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Presence of endophytic fungi in **cacao** plantations (*Theobroma cacao* L.), in the state of Tabasco, Mexico

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Citas directas de menos de 40 palabras: Cuando se transcriben textualmente menos de 40 palabras, la cita se coloca entre comillas y al final se añade entre paréntesis el autor, el año y la página. Ejemplo:

> Alineado al Plan Nacional de Desarrollo 2013-2018, (DOF, 2013), el Programa Sectorial de Desarrollo Agropecuario, Pesquero y Alimentario 2013-2018 establece "Construir un nuevo rostro del campo sustentado en un sector agroalimentario productivo, competitivo, rentable, sustentable y justo que garantice la seguridad alimentaria del país" (DOF, 2013).

Citas indirectas o paráfrasis: Cuando se interpretan o se comentan ideas que son tomadas de otro texto, o bien cuando se expresa el mismo contenido pero con diferente estructura sintáctica. En este caso se debe indicar el apellido del autor y el año de la referencia de donde se toman las ideas. Ejemplo:

> Los bajos rendimientos del cacao en México, de acuerdo con Avendaño *et al.* (2011) y Hernández-Gómez *et al.* (2015); se debe principalmente a la edad avanzada de las plantaciones.

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> Ogata N. (2003a).
> Ogata N. (2003b).

Artículo de revista:

> Wang, P., Zhang, Y., Zhao, L., Mo, B., & Luo, T. (2017). Effect of Gamma Rays on *Sophora davidii* and Detection of DNA Polymorphism through ISSR Marker [Research article]. https://doi.org/10.1155/2017/8576404

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> Turner J. (1972). Freedom to build, dweller control of the housing process. New York: Macmillan.

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Characterization of self-guided trails on the Xihuingo Volcano, state of Hidalgo (Mexico)

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ABSTRACT

Objective: hiking allows us to appreciate nature; it is also an environmental education tool, causing social changes which can be directed towards behaviors and decisions in favor of environmental protection. The objective of this study was to generate the necessary information in order to hike the trails of the Xihuingo Volcano (state of Hidalgo, Mexico), self-guided and with safety.

Design/Methodology/Approach: the methodology consisted of monitoring the guidelines established for the preparation of the Topographic guide, as well as those considered in the Excursion information method (MIDE) and the processing of tracks with global positioning system (GPS) receivers, using a geographic information system as interface.

Results: data on local history, culture and biodiversity were obtained. As well as descriptions, time estimates, and profiles of the routes; with the identification of places of interest in maps; and digital files to be used in GPS receivers.

Limitations of the study/Implications: the limitations in the data are related to the precision margin of the GPS receivers during the recording of the information (±3 m). As well as the number of records on biodiversity at the time of consultation.

Findings/Conclusions: data obtained highlight the wide biological diversity located at the site, with important representation of endemic and native species, some of them in status of ecological risk. These species can be the basis for the creation of a tourism project for the benefit of the local people. Such a program would face the ecological problems derived from mining, illegal logging, and grazing observed in the area, as a strategy for the conservation of the natural environment.

Keywords: conservation, nature, hiking.

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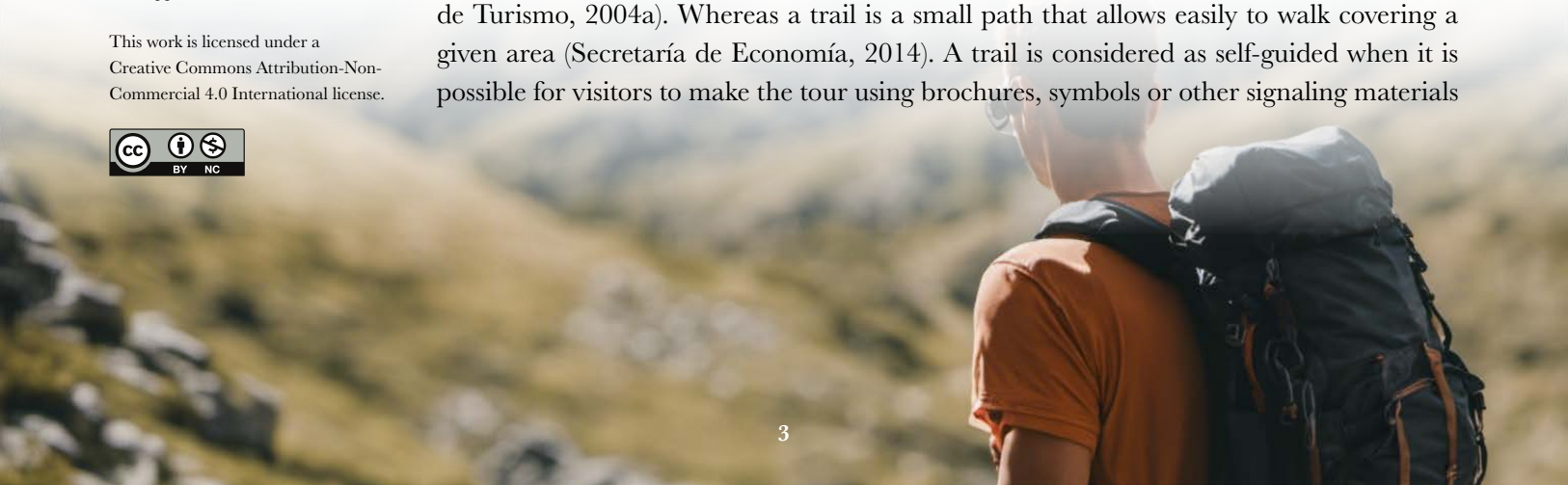
INTRODUCTION

Hiking is a sporting activity that consists of walks following an itinerary (Real Academia Española, 2019); it allows people to open their mind and get in tune with nature (Secretaría de Turismo, 2004a). Whereas a trail is a small path that allows easily to walk covering a given area (Secretaría de Economía, 2014). A trail is considered as self-guided when it is possible for visitors to make the tour using brochures, symbols or other signaling materials



(SECTUR, 2004b). In sum, a topoguide is a document that describes one or several trails (Maza, 2002).

The Excursion information method –MIDE is a system to assess and express the technical and physical demands of the routes (París, 2002). The information regarding trails is analyzed and presented by various authors and institutions with an approach focused on the MIDE (Arqueofuer, 2011; Consorcio Camino del Cid, 2019). Which includes elements of the topographic guide (SECTUR, 2004a, 2004b) or else, it is focused on an international classification of high mountain trails (Neyra, 2012).

However, in general, a standardized methodology has not been defined for obtaining and processing the data necessary for the comprehensive description of the middle and low mountain trails within the national context. Thus, this study constitutes an opportunity to explore such a possibility. The objective was to generate the necessary information in order to hike the trails of the Xihuingo Volcano (estado de Hidalgo, Mexico), self-guided and with safety.

