
3	MEX 91-662	8.0	4.5	0.14	0.22	28	0.5	36.2	7.2	32	1.1	1.1	26	Clay
4	MEX 91-662	7.4	3.1	0.11	0.15	46	0.5	22.6	5.6	27	4.8	4.8	32	Clay loam

Table 2. Mean ($\bar{x}$), standard deviation (s) and optimal sample sizes ($\hat{n}$) of *Aeneolamia albofasciata* eggs in sugarcane plots obtained with Frame15 (M_{15}) and Frame30 (M_{30}).

Plot	Variety	$\bar{x}_{F15}$	s_{F15}	$\hat{n}_{F15}$	$\bar{x}_{F30}$	s_{F30}	$\hat{n}_{F30}$
1	MEX 69-290	2.7121	1.7167	40	4.4274	3.5881	65
2	MEX 69-290	3.4994	1.8124	26	6.6557	3.9214	34
3	MEX 91-662	2.7484	2.0836	57	4.4063	3.4517	61
4	MEX 91-662	4.4485	2.2297	25	7.8406	4.5478	33

Table 3. Variance/mean ratio, Mosirita index (I_δ) and Lloyd's mean agglomeration index (I_A) to determine the degree of aggregation of *Aeneolamia albofasciata* eggs in sugarcane plots obtained with Frame15 (M_{15}) and Frame30 (M_{30}).

Plot	Variety	$\frac{s^2}{\bar{x}_{F15}}$	$I_{\delta_{F15}}$	$I_{A_{F15}}$	$\frac{I_{A_{F15}}}{\bar{x}}$ (%)	$\frac{s^2}{\bar{x}_{F30}}$	$I_{\delta_{F30}}$	$I_{A_{F30}}$	$\frac{I_{A_{F30}}}{\bar{x}}$ (%)
1	MEX 69-290	1.086	1.041	1.031	38	2.907	1.439	1.430	32
2	MEX 69-290	0.938	0.989	0.982	28	2.310	1.201	1.196	17
3	MEX 91-662	1.579	1.222	1.210	44	2.703	1.394	1.386	31
4	MEX 91-662	1.117	1.032	1.026	23	2.637	1.212	1.208	15

s^2=variance; $\bar{x}$ =mean; I_A=aggregation index.

The time to obtain 40 samples with Frame30 was 5.89 h, while to obtain the samples with Frame15 it was 2.44 h; in addition, the sampling effort (E_M) obtained with Frame15 was lower than with Frame30 (Table 4). Therefore, the use of Frame15 was more efficient in function of the standard error and the sampling effort. This agrees with what was reported by King (1975), who demonstrated that the use of small samples is as efficient to estimate spittlebug eggs as the samples of larger size.

The plot and the sampling frame

Table 5 presents the separation of Tukey's means for the number of spotted spittlebug eggs, the plots used and the sampling frames. The factorial analysis (PROC GLM, SAS) showed significant differences between the frames ($P<0.0001$) and between the plots ($P<0.0001$), but not between the interaction frames-plot ($P=0.1509$). The differences between plots can be due to local and ecological effects, as well as to differences in

Table 4. Comparison of the effectiveness between Frame15 (M_{15}) and Frame30 (M_{30}) to obtain samples of *Aeneolamia albofasciata* eggs in sugarcane plots, based on the standard error ($S_{\bar{x}}$) and the sampling efficiency (E_M).

Plot	Variety	$S_{\bar{x}F15}$	$E_{M_{F15}}$	$S_{\bar{x}F30}$	$E_{M_{F30}}$
1	MEX 69-290	0.307	0.751	1.877	11.058
2	MEX 69-290	0.361	0.881	2.273	13.389
3	MEX 91-662	0.528	1.289	1.725	10.164
4	MEX 91-662	0.627	1.532	3.112	18.33

management. In the four plots of this study, oviposition happened in slightly alkaline soils and with clay contents higher than 36%, but the differences in the soil are so small in texture, humidity, or pH, that they could hardly be attributed to these factors.

Sampling dates and egg density

The analysis of measurements repeated in time (PROC MIXED, SAS) of the total eggs extracted did not show significant evidence of the effect of the sampling date (P=0.4568) or of the interaction sampling date-sampling frame (P=0.2659), although it reiterates the differences found between sampling frames (P<0.0001) (Table 6).

According to García-García *et al.* (2006), one month after the rainy season begins, a large amount of eggs can be found from the mixture of different generations; that is, those recently oviposited, as well as eggs with different degrees of diapause (Morales and Gallardo, 1996). The samplings were carried out at the end of the months of July and August, during the rainy season, time when the spotted spittlebug population was already established in the entire crop.

These biological characteristics explain the average number of eggs found in 250 g of soil analyzed with both frames (Table 6). Therefore, a greater agglomeration was observed, with lower variance and standard error in the number of spotted spittlebug eggs found in the area defined by the Frame15, as indicated by the aggregated disposition found both by the variance/mean ratio, the Morisita index, and Lloyd's mean conglomeration index.

Table 5. Means separation and Tukey grouping ($p \leq 0.05$) of the variables variety and plot in sampling of *Aeneolamia albofasciata* eggs in sugarcane, with sampling frames Frame15 and Frame30.

Sampling Frame	$\bar{x}$	Plot	$\bar{x}$
Frame15 (A)*	3.3521	4 (MEX 91-662) (A)*	4.4486
		2 (MEX 69-290) (A)	3.4995
		3 (MEX 91-662) (B)	2.7485
		1 (MEX 69-290) (B)	2.7122
Frame30 (B)	5.8325	4 (MEX 91-662) (A)	7.8407
		2 (MEX 69-290) (A)	6.6557
		1 (MEX 69-290) (A)	4.4275
		3 (MEX 91-662) (A)	4.4064

* Different letters in the same column denote statistical significant differences.

Table 6. Mean, standard deviation and Tukey grouping ($p \leq 0.05$) of total *Aeneolamia albofasciata* eggs by sampling frame (Frame15 and Frame30), through time, year 2012.

Sampling Frame	$\bar{x} \pm s$	Date	$\bar{x} \pm s$
Frame15 (A)*	3.3521 ± 2.0774	1 (jul/23) (A)	3.4994 ± 1.8124
		2 (aug/11) (A)	3.3590 ± 1.9255
		3 (aug/30) (A)	3.9327 ± 2.1469
Frame30 (B)	5.8325 ± 4.1374	1 (jul/23) (A)	6.6557 ± 3.9214
		2 (aug/11) (A)	5.5513 ± 4.3801
		3 (aug/30) (A)	5.4653 ± 3.7266

* Different letters in the same column denote statistical significant differences.

CONCLUSIONS

The use of a sampling frame for spotted spittlebug eggs of 15×15×5 cm (Frame15) reduces the volume of soil extracted and decreases the sampling effort. Its use allows obtaining more accurate samples and with lower variation than those obtained with the traditional sampling frame of 30×30×5 cm (Frame30). The estimation of the sampling size was also lower with Frame15. Therefore, the use of Frame30 can be substituted by Frame15 in sugarcane agriculture in Veracruz, Mexico.

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Colegio de Postgraduados

Yield, quality, and phytochemicals of two strawberry cultivars in response to foliar calcium nanofertilization

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ABSTRACT

Objective: to evaluate the foliar application of Calcium (Ca^{2+}) nanofertilizer on the yield, fruit quality, total phytochemicals and capacity of two strawberry cultivars 'Monterrey' and 'Albion' of *Fragaria*×*annanassa* Duch.

Design/Methodology/Approach: three foliar calcium treatments were established (two commercial foliar fertilizers, one nanofertilizer). The doses used were 2.5, 5 and 7 mEq L^{-1} and were applied at the stages of beginning of flowering, full flowering, end of flowering, fruit setting and full production. The design of the experiment was randomized blocks in four replicates, each replicate consisted of a 1 m^2 area, with 16 plants. An analysis of variance was performed and the Tukey's test ($p \leq 0.05$) was applied for comparison of means.

Results: the results indicated that doses of 2.5 mEq L^{-1} for the 'Monterrey' cultivar and 5 mEq L^{-1} for the 'Albion' cultivar favored increases in total phenols, total flavonoids, total anthocyanins, antioxidant capacity and higher yields, by obtaining 13 kg m^{-2} and 12.59 kg m^{-2} from those cultivars, respectively.

Findings/Conclusions: this indicates that the use of nano-calcium as a foliar fertilizer could be a suitable alternative that helps improving the bioactive compounds and yields of strawberry fruits.

Keywords: *Fragaria*×*annanassa* Duch, phenols, flavonoids, anthocyanins, DPPH.

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INTRODUCTION

The strawberry (*Fragaria*×*ananassa*) crop is popular around the world, occurring in China, the United States and Mexico (FAOSTAT, 2022). Mexico ranks third in the world with a cultivated area of 17 400 hectares and a production of 861 337 Megagrams (SIAP-SADER, 2020), produced mainly in the states of Michoacán, Baja California, Baja California Sur, Guanajuato, Jalisco, and Tlaxcala. A successful strategy has been the foliar application of macronutrients and micronutrients to improve nutrition, increasing quality and disease resistance (Zouari *et al*., 2016; de Dios-Delgado *et al*., 2006). However, these practices at present are not particularly successful at improving simultaneously in plants, the uptake, transport, and efficiency in the use of those nutrients (Adnan *et al*., 2020).

The shelf life of strawberries is one of the problems to be solved in this crop and is reported to be directly related to problems from calcium (Ca^{2+}) deficiency. Since this element, being found in low concentrations, affects the thickness of the cell wall, turgor and the interaction in adhesion of pectate lyases between cells (Lanauskas *et al.*, 2006); all of these are characteristics that impact fruit quality. One of the characteristics of Ca^{2+} is immobilization, so a relevant strategy can be presented with technological advances at the nanoscale. Specifically, foliar nanofertilizers that present a targeted and immediate absorption through the stomatal cells in plant tissues (Kopittke *et al.*, 2019).

This is due to the size of its nanostructures (100 nanometers, nm) which facilitate their intake speed and favorably stimulate physiological and biochemical responses (Lira-Saldívar *et al.*, 2018). This macroelement is a secondary messenger and important precursor in the composition of cell walls. Also it is a promoter of the firmness of the fruits because of its role in the formation of structural polysaccharides. Therefore, complementary studies are required to compare the action mode of Ca^{2+} foliar nanofertilizers vs. conventional sprays in organoleptic qualities such as quality and phytochemicals; phenols, flavonoids, anthocyanins, and antioxidant capacity.

MATERIALS AND METHODS

The experiment was established in March 2022, at the Faculty of Agrotechnological Sciences Campus Cuauhtémoc, of the Autonomous University of Chihuahua. The plants of the cultivars 'Monterrey' and 'Albion' had been established for a year in microtunnel conditions. Vegetative materials were placed in beds, which were delimited by a space of 0.80 m between lanes, 12 m long by 1.20 m wide, at a height of 0.40 m. Black plastic mulch was established on the beds and then perforations were made every 0.25 m to carry out the planting. A drip irrigation system was installed, two drip-lines were used per bed between two plant rows. Each drip-line had drippers every 15 cm, with an average flow of 2 L h^{-1}.

The design of the experiment was randomized blocks in four replicates, each replicate consisted of a 1 m^2 area which included 16 plants. The plants of all treatments were fertigated with a "Triple 18" solution at a dose 150 mg L^{-1} in Spring after the onset of vegetative growth, and 220 mg L^{-1} in the flowering stages and full production. Likewise, minor elements such as boron, zinc and copper were also applied at a dose of 40 mg L^{-1}. Foliar sprays consisted of three commercially available calcium preparations, one of them a nanofertilizer (Table 1). Those were applied during early flowering, full flowering, late flowering, fruit set and full production, to improve the quality traits of the fruit. The experiment included three treatments and one control (Table 1). The physicochemical and phytochemical parameters described in Tables 2 and 3, respectively, were evaluated post-harvest.

Data analysis

An analysis of variance was performed to identify significant variables due to treatments. Also, the multiple comparison of means was done with the Tukey's test ($p \leq 0.05$), corresponding to 95% confidence level, using the statistical software Minitab© 19.

Table 1. Description of treatments with its active Calcium ingredient, chemical composition and doses applied.

Treatments	% Active ingredient Ca	Chemical composition and density	Dose
Control	Non calcium	-	-
Calcium oxide: SuperCALCIO®	20% Ca	Mg=0.10 % Carboxylic acid=0.30 % Amino acids=5.00 % B=200 ppm ***Density=1.244 a 1.284 g/mL	2.5, 5.0 y 7 mEq/L
Calcium oxide: CALBIT C®	15% Ca	Cd=≤0.5 mg/ kg Pb=≤5 mg/ kg Hg=≤0.5 mg/ kg As=≤10 mg/ kg ***Density=1.45 g/cc	2.5, 5.0 y 7 mEq/L
Nanofertilizer: PHC® NANO Ca	41% Ca	***Density=1.65 g/cm	2.5, 5.0 y 7 mEq/L

Table 2. Physicochemical quality traits in two strawberry cultivars, '*Monterrey*' and '*Albion*'.