MATERIALS AND METHODS

The general description was made based on bibliographic and cartographic information, and direct observations on the site. The elevation profiles of the trails were generated with the Profile Tool, in Quantum GIS. The biological information was obtained by consulting the files of the National Biodiversity Information System of the National Commission for the Knowledge and Use of Biodiversity (CONABIO, 2018). Those files were correspondent to different biological groups and their registered locations within the buffer areas of the volcano.

The information about MIDE (París, 2002) was obtained from the data associated with each track consulted in Google Earth Pro, and the physical characteristics observed in each trail. The tracks of the routes were recorded with a Garmin™ Map64s GPS receiver and were rectified with Google Earth Pro, following the recommendations of the Spanish Federation of Mountain and Climbing Sports (2018), which were later used to prepare the map. From the review of different documents that deal with signaling issues (SECTUR 2004b, 2004c; FEDME, 2018; Tacón and Firmani, 2004), a series of elements is proposed for implementation at a local scale.

RESULTS AND DISCUSSION

General information

The general description (history, climate, services, etc.) and the map coincided with the way in which other authors present them (SECTUR, 2004a, 2004b; Arqueofuer, 2011; Neyra, 2012; Consorcio Camino del Cid, 2019). These data offer a site overview.

Profile and routes of the tours

The profile shows the distance traveled and the altitude, locating sites of interest during the journey (Figure 1). They coincide with the way in which other authors present them (SECTUR, 2004a; Arqueofuer, 2011; Neyra, 2012; Senderos GR, 2015; Consorcio Camino del Cid, 2019; Cabildo La Palma, 2021). The profile shows upwards disposition,

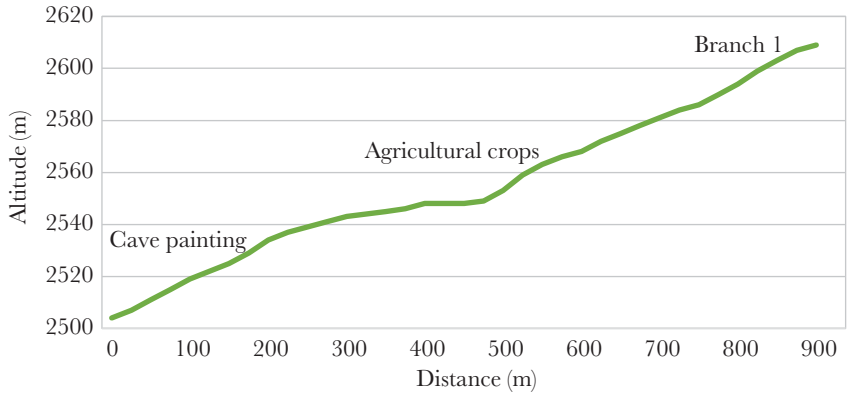


Figure 1. Elevation profile of the "Sendero Microsmos" (one trail). Elaborated by the authors, based on Maza (2002).

towards the summit of the Xihuingo volcano. In addition, 4 tracks were obtained in GPX and KMZ formats. Only in the case of Senderos GR (2015) there are other files of those types.

Biological information

In total, 484 species were found (CONABIO, 2018), of which 78 are considered to have some status of ecological risk (Secretaría de Medio Ambiente y Recursos Naturales, 2019; International Union for Conservation of Nature 2020; Convention on International Trade in Species Threatened Wild Fauna and Flora, 2020). Biodiversity information was found only in two publications (Arqueofuer, 2011; Neyra, 2012).

Excursion Information Method –MIDE

The travel times of the four trails range from 20 min to 2 h 50 min, Trails are of the crossing type, and intermediate level, in terms of environmental severity (Table 1). Some authors use this method to assess trails (Arqueofuer, 2011; Consorcio Camino del Cid, 2019). While SECTUR (Mexico) (2004a) considered only some of these data, and Neyra (2012) opted for an international classification for high mountain trails.

Table 1. Descriptive and quality traits of the trail "Sendero Microcosmos" according to the Excursions Information Method –MIDE.

Microcosmos.			
MIDE		**13-061-0086-R(C) Microcosmos**	
Schedule	30 min	2	Severity of the natural environment
Ascent slope	123 m	3	Guidance on the itinerary
Descent slope	18 m	2	Difficulty in movement
Horizontal distance	924 m	1	Amount of effort
Type of tour	crossing		

Graduation from 1 (easy/minimum) to 5 (difficult/maximum).
Own elaboration based on MIDE (2002).

The qualification scale goes from 1 (easy/minimum) to 5 (complex/maximum). Elaborated by the authors, based on París (2002).

CONCLUSIONS

It is possible to apply the established guidelines for the elaboration of a Topographic guide, based on the Excursion Information Method and the processing of tracks for self-guided hiking along the trails on the Xihuingo Volcano.

The information and infrastructure associated with the trails can contribute to a safe, effective route with low environmental impact. Also, they can be the basis for the integration of a tourism project based on hiking, which can be replicated in other contexts. The conservation of the natural environment is important due to the number of native and endemic species, some of them present with status of ecological risk. Other conservation factors are representativeness at the state scale, diversity of types of vegetation and the environmental services provided. In addition to the metaphorical and spiritual meaning that the volcano site has represented over time since its discovery.

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Effect of plant extracts on the inhibition of the mycelial growth of *Penicilium citrinum* Link

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ABSTRACT

Objective: To evaluate in vitro plant extracts of three plant species —mistletoe (*Psittacanthus* spp.), guinea hen weed (*Petiveria alliacea*), and ginger (*Zingiber officinale*)— with the aim of determining their inhibitory effect on the mycelial growth of *Penicilliun citrinum* isolated from coffee beans.

Design/Methodology/Approach: Filtrations were carried out under aseptic conditions using a vacuum system and were added to the Potato Dextrose Agar medium. Once it had solidified, a 5-mm disc of *P. citrinum* was placed in the center of the Petri dish.

Results: The ethyl plant extracts, like the chemical product, showed a 100% inhibition on the pathogen development.

Findings/Conclusions: Ethyl plant extracts can be an agroecological alternative for the control of *P. citrinum*.

Keywords: Mistletoe, guinea hen weed, ginger, antifungal, coffee.

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INTRODUCTION

Coffee (*Coffea arabica* L.) is one of the most important crops in Mexico. Its economic value lies on the generation of foreign currency, it has social value as a generator of jobs and family labor, and its environmental value is mainly the result of its cultivation under shade conditions, the promotion of carbon capture, and the preservation of biodiversity (Escamilla, 2017; CEDRSSA, 2019). In Mexico, 947,092 tons of green coffee are grown in 710,897 hectares. Chiapas is one of the main coffee producing states: 253,457 ha are used to produce 384,549 tons of coffee (SIAP, 2022).

Fungal problems may cause the deterioration of coffee beans in the warehouse; these problems impact the flavor and aroma of the coffee cup. Some species of the genera *Aspergillus*, *Penicillium*, and *Fusarium* have also been related to the production of mycotoxins —toxigenic secondary metabolites that can cause diseases in humans and animals. Ochratoxins and aflatoxins —two of the main toxins that have been reported— are known to have highly carcinogenic effects (Gruber *et al.*, 2017; Garrido *et al.*, 2018; Davidovich *et al.*, 2019).

The use of synthetic fungicides is currently the main method used to control pathogens; however, they can have negative impacts on the environment and also cause health problems to workers and consumers. Likewise, the effectiveness of fungicides can cause resistance

in phytopathogenic microorganisms (Tamilselvi and Arumugam, 2017; Samsidar *et al.*, 2018). Therefore, it is necessary to look for friendlier alternatives (*e.g.*, extracts of plant origin) that are less toxic than pesticides and that do not harm the health of consumers and farm workers (Tamilselvi and Arumugam, 2017; Samsidar *et al.*, 2018). Furthermore, the use of a large number of plants in Mexico can be researched for the control of plant diseases (CONABIO, 1998).

Mistletoe (*Psittacanthus* spp.) is a hemiparasitic plant that is distributed in Mexico and affects numerous fruit and forest species (Balladores, 2017; Castillo, 2018; CONABIO, 2022). Numerous studies have been carried out about the capacity of this plant to control diseases in humans, but to date this capacity has not been tested against phytopathogenic fungi.

Known in Mexico as *hierba de zorillo* ("skunk weed"), due to its content of sulfur compounds, Guinea hen weed (*Petiveria alliacea*) is widely distributed in Mexico, Central, and South America (Sariego 2013; CONABIO, 2022). It has been evaluated for the control of insects, nematodes, bacteria, and fungi of genus *Aspergillus* (Barrera *et al.*, 2017; Bracho *et al.*, 2019; Pinargote *et al.*, 2019; Oredoyin *et al.*, 2020).

Ginger (*Zingiber officinale*) has been used to control various diseases caused in plants by such fungi as: *Moniliophthora roreri* (Cif & Par), *Fusarium verticillioides*, *Aspergillus flavus*, *Penicillium* spp., *Sclerotinia sclerotiorum*, *Sclerotium rlfsii*, and *Colletotrichum gloesporioides* (Santana *et al.*, 2009; Darshana *et al.*, 2014; Joya *et al.*, 2015; Rodrigues *et al.*, 2017; Bracho *et al.*, 2019; Costa *et al.*, 2020; Pérez *et al.*, 2021).

Consequently, extracts of mistletoe (*Psittacanthus* spp.), guinea hen weed (*P. alliacea*), and ginger (*Z. officinale*) were evaluated under in vitro conditions to analyze their inhibitory effect on the mycelial growth of *Penicilliun citrinum* isolated from coffee grains.

MATERIALS AND METHODS

The isolation of *P. citrinum* started with the collection of samples of coffee beans stored in Villaflores, Chiapas, Mexico. The samples were disinfested with 1% sodium hypochlorite for 3 min and placed in dishes with PDA culture medium for 4 d. The *P. citrinum* colonies were transferred and purified. Molecular identification was carried out using the DellaPorta Method (1983) to extract the DNA from monosporic cultures and the Polymerase Chain Reaction (PCR) was performed, using the ITS4 and ITS5 primers (White *et al.*, 1990). The amplified fragments were visualized via agarose gel in TAE. The fragments were purified with the QiaQuick Purification Kit (QiaGene) and submitted for sequencing. The amplified fragment was sequenced and compared with the NCBI (National Center for Biotechnology Information) gene bank database.

Evaluation of extracts from three species

Twelve treatments were evaluated (Figure 1, Table 1). The extracts were obtained from 100 g of leaves and roots of each of the species studied (guinea hen weed, mistletoe, and ginger), which were washed and disinfected with 1% sodium hypochlorite. Subsequently, they were macerated with 1.0 L of water and diluted with 70% alcohol. Then they were placed in flasks at room temperature and stirred for 24 h. Subsequently, they were filtered

Figure 1. Species used for plant extracts. a) Mistletoe, b) Guinea hen weed, c) Ginger.

Table 1. Treatments evaluated (20% plant extracts) for the control of *P. citrinum*.

N.	Treatment
T1	Aqueous plant extracts (PE) of mistletoe flowers
T2	Aqueous PE of mistletoe leaves
T3	Ethyl PE of mistletoe flowers
T4	Aqueous PE of guinea hen weed leaves
T5	Ethyl PE of guinea hen weed roots
T6	Ethyl PE of guinea hen weed leaves
T7	Ethyl PE of mistletoe leaves
T8	Ethyl PE of ginger roots
T9	Aqueous PE of ginger roots
T10	Aqueous PE of guinea hen weed roots
T11	Copper oxychloride
T12	Potato Dextrose Agar

with Whatman® number 4 filter paper and put through a vacuum system using a 0.2-μm milipore syringe filter. The extracts obtained were added to the 20% Potato Dextrose Agar culture medium. Once solidified, a 5-mm disc of *P. citrinum* was placed in the center of the Petri dish. The mycelial growth of *P. citrinum* was measured with a digital vernier, nine days after the start of the experiment (Bell *et al.*, 1982; Gamboa *et al.*, 2003). A completely randomized design with four repetitions was used. The data were subjected to an analysis of variance with the SAS software (version 9.0).

RESULTS AND DISCUSSIONS

Fungus Identification

A non-cottony, bluish green growth with abundant powdery sporulation was observed adhered to the PDA. On the reverse side of the colony, a yellowish-white color was recorded.

Under the microscope, small, globose, unicellular conidia, simple conidiophores, and chains could be seen (Barnett and Hunter, 1998). Regarding the molecular identification, the sequence of the ITS 4 region was compared with the NCBI and showed maximum identity (99.40%) with the KM278105.1 sequence.

The results of the mycelial growth of *P. citrinum* on the ethyl and aqueous plant extracts of mistletoe, guinea hen weed, and ginger at 20% showed that mycelial growth was observed in the aqueous plant extracts; however, a total growth inhibition was reported with ethyl extracts, which shows its viability as a control option (Figure 2). According to the analysis of variance, the results showed no significant differences, neither between the ethyl treatments and copper oxychloride, nor between the aqueous treatments and the PDA. In contrast, there was a significant difference between the aqueous and PDA extracts versus the ethyl extracts and copper oxychloride (Table 2).