Physicochemical Quality Parameters	Metodología	Unidad de medida
Yield	It was expressed as the sum of the unit weight of the fruits during the production cycle lasting 25 weeks; starting in the month of June and ending in October. Weighing in their entirety the fruits of the 16 plants per 1 m^2.	Kg/m^2
Fruit weight	The number of fruits was calculated by dividing the total weight. The harvested fruits were weighed on a Trupper digital scale (BASE-5EP China mod).	g
Firmness	It was measured using a texture analyzer (Texture Analizer CT3 Brookfield Engineering Laboratories, MA, USA), equipped with a 4 mm conical tip; which compressed the fruit to 8 mm at a speed of 5 mm/s, two perforations were made per fruit and the results were expressed in newtons (N).	N
Hydrogen potential	The juice of 10 strawberries was extracted using a manual juicer. Subsequently, the pH was determined from the mixture of the juice obtained, with an OHAUS potentiometer pH meter (Starter 3100).	pH
Titratable acidity	It was determined based on the citric acid content (%) according to the volumetric method of AOAC 935.57 (1990), in a sample of 5 fruits per treatment, 20 g of pulp were weighed individually, homogenizing with 100 mL of distilled water, subsequently the mixture was filtered and a 5 mL aliquot was taken, which was titrated with 0.1N NaOH, using 2 drops of phenolphthalein in 1% alcohol solution as an indicator.	citric acid (%)
Soluble solids	Soluble solids concentrations were determined in an Abbe refractometer at 20 °C.	°Brix

Table 3. Phytochemical parameters and antioxidant capacity in two strawberry cultivars, '*Monterrey*' and '*Albion*'.

Parameters of phytochemicals and DPPH	Methodology	Units
Total phenols	Five grams of strawberry were homogenized with Ultraturax (Ultra-Turrax, Model Miccra D-9 KT, Digitronic 132 GmbH, Bergheim, Germany). After that, the extract was measured with the Folin - Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using gallic acid as a standard.	Milliequivalents of gallic acid (GAE)/100 g fresh weight
Total flavonoids	The total flavonoid content was measured using a colorimetric assay, according to the method of (Zhishen *et al.*, 1999). Flavonoids were extracted 5% $NaNO_2$ 10% $AlCl_3$ and 1 mol L^{-1} NaOH and measured spectrophotometrically at 510 nm using quercetin as a standard.	Milliequivalents of quercetin (QE)/100 g of fresh weight
Total anthocyanins	The total anthocyanin content was determined using the pH differential method (Lee *et al.*, 2005).	Milliequivalents of Cyanidin-3-glucoside (C3G)/100 g fresh weight
Antioxidant Capacity	Antioxidant capacity was evaluated by radical scavenging activity using DPPH (2-diphenyl-1-picryhydrazyl) as detailed by Velderrain-Rodríguez *et al.* (2018).	Milliequivalents of trolox (TE)/100 g fresh weight

RESULTS AND DISCUSSION

Yield and fruit weight

The PHC® NANO Ca treatment obtained the best yields, the '*Monterrey*' cultivar produced 13 kg m^{-2} at a 2.5 mEq L^{-1} dose, while for the '*Albion*' cultivar the optimal dose was 5 mEq L^{-1} with 12.59 kg m^{-2} (Table 4). Similar data have been reported in other species and it is mentioned that the significant differences among treatments are related to concentration levels, rather than to the type of fertilizer applied. Among those studies, a trial that evaluated apple production showed improvements in the amount of fruits from trees treated with nano-Ca^{2+} 2% (Ranjbar and Ramezanian, 2020). Similarly, applications of calcium and boron resulted favorable in pomegranate fruits, promoting yield increase (El-Salhy *et al.*, 2022).

Table 4. Fruit yield (kg m^{-2}) of two strawberry cultivars ('*Monterrey*' and '*Albion*') under foliar fertilization, with nano-Ca^{2+} and two other conventional commercial products.

	Foliar fertilization treatments with Ca^{2+}					
	superCALCIO®		CALBIT C®		PHC® NANO Ca	
Dose (mEq/L)	*Monterrey*	*Albión*	*Monterrey*	*Albión*	*Monterrey*	*Albión*
2.5	8.78 cd	11.46 ab	8.10 d	10.18 bc	13.00 a	11.79 ab
5	9.55 c	9.84 c	10.55 bc	8.89 c	11.66 ab	12.59 a
7	8.06 bc	8.26 bc	8.56 bc	7.47 c	10.05 a	9.17 ab

* Different letters within columns indicate statistical difference (Tukey, $p \leq 0.05$).

Despite no significant differences were observed in fruit development and weight (Table 5); a higher fruit development was obtained in August with nano-calcium foliar fertilization treatments. The average value of the three doses (2.5, 5 and 7 mEq L^{-1}) evaluated was 24.22 g for the cultivar '*Monterrey*' and 23.96 g for '*Albion*'. This indicates that even though the average number of fruits in the production season (June-October) did not represent statistically significant differences in weight, it did have an impact on overall fruit weight in the month of maximum production (Figure 1).

Physicochemical Quality Traits

Firmness, soluble solids, titratable acidity and pH were all statistically similar, with no differences among treatments and cultivars studied ('Monterrey' and 'Albion') (Table 6). This coincides with studies implemented on pomegranate fruits, where El-Salhy *et al.* (2022) reported that nano-calcium fertilizers, alone or in combination, did not present benefits

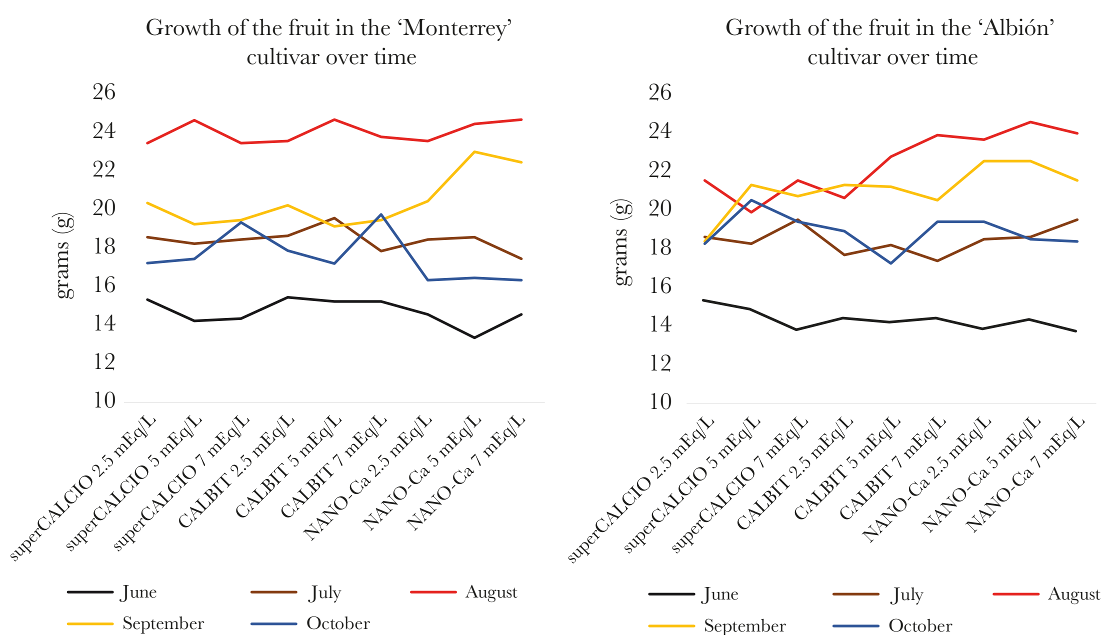

Figure 1. Fruit growth over time in two strawberry (*Fragaria*×*annanassa* Duch) cultivars, '*Monterrey*' and '*Albion*'. Different letters among Ca^{2+} treatments indicate statistical difference (Tukey, $p \leq 0.05$).

Table 5. Fruit weight (g) of two strawberry cultivars '*Monterrey*' and '*Albion*' under foliar fertilization, with nano-Ca^{2+} and two conventional commercial products.

	Foliar fertilization treatments with Ca^{2+}					
	superCALCIO®		**CALBIT C®**		**PHC® NANO Ca**	
Dose (mEq/L)	*Monterrey*	*Albión*	*Monterrey*	*Albión*	*Monterrey*	*Albión*
2.5	18.98 a	18.38 a	19.15 a	18.54 a	18.67 a	19.53 a
5	18.57 a	18.98 a	19.16 a	18.68 a	19.16 a	19.65 a
7	19.00 a	18.94 a	19.21 a	19.07 a	19.09 a	19.37 a

*Different letters within columns indicate statistical difference (Tukey, $p \leq 0.05$).

Table 6. Physicochemical quality traits of two strawberry cultivars ('*Monterrey*' and '*Albion*') fruits, foliarly fertilized with nano-Ca^{2+} and two conventional commercial products.

Foliar fertilization	Variety	Dose (mEq/L)	Firmness (N)	Soluble solids (°brix)	Titratable acidity (citric acid)	pH
superCALCIO®	Monterrey	2.5	4.81 a	10.52 a	1.72 a	3.5 a
		5.0	5.04 a	10.11 a	1.88 a	3.6 a
		7	4.92 a	10.89 a	1.96 a	3.5 a
	Albión	2.5	4.63 a	10.90 a	1.78 a	3.6 a
		5.0	5.13 a	10.02 a	1.92 a	3.4 a
		7	5.06 a	10.14 a	1.77 a	3.5 a
CALBIT C®	Monterrey	2.5	4.88 a	10.08 a	1.66 a	3.5 a
		5.0	4.83 a	10.04 a	1.88 a	3.5 a
		7	5.12 a	10.07 a	1.91 a	3.6 a
	Albión	2.5	4.79 a	10.12 a	1.85 a	3.5 a
		5.0	5.27 a	10.98 a	1.97 a	3.5 a
		7	5.17 a	10.03 a	1.95 a	3.5 a
PHC® NANO Ca	Monterrey	2.5	4.95 a	10.02 a	1.94 a	3.4 a
		5.0	4.76 a	9.96 a	1.77 a	3.4 a
		7	4.83 a	10.74 a	1.93 a	3.4 a
	Albión	2.5	5.12 a	9.87 a	3.98 a	3.5 a
		5.0	5.18 a	10.02 a	3.87 a	3.5 a
		7	4.86 a	10.01 a	3.59 a	3.5 a

in the quality traits of that crop. Which also coincides with Davarpanah *et al.* (2018) who, when studying nano-Ca^{2+} fertilization, reaffirmed that fruit quality was similar in terms of titratable acidity, maturity and total sugars.

Overall, nanotechnology has been useful in improving crop growth, yield and quality; however, Zahedi *et al.* (2020) stated that more research should be done on the effects of nanofertilizers on fruit trees and more cultivars should be studied because the responses are very different among species and cultivars. Although foliar application of Ca^{2+} can be potentially effective in increasing the concentration of Ca^{2+} in fruit, spray of Ca^{2+} has been shown to have low efficiency in many cases. This has been attributed to limitations in Ca^{2+} absorption, intake by the fruit, epidermal characteristics, cuticle presence and composition, and may also be related to low rates of Ca^{2+} allocation through the phloem (Davarpanah *et al.*, 2018).

Transpiration in plant organs tend to accumulate high levels of calcium, which is usually stored within vacuoles or is deposited in leaf trichomes to impose immobility through the phloem, leading to low calcium levels in leaves and fruits (Kumar *et al.*, 2015). The current study showed that applications with calcium nanofertilization had no significant influence on the quality of strawberry fruits, which could lead us to suppose that calcium in vacuoles imposes low mobility through phloem, causing nutritional disorders in fruits. It should be noted that reports in literature still do not show clarity on the effects of nanofertilizers on fruit quality. Other proof of this is reported in a study by Bayat (2016) on pepper fruits where they highlighted that fruits treated with nano-calcium had lower levels of total soluble solids, lower pH and weight loss.

Phytochemical parameters and antioxidant capacity

Calcium is an essential structural, metabolic, and signaling element, which is required for nutritional signaling purposes (Demidchik *et al.*, 2018; Thor, 2019). Therefore, it shows a dual function, as a secondary messenger involved in plant growth and responses to biotic and abiotic stress (Xu *et al.*, 2015; Zhang *et al.*, 2017). This can trigger secondary metabolism responses, synthesizing bioactive compounds such as phenols and their derivatives.

Total phenols

Applications of the Ca^{2+} nanofertilizer increased the content of phenolic compounds in strawberry fruits (Figure 2). The varieties behaved somewhat differently; in the 'Monterrey' cultivar, the treatment of nanofertilizer-Ca^{2+} (PHC® NANO Ca) at a 2.5 mEq L^{-1} dose achieved the highest value of total phenols 302. 84 mg GAE per 100 g of fresh weight. On the other hand, the remaining two treatments with traditional foliar calcium fertilization achieved values of 186. 80 and 196. 23 mg GAE per 100 g of fresh weight for superCALCIUM® and CALBIT C® respectively. In the results for the 'Albion' cultivar, values were increased in the treatment at a 5 mEq L^{-1} dose, which yielded 329. 42 mg GAE per 100 g of fresh weight with PHC® NANO Ca (Figure 2).

It should be noted in the results of this study that for both varieties, the PHC® NANO Ca (nano-Ca^{2+}) treatment was superior. This is mainly due to the effect of the calcium nanofertilizer, which increased phenolic compounds by up to 50%, confirming the positive effect on the phytochemical composition of the fruits. The role of Ca^{2+} in phenol metabolism has been described by Ngadze *et al.* (2014) arguing an increase in the enzyme Phenylalanine Ammonium Liase (PAL), which could be due to a response elicited by a series of transduction signals that result in increased activity of the enzymes Polyphenol Oxidase (PPO) and Peroxidase (POD).

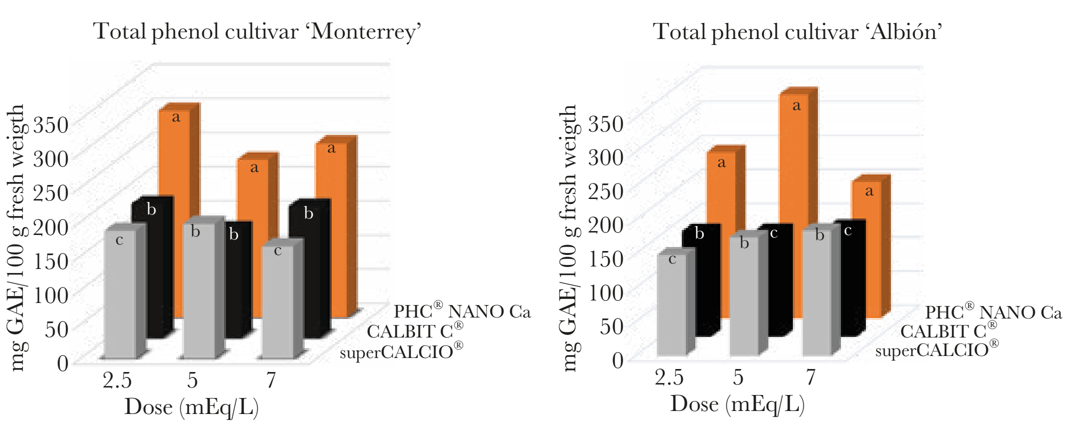

Figure 2. Total phenols in two strawberry (*Fragaria×annanassa* Duch) cultivars '*Monterrey*' and '*Albion*'. Different letters among Ca^{2+} treatments and doses indicate statistical differences (Tukey, $p \leq 0.05$).

Calcium activates PPO and modifies the conformational state of the enzyme, thus enhancing its activity (Ngadze *et al.*, 2014). Results of the current study are in agreement with the findings of Akladious and Mohamed (2018) who demonstrated a direct role of calcium in phenol synthesis. Since the essential role that phenol metabolism plays in many resistance responses to different stresses, rapid and effective manipulation of the metabolic process would improve plant resistance to adverse conditions. It should be noted that in this study, the treatments superCALCIO® and CALBIT C®, which are traditional foliar calcium fertilizers, acted differently on the varieties studied; the three doses evaluated (2.5, 5 and 7 mEq L^{-1}) generated different trends (Figure 2).

Total Flavonoids

In both cultivars, an increase in total flavonoids was generated, standing out the treatment with nano-Ca^{2+}. The 'Monterrey' cultivar with doses of 2.5 mEq L^{-1} obtained values of 30.33 mg QE per 100 g fresh weight, compared to 11.83 and 11.22 mg QE per 100 g fresh weight with the conventional calcium fertilizers superCALCIO® and CALBIT® respectively (Figure 2). Similarly, the 'Albion' cultivar reached 27.36 mg QE per 100 g fresh weight, the highest value with the 2.5 mEq L^{-1} dose. While the superCALCIO® and CALBIT® treatments recorded 11.25 and 12.08 mg QE per 100 g fresh weight respectively (Figure 3).

Flavonoid compounds are among the most important antioxidant substances that initiate a number of secondary metabolites produced by the shikimic acid or malonic acid cycles, which serve as cell signaling agents (Michalak, 2006). The contribution of Ca^{2+} as a cation with multifunctional capacity as a secondary messenger in different responses is evident, antioxidants are free radical scavengers. Therefore, the antioxidant action of flavonoids depends on the availability of free OH groups. Enhanced flavonoid levels with nano-Ca^{2+} doses could be a form of defense (*i.e.*, oxidative loading).

The impact of Ca^{2+} is important according to Ma *et al.* (2019) who stated that the application of Ca^{2+} significantly improved phenol concentrations and enzyme activity used in phenol metabolism (PAL, PPO and POD). In addition, the increase in flavonoid

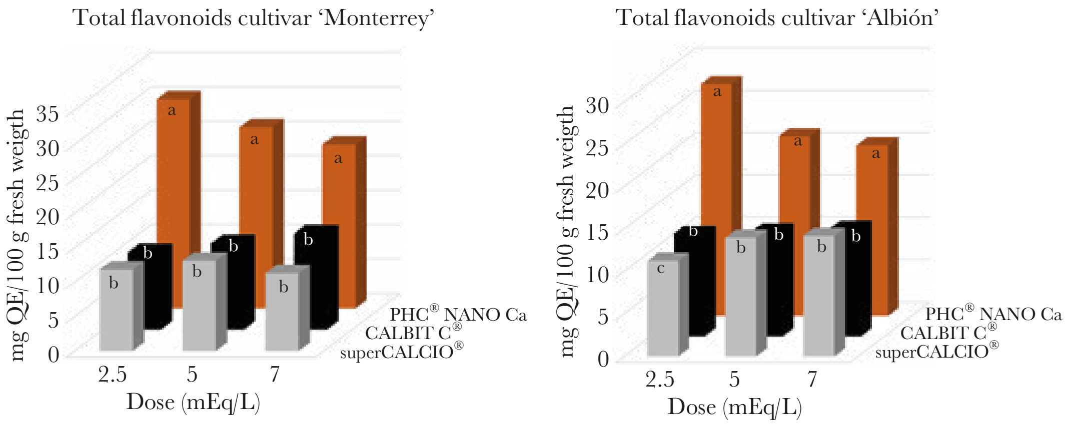

Figure 3. Total flavonoids in '*Monterrey*' and '*Albion*' strawberry cultivars. Different letters among Ca^{2+} treatments and doses indicate statistical differences (Tukey, $p \leq 0.05$).

compounds could be termed as enhanced phenylalanine ammonia-lyase activity, which is crucial for phenol production (Ma *et al.*, 2019). In this regard, foliar sprays of Ca^{2+} on wheat plants showed increases in flavonoid concentrations. Those findings suggest that Ca^{2+} may stimulate the production of secondary metabolites, which function as oxygen scavengers to minimize oxidative stress, thus boosting wheat growth and yield (Sadak *et al.*, 2023).

Total Anthocyanins

In addition to phenolic compounds and flavonoids, foliar fertilization with nano-Ca^{2+} also promoted the accumulation of water-soluble pigments such as anthocyanins. Results showed an average for the three doses of 35.31 mg C3G per 100 g fresh weight in 'Monterrey' and 36.10 mg C3G per 100 g fresh weight in the cultivar 'Albion'. It should be noted that the varieties and doses with the PHC® NANO Ca treatment of nano-Ca^{2+} maintained similarity in their behavior, finding no statistical differences (Table 7). For the rest of the studied treatments of commercial Ca^{2+} fertilization, it was possible to be certain of similar behaviors; these treatments did not affect this studied variable. It is important to note that anthocyanins are one of the main groups of polyphenols in strawberries (40.3% of total phenols) (Nowicka *et al.*, 2019). Hence, their outstanding contribution to the antioxidant properties they confer on these fruits. Therefore, increasing these pigments through foliar fertilization with nano-Ca^{2+} is important.

Table 7. Phytochemical composition of total anthocyanins and antioxidant capacity of strawberry fruits, foliarly fertilized with nano-Ca^{2+} and two conventional commercial products.

Foliar fertilization	Variety	Dose (mEq/L)	Total anthocyanins (mg C3G/100 g^{-1} fresh weight)	Antioxidant capacity (DPPH) (mg TE/100 g^{-1} fresh weight)
superCALCIO®	Monterrey	2.5	27.82 bc	261.70 ef
		5	25.67 bcd	235.03 g
		7	26.41 bcd	247.80 fg
	Albión	2.5	24.95 cd	157.05 i
		5	27.75 bc	165.53 hi
		7	29.09 b	186.51 h
CALBIT C®	Monterrey	2.5	28.00 bc	167.58 hi
		5	23.20 d	177.58 hi
		7	24.39 cd	158.41 i
	Albión	2.5	27.58 bc	176.76 hi
		5	25.98 bcd	187.50 h
		7	23.99 cd	167.02 hi
PHC® NANO Ca	Monterrey	2.5	34.75 a	377.97 a
		5	35.33 a	315.53 b
		7	35.87 a	277.25 de
	Albión	2.5	35.16 a	292.67 cd
		5	35.98 a	306.14 bc
		7	37.17 a	315.92 b

* Different letters within columns indicate statistical differences (Tukey, $p \leq 0.05$).

Results in this study differ from those reported by Zakaria *et al.* (2018) who indicated that strawberry fruits obtained from plants sprayed with Nano-Ca at 15 or 30 mg L^{-1} doses showed the lowest values of anthocyanin concentrations. In this regard, those authors also argued that applications with nano-Ca generated a decrease in color development, thus fruits became less red, and were related to an increase in firmness. In other words, the firmer the fruits, the less red they were.

It should be noted that in this study there was no impact on firmness, which could explain why the results of total anthocyanins are opposite to what was reported by Zakaria *et al.* (2018). Another study implemented by El-Ramady *et al.* (2021) in apple cultivation showed that nano-Ca treatments significantly improved the total anthocyanins content, similar results to which is found in this research.

Antioxidant capacity

Table 7 shows that strawberry fruits foliarly fertilized with nano-Ca^{2+} (PHC® NANO Ca) at doses of 2.5 mEq L^{-1} presented significantly higher values according to the DPPH tests. The lowest values corresponded to the 'Albion' cultivar with the superCALCIUM® treatment. Data ranged from 157.05 to 377.97 mg TE per 100 g fresh weight, which means a 41% increase with nano-Ca^{2+}. In addition, this behavior of antioxidant activity is in agreement with the results of total phenols and total flavonoids in this study; therefore, they are responsible for conferring this activity to strawberry fruits.

CONCLUSIONS

The use of nano fertilizers in agriculture is offering great opportunities to improve plant nutrition, thus increasing yields. Nanomaterials, including nanoparticles, are characterized by rapid absorption and controlled delivery of nutrients to plants. The mechanisms of transport and assimilation are still unknown, although higher yields and an increase in the responses of antioxidant components were found.

Among these, phenols, flavonoids and total anthocyanins with nano-Ca^{2+} foliar fertilization stood out for both cultivars studied. Physicochemical quality traits such as firmness, soluble solids, titratable acidity, and pH did not show differences. They behaved similarly to conventional commercial Calcium foliar fertilizers.

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Milking to 2030: economic and sustainability prospective of the Mexican dairy sector

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ABSTRACT

Objective: This study aims to assess the productive and economic performance of the Mexican milk sector, particularly focusing on small and medium-scale dairy farms, and examining pessimistic, base, and optimistic scenarios.

Design/methodology/approach: Employing a statistical univariate method with time series analysis (ARIMA), we analyzed productive efficiency and price behavior in Mexican dairy systems. Deterministic and stochastic estimations for production volume, milk price, and cattle inventory from 2021 to 2030 were established using confidence intervals to construct pessimistic (lower interval), base (mean), and optimistic (upper interval) scenarios.

Results: The evaluated period witnessed an estimated 10.27% increase in production, equivalent to 576 million liters of milk, with an average annual growth rate of 1.0922%. Milk prices displayed an upward trend, with average prices of $0.66, $0.69, and $0.72 under pessimistic, base, and optimistic scenarios, respectively. In 2030, a 22% price increase compared to 2021 was observed. Considering a base price of $0.45 USD per liter in 2030, costs under pessimistic, base, and optimistic scenarios were $1,658.21, $1,756.43, and $1,855.31, resulting in profits of $1,160.75, $1,229.50, and $1,022.45 from milk sales. Cattle inventory exhibited an upward trend, paralleling milk volumes and prices.

Limitations on study/implications: The study's use of a univariate method may incompletely capture market dynamics complexity, potentially underestimating the impact of external market forces and global economic conditions on milk prices.

Findings/conclusions: To secure forecasted milk volumes in base and optimistic scenarios, maintaining and enhancing good management practices is crucial. Additionally, addressing the imperative to augment production efficiency and improve environmental sustainability and animal welfare is essential.

Keywords: Deterministic and stochastic; Productivity; ARIMA; Livestock.

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INTRODUCTION

In 2016, highlighted the importance of small-scale agricultural and livestock systems in Latin America which contributed with between 50% and 80% of the total consumed products (FAO, 2016). Small-scale farms accounted for 80% of the inventory, generating 30% to 40% of the total Agricultural Gross Domestic Product (GDP). Such activities are based on low input levels and reduced production levels (FAO, 2022). However, with tools such as breeding, genetic improvement, animal health, milking hygiene, nutrition, animal welfare, and an effective socio-economic management, the outlook could be promising.

In Mexico, the dairy cattle population is distributed as follows: 85% belong to semi-intensive household systems, 12% to tropical systems, and 3% to intensive systems (INEGI, 2018; Loera and Banda, 2019).

The main factors that have an impact in milk production and the development of dairy cattle replacements are genetics, environmental conditions, management (Espinoza, 2012), and feeding strategies (Sainz-Ramírez *et al.*, 2021a). Additionally, dairy farms are exposed to a variety of risks stemming from a limited knowledge in disease prevention, management, and control. Such risks could reduce not only milk yields, but also fertility and the longevity of dairy cows. For example, reports worldwide losses from mastitis amounting to more than 40 billion USD per year (Azooz *et al.*, 2020). Since the latter health problem affects the mammary gland, a significant decrease in production is expected with and impact in milk quality, animal welfare, livestock performance, milk disposal, and antimicrobial use in the production unit, which increase operation costs (Neculai-Valeanu and Ariton, 2022). A complex panorama is also expected for dairy systems when considering global factors such as climate change and stricter environmental policies, resulting in a reduction of grains and forages that could affect the capacity to sustain livestock production (Sánchez *et al.*, 2020) and supply chains.