According to Tortora *et al.* (2007), Marín *et al.* (2013), and Balladores (2017), *Psittacanthus* contains viscotoxins, flavonoids, coumarins, lecithins, anthraquinones, tannins, triterpenes, alkaloids, polysaccharides, and phenolic compounds. The bacterial activity of the last substance damages the lipids of the plasma membranes, causing the loss of cellular content, which may be related to the results obtained for the growth inhibition of *P. citrinum*.

P. alliacea contains flavonoids, phenols, tannins, saponins, quinones, triterpenes, and alkaloid steroids related to the control of diseases in plants (Montes, 2009; Ochoa *et al.*, 2013). The biological activity of the alkaloids and quaternary ammonium salts found in

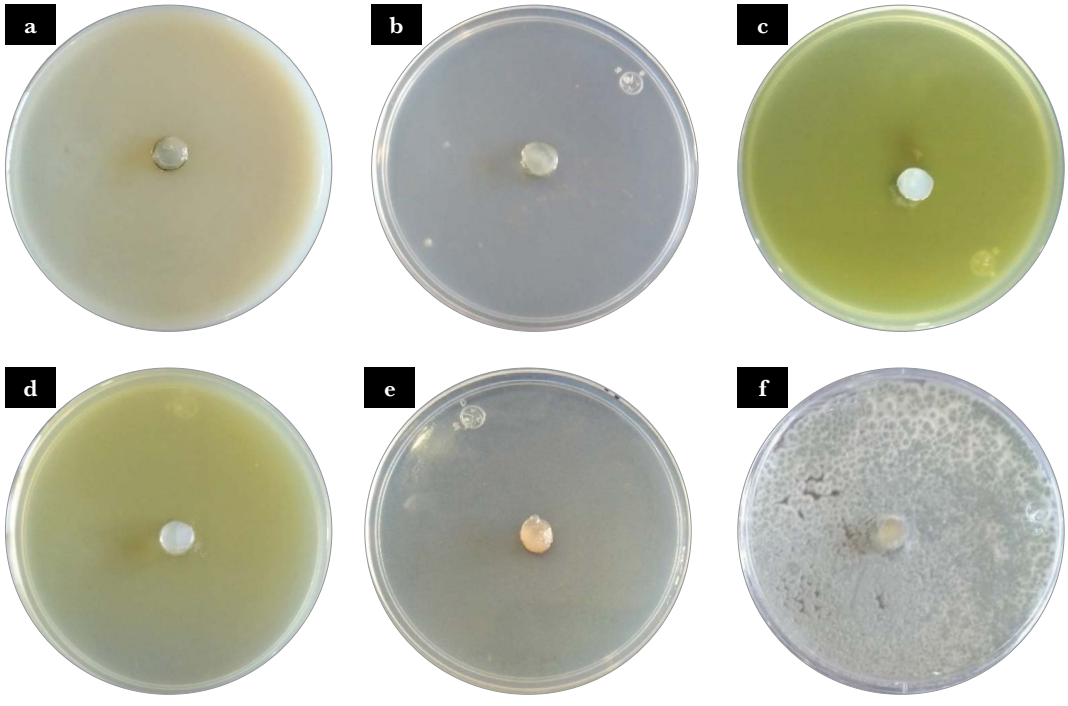

Figure 2. *P. citrinum* in 20% ethyl extracts of mistletoe, guinea hen weed, and ginger: (a) mistletoe flowers, (b) guinea hen weed roots, (c) guinea hen weed leaves, (d) mistletoe leaves, (e) ginger roots, (f) Potato Dextrose Agar.

Table 2. Effect of 20% plant extracts on the mycelial growth of *P. citrinum*.

Treatment	Growth (cm)
T1 Aqueous plant extracts (PE) of mistletoe flowers	7.31±0.58 a
T2 Aqueous PE of mistletoe leaves	6.99±0.74 a
T3 Ethyl PE of mistletoe flowers	0.00±0.00 b
T4 Aqueous PE of guinea hen weed leaves	7.12±0.74 a
T5 Ethyl PE of guinea hen weed roots	0.0 0±0.00 b
T6 Ethyl PE of guinea hen weed leaves	0.00±0.00 b
T7 Ethyl PE of mistletoe leaves	0.00±0.00 b
T8 Ethyl PE of ginger roots	0.00±0.00 b
T9 Aqueous PE of ginger roots	6.68±0.54 a
T10 Aqueous PE of guinea hen weed roots	7.07±0.67 a
T11 Copper oxychloride	0.00±0.00 b
T12 Potato Dextrose Agar	6.55±0.71 a

EV. Vegetable Extract. Means with the same letter are not significantly different, Tukey ($p<0.05$).

ginger helps to control the *Rhizoctonia solani* fungi; additionally, they have a significant participation in the defense of plants against pathogens (Casanova *et al.*, 2004; Him de Fréitez, 2006; Andamayo, 2020). Specifically, as Salch (1997) and Cushnie and Andrew, (2005) also conclude, the plant extracts evaluated contain: flavonoids, phenols, alkaloids, tannins, quinones, and saponin, which seem to be related to the inhibition of the mycelium growth of *P. citrinum*, since they are compounds with high antimicrobial, fungal, antiviral, miticide, and insect repellent activity.

CONCLUSIONS

The 20% ethyl extracts of mistletoe, guinea hen weed, and ginger effectively inhibited the mycelial growth of *P. citrium*. The results of this study indicate that the extracts from the evaluated plant and their parts are taking shape as an effective agroecological alternative for the control of phytopathogens. More detailed studies should be carried out, both regarding biochemical level and extraction methods, in order to determine which molecules and concentrations make up the extracts and which are responsible for antifungal activity against *P. citrinum*. The ultimate aim would be to identify the most effective dosage in the field.

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Colegio de Postgraduados

Determination of optimal areas for the establishment of buffalo herds and German grass in Tabasco, Mexico

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ABSTRACT

Objective: To determine optimal (suitable) areas for the establishment of buffalo (*Bubalus bubalis*) herds fattened with German grass (*Echinochloa polystachya* Kunth Hitchc), based on the biophysical environmental conditions that favor the comfort of the animal species and the best development of the plant species.

Design/Methodology/Approach: An analysis of the bioclimatic parameters for water buffalo and the agroclimatic parameters for German grass was carried out in the state of Tabasco, Mexico. A comparative table of the optimal biophysical variables of water buffalo and German grass was developed from the digital soil geographic databases and the climatological normals recorded in the state of Tabasco. Edaphoclimatic maps were developed to establish buffalo herds associated with German grass, based on a cartographic cross-checking.

Results: The soil-climatic aptitude map of both species was developed at a scale of 1:135,000.

Study Limitations/Implications: Given its recent introduction, there is a lack of basic information on the edaphoclimatic conditions suitable for water buffalo in the state of Tabasco.

Findings/Conclusions: The areas for the establishment of buffalo herds and German grass were identified. Regarding their potentiality, 4.29% of the state of Tabasco is suitable, 56.67% was classified as moderately suitable, and 38.48% is not suitable.

Keywords: *Bubalus bubalis*, geographic information system, map algebra.

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INTRODUCTION

Since water buffalo has only been recently introduced to Tabasco, Mexico, there is no deep knowledge about the effect of this species (Suazo Cortez, 2011). Breeding herds of water buffalo have been implemented in Tabasco; this animal is characterized by a corpulent, stocky body, and a large abdomen. Water buffalo achieve good yields with

diverse forages, regardless of their bromatological quality (Pérez, 2007) and the areas where they are established.

Tabasco is located in the humid tropical zone, with regions below sea level (García, 2004); therefore, it has the flood plain and climate conditions necessary for the establishment of buffalo herds. Buffalo is a species with comparative advantages over cattle, since it develops faster, consequently reducing costs (Álvarez *et al.*, 2005; Zava, 2012).

Animals need optimal environmental conditions for their production and comfort. Therefore, to successfully establish the species, the areas in the state of Tabasco which meet these requirements should be determined. Since these aspects are currently being omitted in the state by those interested in establishing buffalo herds, they could be introduced to places that are not optimal for the animals. Consequently, optimal areas were located for the establishment of buffalo herds associated with German grass, which has been identified as a species with propitious characteristics for the development of the animal (Camarao *et al.*, 2004). Likewise, Meléndez-Nava (2012) has argued that German grass is a species that reaches its maximum use under conditions of permanent flooding —which is likewise favorable for water buffalo. Therefore, the objective of this research was to determine which areas are suitable to establish herds of buffalo (*Bubalus bubalis*) for fattening purposes, associated with German grass (*Echinochloa polystachya*), based on the environmental conditions that favor the comfort of both species.

MATERIALS AND METHODS

The study area covered the state of Tabasco, Mexico (17° 15' 03" and 18° 39' 03" N, and 90° 59' 15" and 94° 07' 48" W). It has a total area of 24,661.0 km^2 (INEGI, 1998). It is bounded by the Gulf of Mexico to the north, the state of Campeche to the northeast, the Republic of Guatemala to the southeast, the state of Chiapas to the south, and the state of Veracruz to the west (Figure 1).

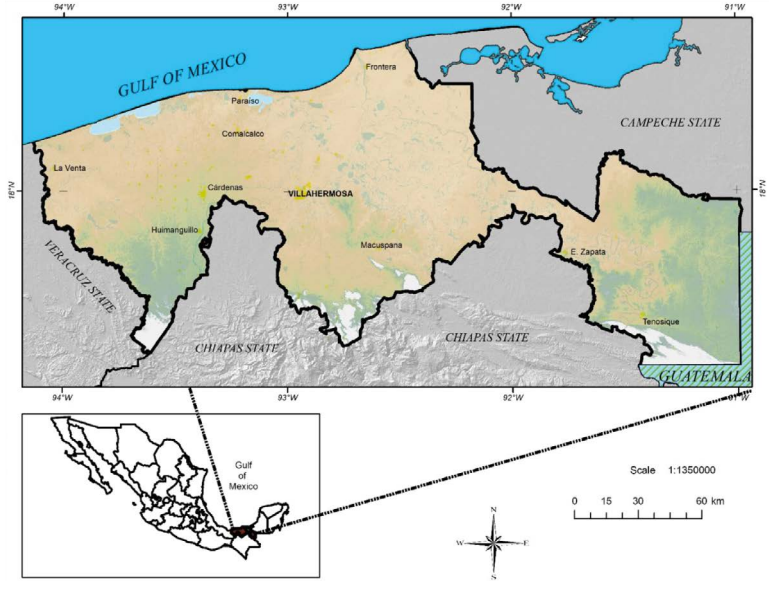

Figure 1. Location of the State of Tabasco, Mexico (study area).

Bioclimatic conditions for water buffalo and agroclimatic conditions for German grass

The main bioclimatic and agroclimatic variables were considered to determine the areas with optimal potential. For this purpose, documentary information was compared in the main research centers of the region, building a database that was systematized with Microsoft Excel 2010. The categories were: name of the author, title, year of publication, origin of the information, and suggested ranges for each variable under study. The database was used to classify the variables by optimal ranges for each species; the arithmetic mean was obtained for the abovementioned ranges. The edaphic variables indicated in Table 1 were computed using Microsoft Excel 2010 and the optimal values for the state of Tabasco were used to classify the soil types in three categories (Palma-López *et al.*, 2007): suitable (Su), moderately suitable (MSu), and not suitable (NSu).

Bioclimatic and agroclimatic analysis

The climate was analyzed using historical records (1951 to 2010) of the climatological normals of the state of Tabasco (Servicio Meteorológico Nacional [SMN], 2022). The classification of the geographical location according to the name, temperature, and precipitation of the climatological stations was incorporated into the GIS software (ArcGis version 10.1); the IDW (Inverse Distance Weighted) method (ESRI, 2022) was used to interpolate the isohyet and isotherm maps. Once the coverage was obtained, the areas with the optimal ranges for the establishment of buffalo herds associated with German grass (B-G) were identified, based on which maps of the areas with bioclimatic and agroclimatic potential were developed.

Soil analysis

The evaluation of the soil resource for the water buffalo was based on the units of the FAO/UNESCO system (IUSS Working Group WRB, 2015), using four variables: depth, slope, drainage, and altitude. Meanwhile, seven variables were taken into consideration in the case of German grass: depth, fertility, texture, pH, slope, drainage, salinity, and aluminum toxicity. Consequently, the limiting elements allowed an approximation of

Table 1. Bioclimatic and agroclimatic variables used for the zoning of water buffalo and German grass.

Species	Soil variable	Climatic variable	Geographic variable
Buffalo	• Depth (m) • Texture (%) • Slope (%) • Drainage (m)	• Total annual precipitation • Average annual temperature • Average minimum temperature • Average maximum temperature	Altitude (msnm)
German Grass	• Depth (m) • Fertility • Texture (%) • pH • Slope (%) • Drainage • Salinity • Aluminum toxicity		

regions suitable for the B-G binomial. The subsequent identification of the pedological units —based on the 1:250,000 scale mapping of the state of Tabasco (Palma-López *et al.*, 2007)— enabled the selection of suitable soils (Su). The selection of the edaphic units led to the development of maps with edaphic potential for the establishment of the B-G binomial.