While (OECD/FAO, 2021) indicates that agricultural production will decrease in the next decade compared to the current one (1.4% per year *vs.* 1.7% before), the main increase in agricultural, livestock, and fishery products will stem from low and middle-income countries, driven by an increase in productivity, investment in agricultural infrastructure, research, and development. In this sense, it is essential to identify the elements of the productive and economic structures that could allow the evaluation, within a planning horizon, of the performance and productive efficiency reflected in economic improvements of the involved systems. Therefore, the objective of this study was to determine the productive and economic performance of the milk sector in Mexico, with an emphasis on small and medium-scale dairy farms and considering a pessimistic, base, and optimistic scenarios.

MATERIALS AND METHODS

The study aimed to analyze the productive and economic aspects of the Mexican milk production system from 2022 to 2030, focusing on variables such as production volume, fluid milk price, and dairy cattle inventory. To achieve this, a univariate time series model, specifically Autoregressive Integrated Moving Average (ARIMA), was employed.

The ARIMA models consisted of autoregressive (AR) and moving average (MA) components, integrating an order of differentiation (d) to ensure stationarity (Kirchgassner, 2007). Stationarity, crucial for the Box-Jenkins methodology (Box *et al.*, 2015), was tested using the Dickey-Fuller (D-F) test: H: Yt∼I(1); H1: Yt∼I(0) (Dickey and Fuller, 1979).

The interactive Box-Jenkins method was then applied to estimate deterministic univariate time series (Box *et al.*, 2015).

In detail, the AR component captured the autoregressive behavior of the time series, while the MA component modeled the moving average. The order of differentiation referred to the transformation needed to make the original time series stationary.

Stationary processes, indicating relatively constant mean and variance, were fundamental for developing ARIMA models. The D-F test confirmed stationarity, allowing for trend estimation and model fitting.

The risk simulation process involved determining 12 types of distributions for data, with subsequent fitting of the best-suited distributions for production volume, dairy cattle inventory, and fluid milk price. Normal distributions were employed for the former two, while a geometric distribution was used for the latter.

Forecasted production volume and price series were simulated using normal and geometric distributions, respectively. Regression models were developed, considering factors such as lags and information criteria for model selection.

Economic analysis focused on fluid milk prices for the entire country from 2022 to 2030, utilizing SIACON-NR (2022) data. Risk factors were determined by establishing confidence intervals from the minimum and maximum series values, constructing pessimistic, base, and optimistic scenarios.

Monetary values were expressed in US Dollars ($USD) using an exchange rate of 1 USD: 19.8030 Mexican Pesos (MXN) as of December 7, 2022. The study included small and medium-scale dairy farms, representing 85% of the dairy cattle inventory and embodying the semi-intensive milk production system in Mexico.

The impact of scenarios on income and profit was calculated, considering the average price paid to the producer as gross income and deducting 70% of total costs, as suggested by relevant literature (Martínez-García *et al.*, 2015; Posadas-Domínguez *et al.*, 2014; Posadas-Domínguez *et al.*, 2018).

Additionally, calculations were performed to determine milk volumes per production unit, per cow per year, and per cow per day, assuming a lactation length of 305 days. This comprehensive approach aimed to provide a robust analysis of the economic and production dynamics within the Mexican milk sector.

RESULTS AND DISCUSSION

The deterministic forecast for 2030 milk volume, represented by the model P_t=94,291.30+0.213 $P_{(t-1)}$+0.051 $P_{(t-2)}$, indicated a clear increasing trend. Figure 1 illustrates the behavior of milk production up to 2030, projecting a 10.27% increase from 2021, equivalent to 1,154 million liters. The average annual growth rate was 1.0922%, with volumes for pessimistic, base, and optimistic scenarios at 11,701, 12,394, and 13,091 million liters, respectively.

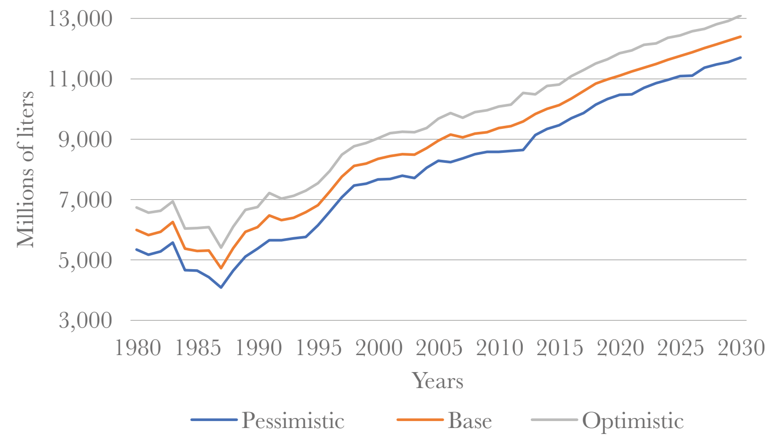

Figure 1. Evolution of the volume of milk produced from 1980 to 2030.

The dairy cattle inventory forecast for 2030, modeled as P_t=27,345.724+0.122 $P_{(t-1)}$+0.164 $P_{(t-2)}$, is depicted in Figure 2. In 2021, the inventory was 2,798,370 cows, producing 3,744; 4,017; and 4,268 liters per cow per year for pessimistic, base, and optimistic scenarios. The estimated inventory in 2030 was 3,142,872 cows, with respective yields of 3,723, 3,944, and 4,166 liters, translating to 12.21, 12.93, and 13.66 liters of milk per cow per day.

The deterministic forecast for 2030 fluid milk price, expressed by P_t=0.082+0.195 $P_{(t-1)}$+0.003 $P_{(t-2)}$+0.230 $P_{(t-3)}$, demonstrated an overall increasing trend (Figure 3). Average prices per liter in pessimistic, base, and optimistic scenarios were \$0.41 USD, \$0.45 USD, and \$0.48 USD, respectively. A forecasted 22% price increase in 2030 compared to 2021 resulted in projected revenues per cow per year of \$1,658.21 USD, \$1,756.43 USD, and \$1,855.31 USD for pessimistic, base, and optimistic scenarios, respectively. Corresponding profit from milk sales and net income varied accordingly.

When considering a base price of \$0.45 USD in 2030, the expected revenues were estimated in \$1,658.21 USD, \$1,756.43 USD, and \$1,855.31 USD per cow per year, under

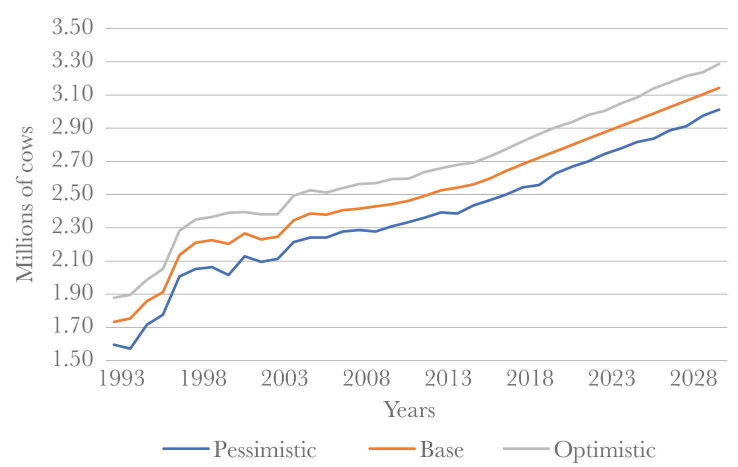

Figure 2. Evolution of the dairy cattle inventory from 1993 to 2030.

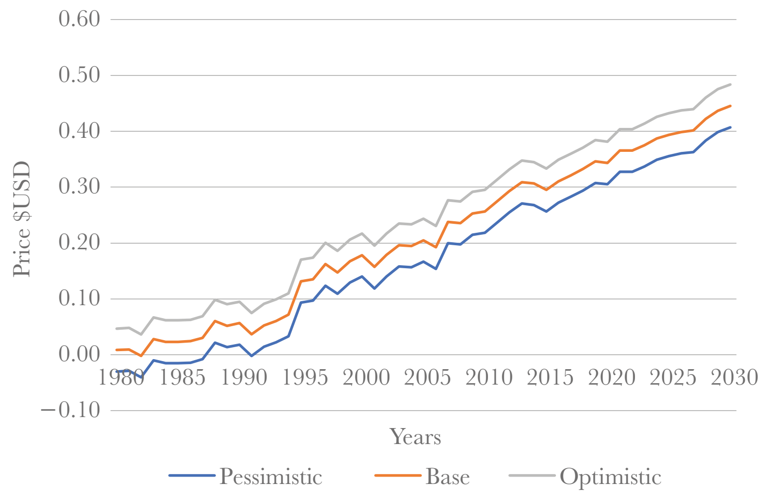

Figure 3. Evolution of price per liter of fluid milk from 1980 to 2030.

the pessimistic, base, and optimistic scenarios, respectively, with a profit from milk sales of $1,160.75 USD, $1,229.50 USD, and $1,298.71 USD, and a net income of $497.46 USD, $526.93 USD, and $556.59 USD, for the respective scenarios.

Milk production in developing regions exhibits significant variability due to diverse infrastructures, technologies, and environmental conditions, with Central America showing lower milk yields per cow compared to North America and Western Europe (FAO/GDP, 2019). While the study projects an increase in production levels in Mexico, aligning with trends in developing countries, this growth is attributed to larger herd sizes rather than improved productivity (OECD/FAO, 2022; Britt *et al.*, 2018). The projected surge in global population emphasizes the importance of enhancing milk production efficiency, considering that dairy farming requires less land for protein production compared to other livestock and agricultural systems.

Dairy farming in Mexico faces challenges like limited access to resources and increasing demand for animal products, operating under diverse environmental conditions (Bennett *et al.*, 2022). A comprehensive evaluation of resource availability is crucial for sustainable dairy production (Bosire *et al.*, 2019). Strategies involving breeding, superior animal selection, and improved farm management are essential for efficiency improvements (Hayes *et al.*, 2013). However, Mexico's current focus on external phenotypic characteristics in cattle selection neglects genetic trend estimations, raising concerns about genetic dependence on foreign material (Domínguez *et al.*, 2003; Toledo *et al.*, 2014).

Small and medium-scale herds in Mexico, predominantly Holstein, may benefit from evaluating alternative genotypes like Jersey for better adaptation to diverse conditions (Larios-Sarabia *et al.*, 2020). However, a declining trend in genetic progress for certain breeds, as identified in previous studies, highlights the importance of restructuring national genetic improvement programs (Boichard *et al.*, 2015; Larios-Sarabia *et al.*, 2020). While global dairy systems move towards specialization and mechanization, this study emphasizes the need to balance resilience and efficiency, considering climatic factors (Cole *et al.*, 2020).

Nutritional management significantly influences milk production, with efficient practices and technologies improving productivity (Hazard, 2004). However, grazing systems, predominant in small-scale herds, pose challenges in effective health control and technology incorporation (Sainz-Ramírez *et al.*, 2021b; Moscovici *et al.*, 2021). Addressing these challenges, along with focusing on environmental sustainability and efficient resource use, is essential for the future of dairy farming in Mexico (Medeiros *et al.*, 2022).

The discussion emphasizes the role of genetics, feeding practices, and management improvements in addressing the uncertainties faced by dairy farmers, such as low milk prices and restricted access to resources (Maltz, 2020). Despite the trend of larger, more efficient farms globally, this phenomenon doesn't necessarily apply to small-scale Mexican dairy farms (Posadas-Domínguez *et al.*, 2014).

Strategies to address challenges include incorporating advancements in breeding, nutrition, and management practices, focusing on disease prevention and improving calf raising facilities (Roland *et al.*, 2016). While milk quality regulations exist, further enhancements in chemical components through feeding strategies must be balanced with economic considerations (Sainz-Ramírez *et al.*, 2021a; Posadas-Domínguez *et al.*, 2018).

The discussion delves into the complexities of the dairy sector in Mexico, considering factors like trade liberalization, price volatility, and environmental impact (Ángeles *et al.*, 2004). The cyclical nature of milk production and its correlation with variables like feed prices and world trade variations highlight challenges for small-scale dairy farms in a globalized market (Navarrete-Molina *et al.*, 2019).

Addressing water scarcity emerges as a critical factor for sustainable dairy farming, suggesting strategies like raising replacements and growing feed inputs in less arid regions (FAO, 2022). The impact of urban growth on rural areas and migration trends among younger generations underscores the need for comprehensive strategies that involve the small and medium-scale dairy sector in shaping the future (Bullock and Crane, 2021; Moreno-Ramos, 2021; Ruiz-Torres *et al.*, 2017).

In conclusion, the projected upward trend in production volume and estimated prices for the semi-intensive milk production system in Mexico necessitates a focus on efficiency improvements, sustainable practices, and strategic planning for the challenges posed by global trends and local conditions. The citations provide a robust foundation for the discussed insights

CONCLUSIONS

The applied methodology estimates milk volume, price, and dairy cattle inventory behavior until 2030, utilizing historical data. Ensuring forecasted milk volumes requires maintaining good practices, enhancing production efficiency, and improving sustainability and animal welfare. Strategies such as implementing milk quality-based pricing, exploring flexible financing for producers, and state investments can mitigate price risks. While reducing dairy cattle inventory seems risky, this study suggests it's feasible in an optimistic scenario without compromising production volumes, challenging conventional strategies in Mexico's fluid milk deficit.

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Colegio de Postgraduados

Bioremediation alternatives for total petroleum hydrocarbon removal in agricultural soil

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ABSTRACT

Objective: The purpose of the present study is to highlight the importance of assessing bioremediation and total petroleum hydrocarbon removal by bioaugmentation and biostimulation on the rhizosphere.