Crossing and cartographic analysis

With the climatic and soil maps of the state of Tabasco, a super positioning of the cartography was carried out with the ArcGis 10.1 software (ESRI, 2022); map algebra was used to generate areas with different edaphoclimatic potential for the B-G binomial.

RESULTS AND DISCUSSION

Climatic requirements for water buffalo

According to the analysis of the existing bibliographic information for the establishment of buffalo herds, the optimal temperatures vary from 27.1 °C to 28.9 °C. The optimal precipitation ranges between 2,000 and 3,314 mm (annual average: 3,313.99 mm), at an altitude of 0-50 m.a.s.l. These results match the data reported by Das *et al.* (1999), who point out that a >30 °C environmental temperature would cause the animal to gasp, because of the heat stroke. The spatial distribution of temperature per suitable municipality in the state of Tabasco indicates that 28.99% of the territory is Su for establishing buffalo herds, while 67.55% is MSu; therefore, it could be inferred that temperature is not a limiting factor for the establishment of buffalo herds, since only 3.45% of the territory was NSu. According to their total annual precipitation, the areas of the state with potential for the establishment of buffalo herds are divided as follows: 68.47% is Su, while 17.00% is MSu, and only 14.53% is NSu. The areas that met the temperature and precipitation conditions were called bioclimatic: 21.22% of the surface of the state were Su for the establishment of the animal, while 61.35% was MSu, followed by a 17.43% of NSu areas.

Soil aptitude for the establishment of buffalo herds

Deep clay soils, with a <20% slope and poor drainage (Palma-López *et al.*, 2007) do not involve any drawbacks for the establishment of buffalo herds. Therefore, three groups of Su soils identified in Tabasco accounted for 44.05% of the total surface of the state that report waterlogging in rainy seasons (an environment necessary for the comfort of buffalo herds), while 20.76% comprise five groups cataloged as MSu and 35.19% were classified as part of the NSu soil group (Table 2).

Edaphoclimatic requirement for the establishment of buffalo herds

Table 3 shows the results of the edaphoclimatic geospatial analysis for the establishment of buffalo herds. Of the total area of the state, 10.37% is Su for the establishment of this species; 54.09% is classified as MSu and therefore some areas can become optimal, if an adequate management of the soil (drainage, slope, etc.) is carried out; and 35.07% was classified as NSu —*i.e.*, unsuitable (or at the very least unprofitable), even with management.

Table 2. Soil groups in the state of Tabasco, with different aptitude for the establishment of buffalo herds.

Code	Group	Aptitude	(%)
GL	Gleysol	suitable	44.05
HS	Histosol	suitable	
VR	Vertisol	suitable	
AC	Acrisol	moderately suitable	20.76
CA	Cambisol	moderately suitable	
FR	Ferrasol	moderately suitable	
FL	Fluvisol	moderately suitable	
RG	Regosol	moderately suitable	
Al	Alisol	not suitable	35.19
AR	Arenosol	not suitable	
LP	Leptosol	not suitable	
LV	Luvisol	not suitable	
PL	Plintisol	not suitable	
SC	Solonchaks	not suitable	

Table 3. Area with different soil-climatic aptitude for the establishment of buffalo herds in the state of Tabasco, Mexico.

Soil-Climatic Aptitude	Area	
	(%)	Ha
Suitable	10.37	256547.02
Moderately	54.09	1338099.44
Not Suitable	35.07	867609.25
Total	100	2473527.41

Figure 2 shows the map of soil-climatic aptitude for the establishment of buffalo herds in the state of Tabasco. The most representative areas are identified in yellow and are cataloged as MSu.

Bioclimatic requirements of German grass

For German grass (*Echinochloa polystachya*), the areas with high climatic and edaphic potential were taken, based on their optimal values, as established by ECOCROP (FAO, 2022).

Characteristics of German grass

German grass is a species that is successfully cultivated from 0 to 100 m.a.s.l., in the lowlands near the Gulf of Mexico (Enríquez *et al.*, 2015). It is a perennial species, which resists frequent and prolonged (up to six-month long) flooding (Enríquez *et al.*, 2015).

It tolerates a wide range of soils, although its growth is jeopardized when the lack of water is evident (Meléndez-Nava, 2012); ideally, this type of grass should be grown under

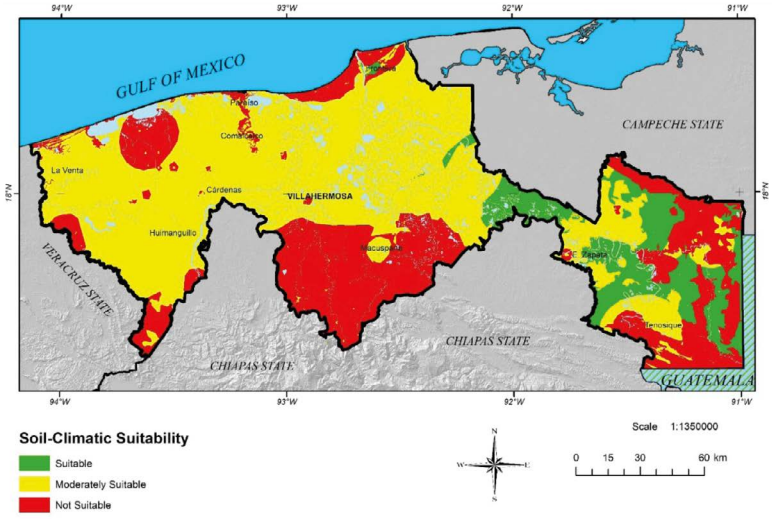

Figure 2. Areas with edaphoclimatic suitability for the establishment of buffalo herds in the state of Tabasco, Mexico.

waterlogging conditions. Meléndez-Nava (2012) mentions that, in Tabasco, German grass has an excellent growth in Gley type soils, adapts to soils with a pH of 4.0 to 8.0, has some resistance to alkalinity, and is highly tolerant to poor drainage —a fact that is also supported by the FAO (FAO, 2022).

Authors such as Enríquez *et al.* (2015) and Meléndez-Nava (2012) indicate that it is tolerant to approximately 2.8 mmhos/cm; nevertheless, according to the latter author, its yields are jeopardized, although they can grow under very high precipitation (>3,000 mm), and overall they do not have a good "growth in drought conditions, unless there is a high-water table or the soil retains reserve moisture" (Meléndez-Nava, 2012, p.187).

The resulting climatic conditions indicated that, according to their temperatures, the zones of the Tabasco territory in which German grass should be established are divided as follows: 80.35% were Su, followed by 17.06% MSu, and only 2.58% NSu. The spatial distribution of the precipitation data indicates that 70.96% of the territory of the state of Tabasco meets Su conditions for the establishment of pasture, while 29.01% is classified as MSu. Just like in the case of the temperature condition, Tabasco does not present limitations for the establishment of German grass, since only 0.01% of the total territory was classified as NSu. Based on the cross-checking of temperature and precipitation information, it was designated as agroclimatic: 54.89% of the state of Tabasco is Su for the establishment of pasture, while 42.50% is MSu and only 2.59% is registered as NSu. Table 4 shows the relative values resulting from the different edaphic aptitudes for German grass. This table shows that 59.77% of the state is edaphically Su, 16.17% is MSu, and 24.05% is NSu.

Figure 3 shows that the geospatial analysis of the agroclimatic and edaphic information indicated that 34.30% of the surface of the state is edaphoclimatically Su for the establishment of German grass, while 39.40% is MSu, and 26.30% is NSu.

Table 4. Area of the different soil aptitudes for German grass (*Echinochloa polystachya*) in the state of Tabasco, Mexico.

Soil Aptitude of german grass	Area	
	(%)	Ha
Suitable	57.77	1478505.76
Moderately	16.17	399986.30
Not Suitable	24.05	595093.4
Total	100	2,473,527.41

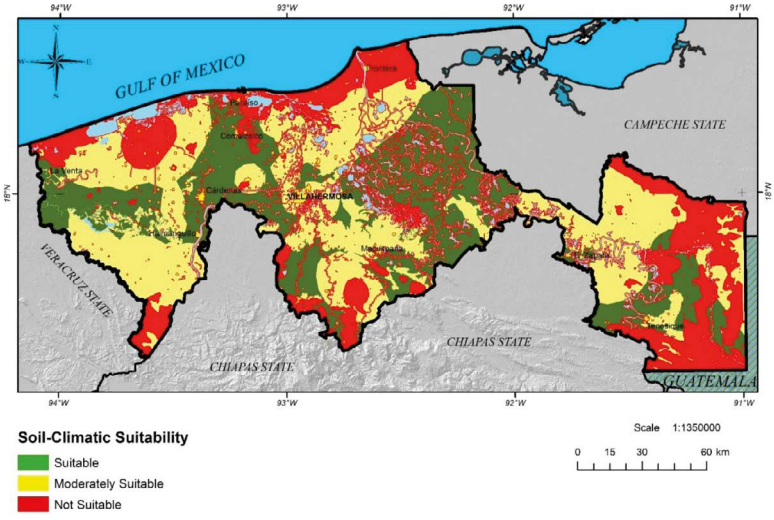

Figure 3. Areas with edaphoclimatic aptitude for the establishment of German grass (*Echinochloa polystachya*) in the state of Tabasco, Mexico.

The cross-checking of the edaphoclimatic information for the B-G binomial led to the differentiation of the following areas: 4.29% of the state area has Su edaphoclimatic potential for B-G; therefore, buffalo herds could be established in these areas and, since the comfort of the animal and its food would be guaranteed, good returns would be obtained. Meanwhile, 56.67% is MSu and establishing a buffalo herd in these areas is feasible; however, some type of soil management would be necessary, mainly to achieve optimal yields. Finally, 38.48% of the state was identified as NSu for the establishment of a productive herd. In Figure 4, the suitable areas are identified in green; they are located in the central part of the municipality of Balancán, southern Jonuta; the municipality of Emiliano Zapata is also found in the central and southern areas of the municipality. The areas with moderately suitable potential are in the central region of the state of Tabasco and include the municipalities of Cárdenas, Huimanguillo, Centro, Centla, and Jonuta, among others. The unsuitable areas for the establishment of the B-G binomial are highlighted in red; they are located mainly in the North and South areas of the state, due to the presence of sandy or stony soils, as well as altitudes higher than the two species under study can resist.

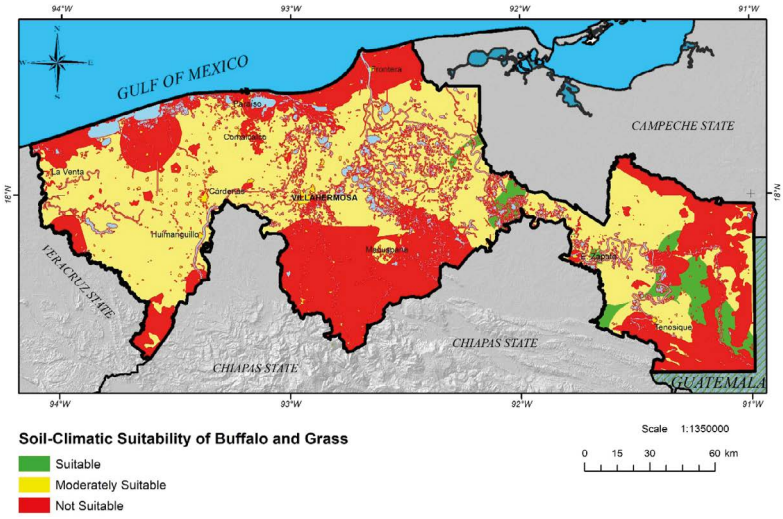

Figure 4. Areas with edaphoclimatic suitability for the establishment of buffalo herds (*Bubalus bubalis*) and German grass (*Echinochloa polystachya*) in the state of Tabasco, Mexico.

CONCLUSIONS

Out of all the whole area of the state of Tabasco, 10.37% and 34.30% are edaphoclimatically Su for the establishment of buffalo herds and German grass, respectively. Meanwhile, 4.29% of the total area of the state of Tabasco was identified as Su edaphoclimatic zones for the establishment of the B-G binomial.

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Colegio de Postgraduados

Estimation of fig (*Ficus carica* L.) yield in fertigation using linear regression

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ABSTRACT

Objective: To estimate the fig (*Ficus carica* L.) yield in fertigation, through the use of linear regression, under the conditions of the Mixteca region of Puebla.

Design/Methodology/Approach: The plants were established in 10-L black polyethylene bags, using pine sawdust as a substrate. The treatments consisted of 6 variations of the following nutrient solution: 1.52 mg L^{-1} of potassium nitrate, 0.08 mL L^{-1} of phosphoric acid, and 0.38 mg L^{-1} of magnesium sulfate. Plant height, number of fruits per plant, and growth speed (cm day^{-1}) were determined. A completely randomized experimental design was used and the levels of nutrient solution and orthogonal contrasts were subjected to a linear regression analysis, through the SAS® On Demand for Academics statistical package.

Results: The linear model describes the behavior of the data with an alpha of 0.01. A 60% nutrient solution level has a greater effect on growth and generates highly significant differences in both the plant height increase rate and plant height variables.

Study Limitations/Implications: This study includes only preliminary results; therefore, a longer period is necessary for data collection. Additionally, the following variables must also be included: yield and the content of nitrogen, phosphorus, potassium, magnesium in plant tissue.