Design/methodology/approach: An 89-day experiment was established with treatments considering plant (corn) establishment crude petroleum (25,000 mg kg^{-1}) bacteria and hydrocarbonoclastic fungi adding nitrogen and phosphorus to agricultural soil. At the end of the experiment, hydrocarbonoclastic fungal and bacterial populations and total petroleum hydrocarbon removal were assessed.

Results: Both microbial groups increased in number and time. The treatment with 120 kg nitrogen ha^{-1} and 12.5 kg phosphorus ha^{-1} allowed the highest population (227×10^3 g^{-1} of colony forming units (CFU) of hydrocarbonoclastic bacteria). A total of 83% petroleum hydrocarbon removal was obtained as established in 89 days.

Limitations on study/implications: The effectiveness of bioremediation can vary significantly in real environments due to factors, such as soil variability, climate.

Findings/conclusions: The previous results highlight the importance of using these bioremediation techniques to eliminate hydrocarbons in contaminated agricultural soils.

Keywords: Environmental impact, contaminated soils, biodegradation, microorganisms, nutrients.

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INTRODUCTION

At global level, the growth of the petroleum industry involves a constant increase in activities, such as exploration, exploitation, refinery, transport, and consumption of the products derived from petroleum (Meyer, 2023; Ortuño, 2021). However, petroleum products generate environmental impact, such as soil, water, and air global warming, causing serious public health besides economic and social problems (Martins *et al.*, 2019).

In many affected sites, the population and natural resources are exposed to continuous risks (Okonofua *et al.*, 2023; Ukhurebor *et al.*, 2021). Agricultural soils contaminated by hydrocarbons show problems in quality and cultivation production, which are due to the loss of beneficial microflora, as well as to the decrease of air and water availability and contaminant toxicity among other factors (Devi *et al.*, 2022).

The alternatives proposed for cleaning the contaminated sites are bioremediation technologies, which by means of the microbial activity convert the contaminants into innocuous compounds, such as carbon dioxide and water Bioremediation implies soil and water decontamination by the interaction of microorganism-soil/water-contaminant (Bhat & Hakeem, 2020; Singh *et al.*, 2020). The biodegradation speed of diverse soil hydrocarbons depends on factors, such as texture, structure, temperature, pH, oxide-reduction potential, oxygen, nutrients, organic matter, salinity, water content, quantity and bioavailability of the contaminants (Cambarieri *et al.*, 2021).

When agricultural hydrocarbon contaminated soils contain few nutrients, the biodegradation processes are significantly reduced because the plants require such elements for their development, above all for their radical system growth, the habitat where microorganisms transform the contaminants (Panwar, R., & Mathur, 2023). Therefore, incorporating essential nutrients in soil is necessary (Martínez *et al.*, 2019). Given the crucial importance of these topics in the framework of the present research, two bioremediation alternatives (bioaugmentation and biostimulation) were carried out to determine the total petroleum hydrocarbon (TPH) removal in agricultural soil with the future goal of restoring their functions in the ecosystem.

It is hypothesized that bioaugmentation and biostimulation will be effective in removing total petroleum hydrocarbons in an agricultural soil and that both alternatives will lead to a significant improvement in soil remediation.

MATERIALS AND METHODS

For the experiment, Mayan crude petroleum was used provided by the Mexican Petroleum Institute (IMP, Instituto Mexicano del Petróleo), characterized as heavy (21° to 22° API, American Petroleum Institute, U.S.A.), sour and with sulfur content (3.4-3.8% in weight) according to the scheme in accordance with the security data page (PEMEX, 2023) and the Mexican regulation NOM-018-STPS-2015. Petroleum is viscous liquid, black, water insoluble, with a boiling point from 71 to 538 °C, flammable, vapor pressure 6.0 lb/plg^2 and a relative density of 0.9269 15.5°C.

Preparation of fungal and hydrocarbonoclastic bacterial inoculum

Two types of inoculums were prepared: one composed of hydrocarbonoclastic bacteria (HC) *Agrobacterium radiobacter* and *Pseudomonas* sp. and the other one by fungus genera *Trichoderma* sp., *Aspergillus* sp. and *Mucor* sp. Both inocula were cultivated in six-250 mL assay flasks of which three of them contained 50 mL of combined Rennie (1981) mineral carbon culture medium, modified by Hernández-Acosta *et al.*, (2003) for HC bacterial growth. The other three flasks used Eggins and Punhg (Leander and Curl, 1972) for fungi. Besides the culture media, 2 mL of petroleum were added to each flask, so the microorganisms used them as a carbon source.

For the HC bacteria, an incubation time of 22 h was considered and eight days for fungi. Subsequently, the culture medium in the flasks were collected to be used as inoculum in the experiment under greenhouse conditions. The inoculum from fungi and bacteria were quantified in colony forming units (CFU) mL^{-1}.

Establishment of the greenhouse experiment

The sterile agricultural soil used previously determined the following properties: texture, organic material, pH, total nitrogen (N), phosphorus (P), potassium (K), and the cationic exchange by the methods established in the Mexican norm NOM-021-SEMARNAT-2000 (Semarnat, 2002). Petroleum (25,000 mg kg^{-1}) was homogenized previously to be transferred into 940-mL amber glass flasks. Each flask had a quantity of 700 g of crude soil petroleum.

On soil petroleum was used as a reactive degree source of nitrogen to ammonium sulfate [$(NH_4)_2SO_4$] in dosage of 0, 120, 240, and 360 kg N ha^{-1}. The criterium for using these doses was due to the result of the fertility analysis indicating poor N content. Thus, a low dosage was considered and a higher one than that recommended since the plant has the disadvantage of developing adequately due to the contaminated soil. Creole corn (MV06) was used as plant indicator. With respect to P, only one dosage (12.5 kg of P ha^{-1}) of reactive degree monobasic potassium phosphate (KH_2PO_4) was used to cover a minimum demand since corn cultivation requires reduced applications of this nutrient.

Subsequently, two corn seeds were placed at the center of each flask; 5 mL of the HC bacterial inoculum were applied with a charge of 5.0×10^{10} CFU mL^{-1} and 4.0×10^{8} CFU mL^{-1} were used for the HC fungal inoculum. The experimental units within the greenhouse were maintained at 50% humidity with distilled water, the first soil sampling was performed at 65 days, the second one at 79 days, and the last one at 89 days. Sampling intervals were considered to observe significant changes in the soil and soil plant interaction during the growth cycle of corn, allowing for the evaluation of bioremediation techniques.

The experiment was established under a complete randomized design with a factorial arrangement of 4×3 with 4 replicates where the factors were the treatments and incubation days: T1: Plant+25,000 mg kg^{-1} of petroleum+HC bacteria+HC fungi+0 kg N ha^{-1}+12.5 kg P ha^{-1}; T2. Plant+25,000 mg kg^{-1} of petroleum+HC bacteria+HC fungi+120 kg N ha^{-1}+12.5 kg P ha^{-1}; T3: Plant+25,000 mg kg^{-1} of petroleum+HC bacteria+HC fungi+240 kg N ha^{-1}+12.5 kg P ha^{-1}; T4: Plant+25,000 mg kg^{-1} of petroleum+HC bacteria+HC fungi+360 kg N ha^{-1}+12.5 kg P ha^{-1}.

In each sampling, the CFU of the fungal and bacterial HC were determined according to Hernández-Acosta *et al.* (2003), as well as the TPH elimination percentage according to the United States Environmental Protection Agency (EPA) 418.1 method and quantified by infrared spectrophotometry modified by EPA (1986) method.

Data analysis

Normality tests (Shapiro Wilks) and homogeneity of variance (Levene's test) were applied to the data obtained. Once the assumptions were met ($p\geq0,05$), an analysis of variance and multiple comparison of means (ANOVA) was performed by Tukey's method ($p\leq0.05$). To find significant differences among the treatments, a statistical model was used:

$$Y_{ijk}=\mu+T_i+Dj+T_iD_j+\varepsilon_{ijk}$$

Where: Y_{ijk}=obtained response; μ=general media; T_i=treatment i effect; D_j=effect by days; D_jT_i=interaction; ε_{ijk}=observation associated randomized error Y_{ijk}. All the previous was made using InfoStat statistical software, free version (Di Rienzo *et al*., 2017).

RESULTS AND DISCUSSION

The chemical and physical analyses performed on the soil used in the experiment and its comparison with the reference values established in NOM-021-SEMARNAT-2000 showed the following results: sandy loam texture, moderately alkaline pH (7.8), extremely poor organic matter percentage (0.459), poor total N (0.040%) content, high phosphorus concentration (11.04 mg kg^{-1}), low potassium concentration (0.340 Cmol (+) kg^{-1}) and cationic exchange capacity (9.15 Cmol (+) kg^{-1}).

After performing the statistical analyses, significant differences ($p<0.05$) were observed per treatment, days and interaction (Table 1). By analyzing the results per treatments, T1 did not show significant ($p>0.05$) differences in the bacterial CFU per day. The HC bacterial CFU were significantly greater ($p<0.05$) in soil with T2 (120 kg N ha^{-1} and 12.5 kg P ha^{-1}) at 89 days, which also occurred with the fungal CFU. T3 and T4 showed greater bacterial population at 89 days, but not in fungi whose greater populations showed at 79 days.

Table 1. Hydrocarbonoclastic populations and total petroleum hydrocarbon removal at 65, 79, and 89 days.

Treatments	Days	Bacteria HC 1×10^3 CFU g^{-1} soil	Fungi HC 1×10^5 CFU g^{-1} soil	Concentration/Elimination TPH	
				(mg kg^{-1})	(%)*
T1	65	155 a	14 a	15,470 a	38
	79	127 a	5 c	13,875 b	44
	89	147 a	13 b	11,239 c	55
T2	65	93 b	9 b	12,638 a	49
	79	85 c	7 c	12,417 b	50
	89	227 a	14 a	10,442 c	58
T3	65	156 b	18 b	14,625 a	41
	79	84 c	9 c	12,667 b	49
	89	188 a	24 a	5,756 c	77
T4	65	72 c	6 c	9,650 b	61
	79	80 b	35 a	11,833 a	53
	89	104 a	17 b	4,334 c	83
Factors					
Treatments		0.0001	0.0001	0.0001	
Days		0.0001	0.0001	0.0001	
Treatment×Days		0.0001	0.0001	0.0001	

Different letters in the column per treatment indicate that a statistically significant difference exists. Tukey's test for multiple comparison of means ($p<0.05$). * Percentage with respect to the initial petroleum concentration. TPH: total petroleum hydrocarbons, HC: Hydrocarboboclast.

Hydrocarbon degrading microorganisms have different capacities in the contaminated ecosystems to reduce the potential of the dangerous chemicals released in the environment (Domínguez-Sánchez *et al.*, 2018). In general, the bacterial and fungal HC populations in soil increase significantly in time. As observed, this microbial behavior is defined as adaptation or acclimation when the microorganisms adapt to the contaminated medium characteristics, which could trigger their populations, and species selection that degrade the contaminants to which they are exposed, besides adding their microbial composition and physiological capacity to achieve success in bioremediation (Brzeszcz *et al.*, 2023; Atlas & Bartha, 2002).

It should be noted that the treatments where inorganic sources were applied and the corn plant was present, greater hydrocarbonoclastic bacterial and fungal populations were observed when compared to the treatment without fertilizer.

In this respect, Rivera *et al.* (2018) mentioned that in biostimulation in hydrocarbon contaminated soils, nutrient application allows increasing the native microbial activity and new cells. In respect to the plant presence, Shah y Daverey (2020), contaminant degradation on soil suggest increases in the rhizosphere area due to the existing synergism between the microorganisms and the area (Hernández-Valencia *et al.*, 2017). In the present research, the rhizosphere biostimulation allowed the microbial populations to act with greater efficiency in degrading petroleum.

The TPH removal was greater in T4 (360 kg N ha^{-1} and 12.5 kg P ha^{-1}) at 89 days, observing that as the N dosage increased and time passed by, the hydrocarbon concentration decreased (Table 1). The previous result confirmed that the corn rhizosphere biostimulation increases HC bacterial and fungal populations. In this respect, Rodríguez-Gonzales *et al.* (2022) mentioned that applying nutrients as N and P in a contaminated site is important to favor microorganism growth, which are in charge of biodegrading and help in plant growth and development.

On the other hand, Hernández-Valencia *et al.* (2019) investigated biostimulation with nitrogen, phosphorus, detergent, microorganisms and worm compost to mineralize automotive waste oil in soil. After 130 days, the soil had 1,532 mg kg^{-1} of the contaminant, with 98% removal. In an agricultural soil contaminated with motor oil, Juárez-Cisneros *et al.* (2023) applied biostimulation with fungal extract and green manure, reducing the hydrocarbon concentration from 34,500 to 2,066 ppm in 60 days. Subsequently, by phytoremediation, they achieved a final concentration of 86.9 ppm in 120 days.

In contrast to previous work, this research focused on the use of nitrogen and phosphorus as the primary nutrients in an 89 days experiment. Despite these more simplified conditions, a contaminant removal rate of 83% was achieved, which represented considerable savings in time and money.

This study reveals important benefits for the soil, thus contributing to its restoration and health. The high percentage of TPH removal suggests that the addition of hydrocarbonoclast microorganisms, which use hydrocarbons as a carbon source to break down oil molecules, together with the incorporation of nutrients as an energy source, can promote more efficient degradation of hydrocarbons. This strategy, supported by previous studies such as Atlas & Bartha (2002) suggests that microbial biodegradation can be an effective tool to mitigate soil contamination.

CONCLUSIONS

The combined application of bioaugmentation and biostimulation on the corn rhizosphere was efficient in agricultural soil remediation contaminated by removing up to 83% of total petroleum hydrocarbon in a period of 89 days. With these bioremediation alternatives, some soil functions within the ecosystem, such as the increase in microbial populations, soil fertility improvement has a bearing in plant growth development, as Poaceae or Gramineae.

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Scientific research on exotic and native mollusk farming in Mexico according to SCOPUS

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ABSTRACT
Objective: The objective of this study was to evaluate scientific productivity in this field through the database deposited in SCOPUS for Mexico.
Design/methodology/approach: The genera and species of mollusks, currently cultivated or with cultivation potential, marine and freshwater, native and exotic, that have been studied are identified. The SCOPUS search was performed using the scientific name of the corresponding species in the publication title. The number of publications, the institutions that generated them, the SCOPUS theme of the study and the research funders were obtained.
Results: There is an uneven growth, with a greater focus on abalone and octopus, while the genus *Crassostrea*, particularly the species *C. gigas*, leads the production of scientific articles. CIBNOR and IPN are the most relevant institutions in scientific production in molluscan aquaculture in Mexico.
Limitations on study/implications: Despite the interest, advances in areas such as physiology, nutrition and reproduction have not been translated into efficient culture technologies in most cases.
Findings/conclusions: These findings highlight the need to promote research and technological development in the aquaculture of native mollusks in Mexico, as well as to promote collaboration between academic research institutions and the production sector to overcome the challenges in the culture of these species.

Key words: *Crassostrea*, *Magallana gigas*, cultivation, scientometry, production.

INTRODUCTION

Aquaculture plays an important role in food security, as it increases food availability by providing a reliable source of protein and other essential nutrients for human consumption (Hosomi *et al.*, 2012, Garlok *et al.*, 2020, FAO 2022). In Mexico, aquaculture has presented

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a remarkable growth in recent decades, this practice has generated a significant impact on various social aspects of the country, opening a wide range of opportunities and challenges (Sosa-Villalobos *et al.*, 2016, SAGARPA 2017).

Mollusk production is a sector of great importance for Mexico, as it is part of the economic development of the communities that are dedicated to their exploitation, either through cultivation or artisanal fishing, positioning this group of species as potential candidates for technological development (Sarkis, 2008; CONAPESCA, 2012; FAO, 2022). Research in mollusc farming is essential in Mexico to ensure food security, boost sustainable economic development, conserve the marine environment and promote adaptation to climate change (Maeda-Martínez, 2008, Cáceres-Martínez and Vásquez-Yeomans, 2014). In addition, by investing in research and development in this field, the country can strengthen its position in aquaculture nationally and internationally.

Mollusks are a good source of nutrients, such as high biological value proteins, amino acids, essential minerals, and vitamins (Astorga-Espana *et al.*, 2007; Kehinde *et al.*, 2015; Cheong *et al.*, 2017). Bivalve mollusks are considered as a food low in saturated fat and high in essential amino acids and fatty acids, such as omega-3, EPA and DHA (Valenzuela *et al.*, 2011; Joy and Chakraborty, 2017). Consuming mollusks regularly can improve immune response and help avoid some physical ailments, this because of their content of vital nutrients and active secondary metabolites (Benkendorff, 2010). Another importance of mollusk production is the development of other markets such as pearl and nacre shell production (Serna-Gallo *et al.*, 2014); and the potential in environmental services, due to their ability to regulate marine nutrient flows, filter pollutants in the water and their function as marine indicators (Galimany *et al.*, 2017; Smaal *et al.*, 2019).

Globally, the group of mollusks (phylum: Mollusca, Linnaeus 1758) represents 10% of the total marine capture fisheries; Mexico is among the main fishery producers (FAO, 2022). In terms of world production of mollusks by aquaculture, despite the COVID health contingency, a total of 17.7 million tons was recorded, which in monetary terms represents US$29.8 billion; however, Mexico is not among the main producers of these resources (FAO, 2022). Despite this, more than 54 species of mollusks are exploited in the Mexican Pacific alone (Baqueiro, 1984; Maeda-Martínez, 2008).

The universities and public research centers of CONAHCYT have dedicated a great effort in economic and human resources to carry out research and technological development on mollusks with economic importance, with the objective of laying the foundations for their cultivation in Mexican coastlines (Chong-Carrillo *et al.* 2023). Much of the research carried out focuses on native species; however, there are exotic species whose cultivation has spread rapidly by adapting imported technologies (Chong-Carrillo *et al.*, 2023). Even so, there have been no studies that address the scientific production, understood as articles published in specialized journals, generated with native or exotic mollusks in Mexico. In the present work, the mollusk species currently under cultivation were identified, as well as those with potential for cultivation, with the objective of evaluating the behavior of scientific productivity in Mexico deposited in the SCOPUS database.

MATERIALS AND METHODS

To develop the study, the genera, and species of mollusks, currently cultivated or with potential for cultivation, marine and freshwater, native and exotic, of which studies have been carried out that have resulted in scientific publications, were identified. The SCOPUS database was used to obtain this information. The SCOPUS search was performed using only the scientific name of the corresponding species in the title of the publication. This variable alone was used to obtain the number of publications, the institutions that generated them, the SCOPUS subject of the study and the funders of the research. Only Mexican institutions were included, both in the institutions of affiliation and in those that financed the projects that generated the published articles. The data obtained were captured in Excel® spreadsheets for their management and creation of graphs. Likewise, a review of the lines of research involved was made based on the titles of the publications. In the general analysis, the results obtained for all the species previously identified were included. The data on the number of publications over the years were subjected to simple linear regression analysis to evaluate if there is a pattern of increase or decrease, this included the genera *Haliotis*, *Octopus*, *Panopea*, *Nodipecten*, *Argopecten*, *Mytilus*, *Atrina*, *Pomacea* and *Anadara*. Data were analyzed with SigmaPlot 11.0 software.

RESULTS AND DISCUSSION

The mollusk species already cultivated or with potential for cultivation were identified based on research projects developed by several public research centers and from direct information provided by researchers and academics from academic and scientific institutions. All species considered in this study are native except *Crassostrea gigas*, *Crassostrea sikamea* and *Mytilus galloprovincialis*.

Anadara tuberculosa (mangrove cockle)

Anadara tuberculosa is distributed in the mudflats of the mangrove areas of the Pacific coast of tropical America between Baja California and the northern coast of Peru (Lucero-Rincón *et al.*, 2021). Despite being a very popular clam for consumption in Mexico, the research that has been carried out, with a view to its cultivation, is poor. Only twelve articles were detected in SCOPUS, involving Mexican institutions. Figure 1 shows that the interest in developing studies with this species, mainly covers the period from 2008 to 2019, to later disappear.

The Mexican institutions that are most mentioned in the articles are shown in Figure 2. The Centro de Investigaciones Biológicas del Noroeste (CIBNOR), the Instituto Nacional de la Pesca (INAPESCA) and the Centro Interdisciplinario de Ciencias Marinas (CICIMAR) are the research institutions with the highest number of mentions. In relation to the topics covered by the publications, although it is a clam with potential for cultivation in Mexico, most of these are on aspects of population ecology, potential of its filtering capacity to remove effluents and obtaining probiotics. There are no studies on aspects of captive breeding or culture. The only entity that has financed the projects

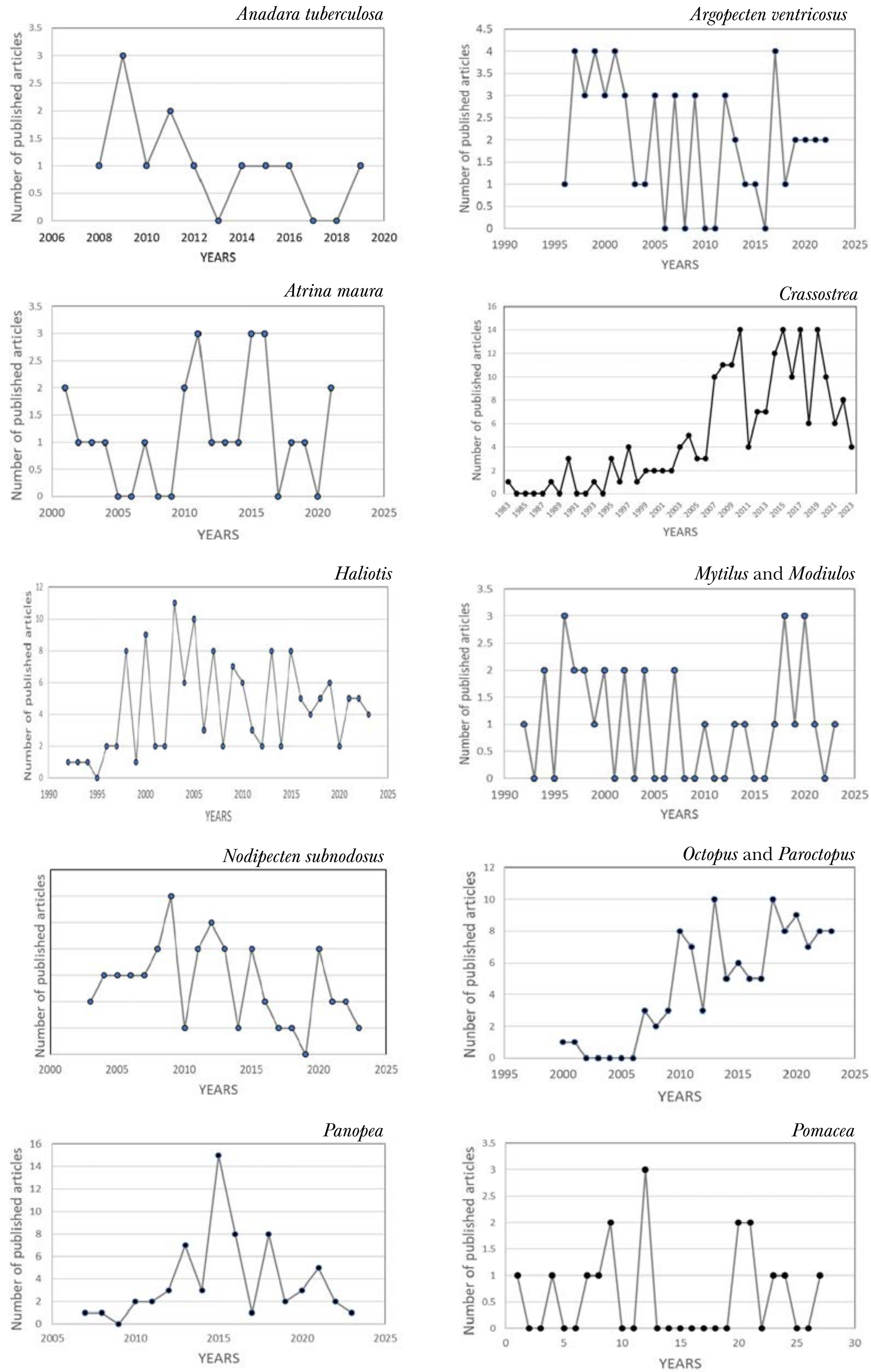

Figure 1. Timelines of the publication of articles on mollusks by Mexican institutions according to SCOPUS.

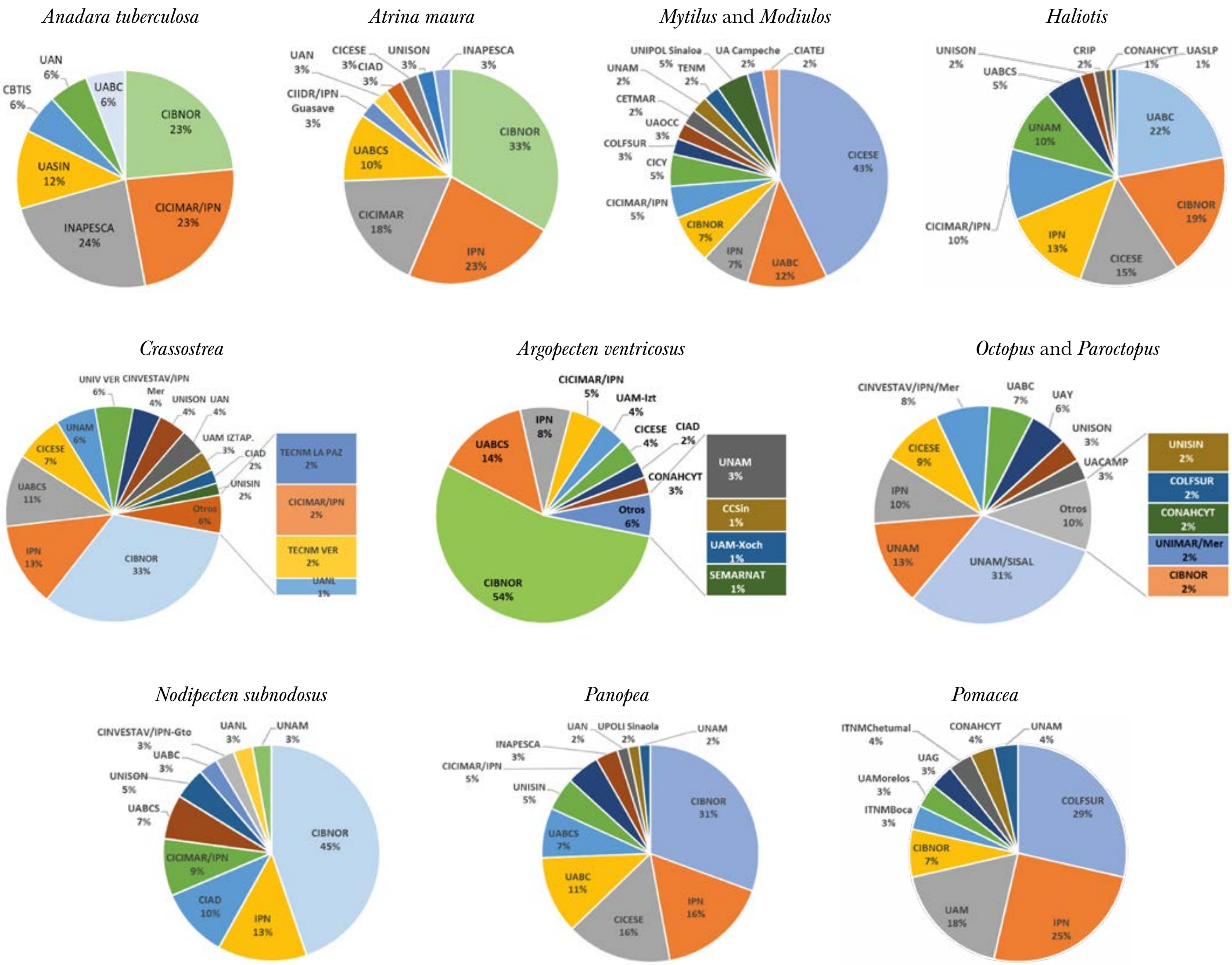

Figure 2. Mexican institutions generating articles on mollusks (in percentage). Nomenclature of institutions in alphabetical order: CBTIS=Bachillerato Tecnológico Industrial y de Servicios, CETMAR=Centro de Estudios Tecnológicos del Mar, CIAD=Centro de Investigación en Alimentación y Desarrollo, CIATEJ=Centro de Investigación y Asistencia en Tecnología del Estado de Jalisco, CIBNOR =Centro de Investigaciones Biológicas del Noroeste, CCSin=Centro de Ciencias de Sinaloa, CICESE=Centro de Investigación Científica y de Educación Superior de Ensenada, CICIMAR/IPN=Centro Interdisciplinario de Ciencias del Mar del Instituto Politécnico Nacional, CICY=Centro de Investigación Científica de Yucatán, CIIDIR/IPN Guasave=Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional unidad Guasave, CINVESTAV/IPN=Centro de Investigación y de Estudios Avanzados del IPN, COLFSUR=Colegio de la Frontera Sur, CONAHCYT=Consejo Nacional de Humanidades, Ciencia y Tecnología, CRIP=Centro Regional de Investigación Pesquera, INAPESCA=Instituto Nacional de Pesca y Acuicultura, IPN=Instituto Politécnico Nacional, ITNMBoca=Instituto Tecnológico Nacional de México Boca del Río, ITNM Chetumal=Instituto Tecnológico Nacional de México campus Chetumal, TECNM=Tecnológico Nacional de México, TECNM La Paz=Tecnológico Nacional de México La Paz, TECNM VER=Tecnológico Nacional de México Veracruz, UANL=Universidad Autónoma de Nuevo León, UABC=Universidad Autónoma de Baja California, UABCS=Universidad Autónoma de Baja California Sur, UA Campeche=Universidad Autónoma de Campeche, UAG=Universidad Autónoma de Guadalajara, UAM Izt=Universidad Autónoma Metropolitana Iztapalapa, UAM Xoch=Universidad Autónoma Metropolitana Xochimilco, UAMorelos=Universidad Autónoma de Morelos, UAN=Universidad Autónoma de Nayarit, UANL=Universidad Autónoma de Nuevo León, UAOCC=Universidad Autónoma de Occidente, UASIN=Universidad Autónoma de Sinaloa, UASLP=Universidad Autónoma de San Luis Potosí, UAY=Universidad Autónoma de Yucatán, UNIMAR=Universidad del Mar, UNAM=Universidad Nacional Autónoma de México, UNAM/SISAL=Universidad Nacional Autónoma de México unidad SISAL, UNIPOL Sinaloa=Universidad Politécnica de Sinaloa, UNISON=Universidad de Sonora, UNIV VER=Universidad Veracruzana, SEMARNAT=Secretaria de medio ambiente y recursos naturales.

that resulted in these publications was the National Council of Humanities, Science and Technology of Mexico (CONAHCYT).

***Argopecten ventricosus* (Catarina scallop)**

Argopecten ventricosus (Waller, 1995) commonly known as the Pacific scallop or catarina scallop, is a species of bivalve of the family Pectinidae, whose habitat is distributed in the eastern Pacific Ocean, from the west coast of North America to Central and South America, including regions such as the Gulf of Baja California, Mexico, to Peru (Bertsch and Aguilar-Rosas, 2016). It has been collected in both brackish marine environments and fully marine areas. In SCOPUS 53 articles were detected, involving Mexican institutions, covering a period from 1996 to 2022. Between 1996 and 2002 there is a higher production than in the rest of the period, although this has been maintained with only a slight decrease in recent years (Figure 1). The institutions that have contributed most to the accumulation of published information are CIBNOR with more than 50% of the total, followed by UABCS and IPN, these three institutions accumulate more than 75% of all the articles registered in SCOPUS (Figure 2).

The topics that produce the most articles cover diverse lines of research: abundance, genetics/genomics, growth, reproduction in captivity, immunomodulation, bioactive compounds, pathology, nutrition, probiotics, fisheries, exposure, and accumulation of heavy metals/biotoxins, ecology, environmental conditions, settlement and morphology, among others. The funding entity that has supported the most projects resulting in publications is CONAHCYT with almost 75% of the funding, followed by CIBNOR with 17% and IPN with 9%.

***Atrina maura* (maura pen shell)**

Atrina maura (Sowerby 1835) is a bivalve commonly known in Mexico as "callo de hacha". This species is distributed from the Baja California peninsula to Peru and is found in protected bays with sandy, silty, or clayey soils (Góngora-Gómez *et al.*, 2016). For this species, 24 articles were identified, involving Mexican institutions. Figure 1 shows the 20-year period that records published articles, from 2001 to 2021. Just a little more than one article per year, at least in the journals included in SCOPUS. This shows that, although it is a species of interest, its scientific approach is poor compared to others. The Mexican institutions that are most mentioned in the articles are shown in Figure 2. CIBNOR is the one that has contributed the most research, followed by IPN, CICIMAR and UABCS. The four of them account for almost 80% of all the publications generated. The topics covered by the publications include studies on reproductive aspects and culture and development both in nature and under laboratory conditions, fisheries, nutrition, environmental conditions, presence of heavy metals and ecology, among others. CONAHCYT and IPN are the entities that have financed most of the projects that resulted in these publications (39% and 23%, respectively), although the Comisión Nacional de Áreas Naturales Protegidas, the Instituto Nacional de Pesca y Acuicultura and CIBNOR are also included.

***Crassostrea* (oysters)**

Crassostrea is a genus of bivalve mollusks, known as oysters, belonging to the family Ostreidae. They are used as food because of their high nutritional value and the ease with which they are obtained.

This section includes the four species of *Crassostrea* that have been studied in Mexico and have resulted in publications in SCOPUS. In order of importance by number of articles published are *C. gigas*, *C. corteziensis*, *C. virginica* and *C. sikamea*. *C. gigas* has been the species of greatest interest to Mexican institutions. Less attention has been paid to the native species (*C. corteziensis* and *C. virginica*), although both account for 50% of the published studies (Figure 1). The other exotic species, *C. sikamea*, is of more recent interest and has generated the least number of articles.

In relation to the Mexican institutions that have conducted the studies and published the results, we found that there are a good number of them. CIBNOR leads the production of articles with 32% of the total, followed by IPN with 12% and UABCS with 9%. These three institutions alone exceed 50% of the articles published according to SCOPUS (Figure 2). The lines of research addressed are practically all those related to aquaculture research: reproduction, genetics, pathology, culture, development, environmental conditions, nutrition, accumulation of contaminants and biotoxins, and ecology, among others. However, *C. gigas* has been the most extensively studied in all of the above aspects. The native species *C. corteziensis* has also been the subject of studies in many of the aforementioned lines. However, in the case of *C. virginica*, also native, there are still few studies that involve its management in the laboratory; however, it is included because it is a species of high economic interest and, in fact, it is already in extensive cultivation in some regions of Mexico. The financing entities have been mainly CONAHCYT with 64%, the rest include CIBNOR, IPN and CICESE as the most important.

***Haliotis* (abalones)**

Abalone is a gastropod mollusk of the family Haliotidae that inhabits rocky areas associated with mats of *Macrocystis pyrifera* and other algae and is widely distributed in temperate and tropical oceans (Gluyas-Millán and Talavera-Maya, 2003). Of this genus, articles produced by Mexican institutions and housed in SCOPUS were identified with the species *H. fulgens*, *H. corrugata* and *H. rufescens*. *Haliotis fulgens* is the species that has generated the most articles with almost 60% of the total. The publication of articles, the sum of the three species, over the period 1992 to 2023, demonstrates a production with ups and downs but which has remained constant for 40 years (Figure 1).

Regarding the Mexican institutions that have carried out the studies and published the results, UABC, CIBNOR and CICESE are the most mentioned and represent more than 50% of the total (Figure 2). The lines of research identified in the articles are diverse and address practically all areas related to aquaculture biology and management: nutrition, fisheries, management and culture, environmental preferences, pathology, genetics/genomics, reproduction, ecology, and morphology, among others. The funding entity has been mainly CONAHCYT with more than 60% of the total number of projects supported, and with CICESE and CIBNOR they account for almost 80% of contributions.

***Mytilus* and *Modiulos* (mussels)**

Mitylids (Mytilidae), commonly known as mussels or choros (in some parts of South America), are a family of bivalve mollusks of great economic and gastronomic interest. Like other bivalves, they are filter-feeding animals that live attached to the substrate. They are mainly marine and live in both intertidal and submerged areas of coasts around the world, although there are species that live in freshwater and mixed or brackish bodies of water (a mixture of freshwater and seawater) (NaturalisEc, 2023).

This study brought together the two species identified with publications in the SCOPUS database: *Mytilus galloprivincialis* (Mediterranean mussel) and *Modiulos capax*, the former is an introduced species, and the latter is native. Paradoxically, although *M. galloprivincialis* is an exotic species, it has generated the most articles (66%) while *M. capax* only 34%. Research on these species has been carried out from 1991 to the present year, with little but constant production of articles (Figure 1). The institutions that are most mentioned as generators of publications are CICESE with a wide advantage over the others, followed in importance by UABC (Figure 2). The lines of research addressed are mostly nutrition, accumulation of heavy metals and biotoxins, reproduction, distribution, larval production, environmental conditions, pathology and culture. Regarding the entities that have provided funding for projects that have resulted in publications, CICESE is the most relevant with almost half of the support granted, followed by CONAHCYT.

***Nodipecten subnodosus* (giant lion's paw)**

Bivalve distributed from Laguna Ojo de Liebre in Baja California Sur to the coasts of Peru (Rombouts *et al*., 1991), large size (up to 175 mm), with symmetrical valves characterized by two auricles and in the center is the hinge. Their coloration varies from opaque purple or white with purple lines, to bright orange and magenta (INAPESCA 2021). Its exploitation is relatively recent, in 1995, its capture was introduced as a replacement for the catarina scallop (*Argopecten ventricosus*) because the latter had suffered overfishing that led to its depletion in the late 1980s (González-Ortíz and Hernández-Alcántara, 2021).

The SCOPUS database identified articles published by Mexican institutions in the period from 2003 to 2023 (Figure 1). During this period there are ups and downs, although the production of articles has remained constant. CIBNOR, like most of the species treated in this study, is the institution that is mentioned most often as the institution generating the publications, with 45% of the total, followed by IPN, CIAD and CICIMAR. However, the sum of the four institutions exceeds 75% of the total number of publications (Figure 2). The lines of research addressed in the publications correspond to reproduction, larval production, nutrition, developmental biology, pathology, development under diverse environmental conditions, among others. The funding entities are CONAHCYT with more than 50% of support for projects that resulted in publications, followed by CIBNOR (33%) and the IPN the rest.

***Octopus* and *Paroctopus* (octopuses)**

For this analysis we combined the publications identified in SCOPUS of four species of octopus, all native to Mexico: *Octopus maya*, *O. vulgaris*. *O. bimaculoides* and *Paroctopus digueti*.

The largest number of articles was concentrated in *O. maya* with 74% of the total, followed by *O. vulgaris* with 19% and the rest, with similar numbers, for the other two species. Regarding the period of publication of articles, these were dissimilar among the species. *Octopus maya* shows publications from 2000 to the present, *O. vulgaris* records publications in a period of 13 years (2010 to present), *O. bimaculoides* a period of nine years, starting in 2009 and ending in 2018 and finally P. digueti, with the shortest period of only four years, from 2020 to 2023. Figure 1 shows the sum of all publications of the four species as a function of time. Publications with *O. maya*, are the ones that contribute the most published research of the total, with 81 articles hosted in SCOPUS, developed by Mexican institutions, out of a total of 109.

The institution that has contributed most to the knowledge of these species is UNAM, mainly in its SISAL Unit, which alone accounts for 31% of the mentions in articles. CICESE registers 9% and IPN also stands out, mainly through CINVESTAV-Mérida (8%) (Figure 2). The most common lines addressed in the study of octopuses have been nutrition, environmental conditions, reproduction, physiology, embryology, exposure to contaminants, abundance, distribution, climate change, fisheries, immunology, pathology, ecology, genetics/genomics, and culture, among others. Of the sponsoring institutions, CONAHCyT has made the greatest contribution, as in all previous cases, with 60% support for projects that have resulted in publications. UNAM also stands out with 35%.

***Panopea* (geoducks)**

In the present analysis we included the two species present in Mexico and for which research has been carried out leading to publications: *Panopea generosa* and *P. globosa*. The clam *P. globosa* is distributed in the Gulf of California and Magdalena Bay, while the clam *P. generosa* is distributed from Alaska, United States of America to Baja California, Mexico (Arambula-Pujol *et al*., 2008; DOF, 2013).

Between 2013 and 2018 there has been a constant publication of articles, however, it is from 2013 to 2018 that there was a significant upturn and then a decline until the present (Figure 1). CIBNOR is the most represented institution in the publications followed by IPN, CICESE and UABC (Figure 2), which account for almost 75% of the published articles. The topics most addressed are ecology, distribution, genetics/genomics, larval production, reproduction, and nutrition, among others. Regarding the entities that have financed the projects that have resulted in the identified publications, CONAHCYT accounts for more than 50%, followed by CICESE and INAPESCA.

***Pomacea* (snails)**

Apple snails or Pomaceous snails are a group of aquatic mollusks belonging to the family Ampullariidae. They inhabit muddy and shallow waters of lakes and rivers, are found in tropical and humid subtropical areas of southeastern Mexico, Central America, South America and the Caribbean Islands, inhabiting rivers, lakes, canals, swamps, and wetlands, preferably in places with abundant vegetation (Castillo-Capitán *et al*., 2020).

This analysis considered the two species with articles in the SCOPUS database: *Pomacea patula* and *P. flagellata*. Only 16 articles were identified, of which 10 correspond to *P.*

patula (63%) and six to *P. flagellata* (37%). These articles have been published from 1995 to 2023. Figure 1 shows that, although the production of articles has been maintained during the period, there is a valley of seven years in which none were published. The Colegio de la Frontera Sur and the IPN are the two institutions that have contributed the most articles to the knowledge of these species, followed by UAM and CIBNOR. These four institutions account for a little less than 75% of all that has been published (Figure 2). The topics covered include cultivation, environmental conditions, bioeconomics, ecology, distribution, exposure to contaminants, physiology, reproduction, cultivation, nutrition, genetics, and morphology, among others. There is no record of financing entities.

The results show that Mexican academic and research institutions have a clear interest in addressing the study of native species, however, technologies derived from basic studies of physiology, nutrition, and reproduction, among others, have only rarely resulted in efficient culture technologies. In fact, there is apparently an impasse in the study of most native species, with few articles published over time, although there are two groups, abalone, and octopus, that demonstrate statistically confirmed growth. However, the genus demonstrating the most growth, with the highest number of published articles is *Crassostrea*. Although the native species (*C. virginica* and *C. corteziensis*) have received attention, *C. gigas* accumulates 46% of the publications and if *C. sikamea* (also exotic) is added, it reaches 50%.

Table 1 shows the total number of articles published and hosted in the SCOPUS platform. The number of publications through the years 1983-2023 records the species of the genus *Crassostrea* as the most studied (with 200 publications), followed by *Haliotis* (141), *Octopus* (109), *Panopea* (64), *Nodipecten* (56), *Argopecten* (53), *Mytilus* (30), *Atrina* (24), *Pomacea* (16) and *Anadara* (9). Of all the groups of mollusks analyzed, only three show a linear growth pattern through the years: *Octopus* (0.838, $P<0.001$), *Crassostrea* ($R=0.772$; $P<0.001$) and *Haliotis* ($R=0.556$, $P<0.001$), the rest of the genera, although they register an increase in the number of publications through the years, these appear with variations without a defined or significant predictive pattern.

Crassostrea gigas, with 92 articles, is the most studied, even more than the native species of the same genus. Twenty-eight percent of the articles generated correspond to *Crassotrea*, 20% to *Haliotis* and 15% to *Octopus*. The latter has shown the greatest increase in recent years (Figure 3). In Figure 4, the production of articles can be observed in a timeline. It is evident that there are three that have maintained almost constant growth (*Crassostrea*, *Haliotis*, *Octopus*) or with dramatic but short increases over time (*Panopea*), while the others have barely maintained a discrete pro production despite the fact that they are all native species (with the exception of the introduced mussel, *M. galloprovincialis*). With respect to one of the most socioeconomically and ecologically important native species in the Yucatan Peninsula and the southern Gulf of Mexico, *O. maya*, UNAM generates almost all the research on the cultivation of the species, while the IPN collaborates to an equal extent with research on exploitation and governance in the use of the species (Santamaria *et al.*, 2023). Since 2007 to date, research on the cultivation of *O. maya* has been generated from the Multidisciplinary Unit of UNAM in Sisal, Yucatan, covering many aspects that are determinant for mastering the technology of the species (*e.g.* Rosas *et al.*, 2014).

Of the institutions that have generated the total number of articles on mollusks, CIBNOR (including all its regional and local units) is positioned as the most relevant with 31.3% of all the mentions that refer to the authors' affiliation. IPN, including all its units (CICIMAR, CIIDIR, CINVESTAV), is in second place with 22.8% and CICESE in third place with 10.3%. Only CIBNOR and IPN exceed 50% of all mentions. Of the latter, of the ten mentioned, eight are universities (including UNAM, also including all its units) (Table 2).

The results show that, in general, bivalve aquaculture in Mexico has focused on the Pacific oyster *C. gigas* (now *Magallana gigas*), which was introduced in the 1970s (Chavéz-Villalba, 2014). It is important to note that not all exotic mollusks have negative effects, some introduced bivalves can even generate welfare to ecosystems by increasing water filtration capacity and decreasing the effects of eutrophication (Burlakova *et al.*, 2023),

Table 1. Number of publications by mollusk genus.

Gender	Publications
Crassostrea	200
Haliotis	141
Octopus/Paroctopus	109
Panopea	64
Nodipecten	56
Argopecten	53
Mytilus/Modiulos	31
Pomacea	16
Atrina	24
Anadara	9
TOTAL	703

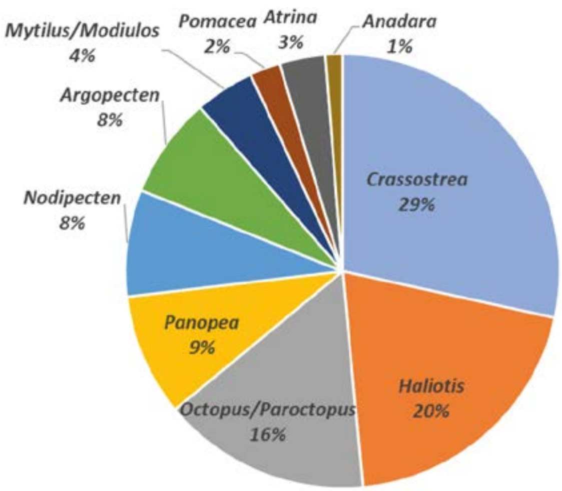

Figure 3. Percentage of publications by mollusk genus produced by Mexican institutions according to SCOPUS.

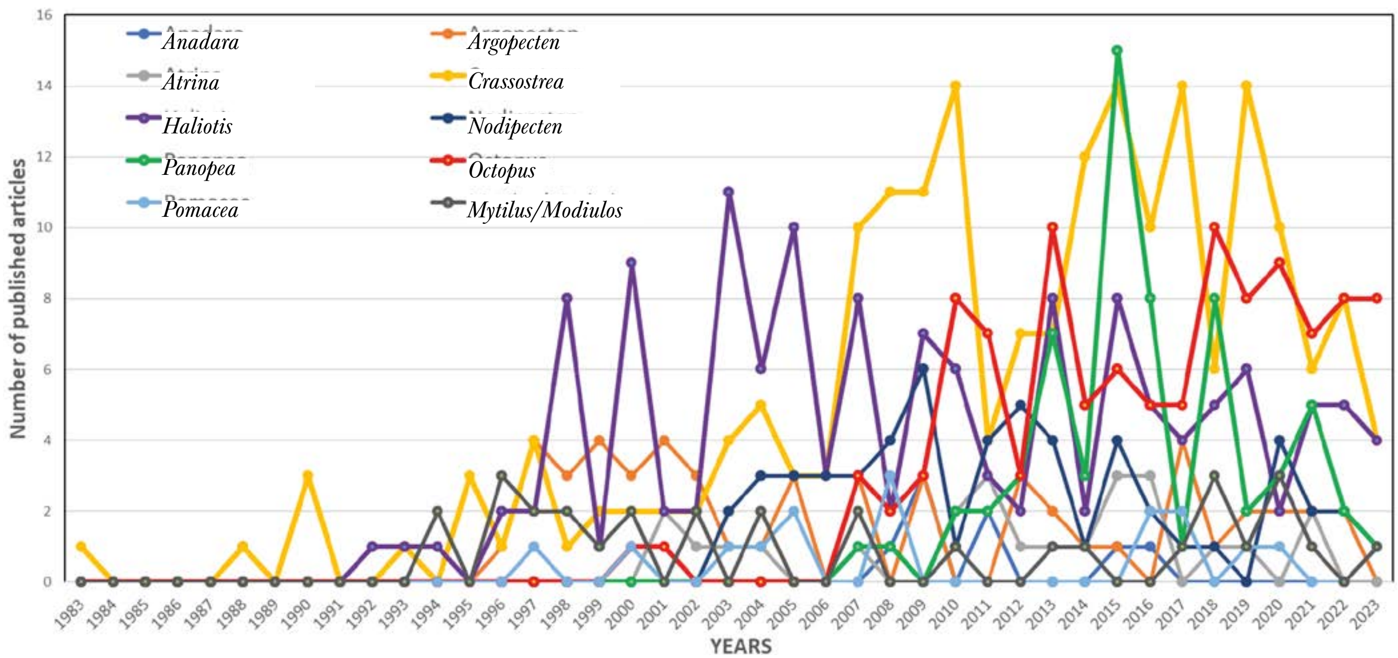

Figure 4. Timeline of publication of articles on mollusks by Mexican institutions according to SCOPUS.

Table 2. Institutions most mentioned in articles on mollusks published by Mexican institutions and hosted in SCOPUS (as a percentage of total articles).

Institutions	%
CIBNOR	31.3
IPN	21.8
CICESE	10.3
UNAM	7.7
UABCS	6.9
UABC	6.2
COLFSUR	3.8
UAM	3.1
UNISIN	2.3
CIAD	1.9
UNISON	1.5
UAN	1.5
TecNM	1.2
UANL	0.4

as well as antiviral medicinal and commercial uses (Khan and Liu, 2019). There is equal information on successful cases of introduced mollusc cultures, such as red or Californian abalone (*H. rufescens*) in Chile, which, when carried out in a responsible and sustainable manner, has allowed technological consolidation and increased the production of other marine crops such as macroalgae (mainly *Macrocystis* spp.) so as not to exploit natural populations (Mardones *et al.*, 2013); such algae generate opportunities to meet other needs because the alginates produced in the processing of algae have nutraceutical properties and important industrial uses (Hernández-Carmona *et al.*, 2012). However, the introduction of exotic species into new habitats should always be carefully evaluated and regulated to minimize potential negative impacts on local ecosystems and species (Gubiani *et al.*, 2018).

In contrast to exotic species, Mexico has native species of bivalve mollusks that are cultivated, including the American oyster *Crassotrea virginica* and the pearl oyster *Pinctada mazatlanica*; in addition, there are a large number of species with cultivation potential (Cáceres-Martínez and Vásquez-Yeomans, 2008), as may be the case of the rock oyster (*Striostrea prismatica*) that requires incentivizing scientific research to realize its technological development, since it is a commercial fishery resource destined for human consumption (Ríos-González *et al.*, 2018).

It is important to recognize that the production of farmed mollusks in Mexico is low compared to world statistics (FAO, 2022). Maeda-Martínez, (2008), comments that this problem can be explained by the lack of trained technicians, the lack of organization and a business culture in the production sector, and the lack of interest of the scientific sector in solving the problems they pose. In this perspective, the analysis of bibliometric indicators of scientometric type on scientific productivity (such as the number of publications, the institutions that generate them, the subject of the study and the funders of the research) constitutes a fundamental tool for developing political and social strategies that contribute to a better diagnosis of scientific productivity in the country (Quintanilla, 2007; Jiménez-Borges *et al.*, 2020). As a sample derived from this type of analysis, we can cite the works of Mohd Noor *et al.*, (2021), Chong-Carrillo *et al.*, (2015), and Kumaresan *et al.*, (2014), whose objectives share that of highlighting trends in research on aquaculture-related species, as well as identifying more developed areas of research and pointing out possible gaps in scientific knowledge on target species.

CONCLUSIONS

The results of this study reveal important trends in mollusk aquaculture research in Mexico. There is a clear interest on the part of academic and research institutions in addressing the study of native mollusks and integrating them into aquaculture production systems. Despite this interest, technologies derived from basic studies in areas such as physiology, nutrition and reproduction have not been translated into efficient culture technologies in most cases. There was uneven growth in aquaculture research on native species, with few publications on aquaculture during the period evaluated, with a greater effort in two groups: abalone and octopus.

With respect to the overall production of scientific articles, the genus *Crassostrea* leads the publication efforts in SCOPUS, with *C. gigas* being the most studied species. And there is a recent trend towards growth in research on the genus *Octopus*, especially on the species *O. maya*. Regarding the role of academic institutions in Mexico, CIBNOR and IPN are the most relevant institutions in scientific production in mollusk aquaculture, generating most of the publications in this field.

Despite having an important diversity of native mollusks with culture potential in Mexico, aquaculture production of these organisms is low when compared to global statistics; this can probably be attributed to the lack of trained technicians, lack of adequate organization in the production sector, and limited interest on the part of the scientific community with respect to consolidating technological processes in mollusk culture. These findings highlight the need to foster research and technological development in native mollusk aquaculture in Mexico, as well as to promote collaboration between academic research institutions and the production sector to overcome challenges in the culture of these species.

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