Findings/Conclusions: Plant growth (height) can be estimated through the application of a linear model. An increasing linear behavior was observed in height with respect to the levels determined 50 days after the experiment was established. There are highly significant differences between the 60% dose and the rest of the treatments.

Keywords: Mixteca, productive reconversion, dry tropics.

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INTRODUCTION

The genus *Ficus* L. comprises approximately 800 species, the most important of which is *F. carica*. Although this crop is mainly grown in temperate regions, it adapts to tropical and subtropical regions. In the Mediterranean, annual production reaches ≈1,064,784 tons, which are harvested from 311,080 hectares. Seventy percent of this production is distributed in Turkey, Egypt, Iran, Greece, Algeria, and Morocco, while the United States contributes only 4% of world production (Moniruzzaman *et al.*, 2020). Fig is considered a

crop with high economic potential, that it thrives in arid and semi-arid conditions, with high temperatures and poor soils. Furthermore, it has been established with densities of 500 to 1,000 trees ha^{-1}, obtaining yields of 5.1 t ha^{-1} to 11.6 t ha^{-1} (Kurubar *et al.*, 2017; Chithiraichelvan *et al.*, 2017). In Mexico, its expansion has been driven by export demand (Del Sol-Rodríguez, 2021). The consumption of figs constitutes an important source of polyphenolic antioxidants, anthocyanins (aglycone 99-85%), and cholesterol-free fats, among other compounds (Ercisli *et al.*, 2012). In addition, the waste generated during fig production constitutes an alternative for the production of biofungicides (Istúriz-Zapata *et al.*, 2019).

Protected agriculture is used for the intensive management of figs: a population density of 1.25 plants m^2 in 40-L containers, localized irrigation, and Steiner fertilization (Mendoza-Castillo *et al.*, 2019). In the state of Puebla, this species is planted in the municipalities of Xochiapulco, Coatzingo, Zacapoaxtla, Izúcar de Matamoros, and Teziutlán (SIAP, 2022).

In the Mixteca region of Puebla, the tropical deciduous forest predominates; its main economic activities are the grazing of goats and cattle, extraction of firewood, production of agricultural instruments, and exploitation of materials for the construction of rural homes. The deterioration of regional ecosystems is very noticeable: there is accelerated soil erosion, loss of biodiversity, fragmentation of ecosystems, and depletion of aquifers (Guízar-Nolazco *et al.*, 2010). Based on all of the previously mentioned factors, the objective of this research was to estimate fig yield in fertigation, through the use of linear regression, under the conditions of the Mixteca region of Puebla.

MATERIALS AND METHODS

The experiment was established at the Instituto Tecnológico Superior de Acatlán de Osorio (18° 12' 08" N and 98° 02' 54" W, at 1,180 m.a.s.l.), located in the municipality of Acatlán de Osorio. The plant material was obtained from the vegetative propagation of mother plants from family gardens. Semi-woody cuttings with an ≈10-cm long vegetative bud were used, adding radix 10000. The rooting substrate consisted of a mixture of Agrolita® substrate with peat moss (2:1 ratio); this stage lasted 66 days. Once they had established roots, the cuttings were transplanted in 10-L black polyethylene bags, using pine sawdust as a substrate. In the conditioning stage, two applications of 100 g of triple 17 were used as fertilizer. The application of the fertilization treatments began 141 days after rooting, using six variations (60%, 50%, 40%, 30%, 20%, and 10%) of the following nutrient solution: 1.52 mg L^{-1} of potassium nitrate, 0.08 mL L^{-1} of phosphoric acid, and 0.38 mg L^{-1} of magnesium sulfate. To decrease the pH of the nutrient solution, 0.91 g L^{-1} of ferrous sulfate was added to the treatments. The nutrient solution was irrigated daily during the application of the treatments.

A completely randomized experimental design was used. Each treatment consisted of three repetitions and each repetition included one plant. Plant height, number of fruits per plant, and growth speed (cm day^{-1}) were measured from the beginning of the treatment application, until 195 days after rooting. In the regression analysis, the levels of nutrient solution (%) were considered as the independent variable and the increase

in height (cm) was considered as the dependent variable. The effect of each level of nutrient solution on the increase in height (cm) in relation to time (days after rooting) was analyzed individually. A linear regression analysis and orthogonal contrasts were used to analyze the data, along with the SAS® On Demand for Academics statistical package.

RESULTS AND DISCUSSION

The effect of the dose levels of the nutrient solution applied to the fig plants on the response variables were determined 50 days after the start of the application. The preliminary results are only shown for the 60%, 50%, 40%, and 30% levels, since no vegetative development was registered during that period for the 20% and 10% treatments.

Linear regression analysis

Based on the results of the linear regression analysis for the nutrient solution levels on height increase variable, the model does describe the behavior of the data ($\alpha=0.01$) for the interval under study. The 60% nutrient solution applied for this interval has a greater effect on growth (Figure 1).

Linear regression analysis for the 60%, 50%, 40%, and 30% levels of nutrient solution

Using the linear regression model to describe the behavior of the increase in height with the application of the 60% dose, a height increase rate of 0.9621 cm day^{-1} was obtained, while the 50%, 40% and 30% levels recorded height rates of 0.7523, 0.3716, and 0.5091 cm day^{-1}, respectively (Figure 2).

According to these results, a decreasing trend is apparent in the growth speed during the period under study, since as the amount of fertilizer applied decreases, a decrease in growth is recorded.

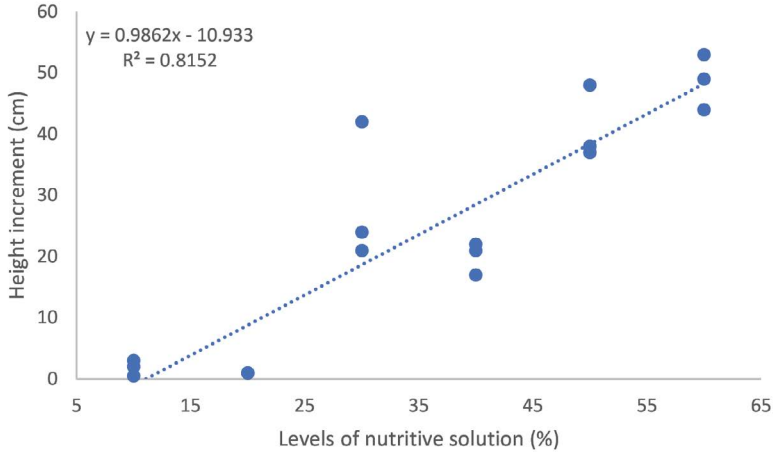

Figure 1. Effect of nutrient solution levels on height increase.

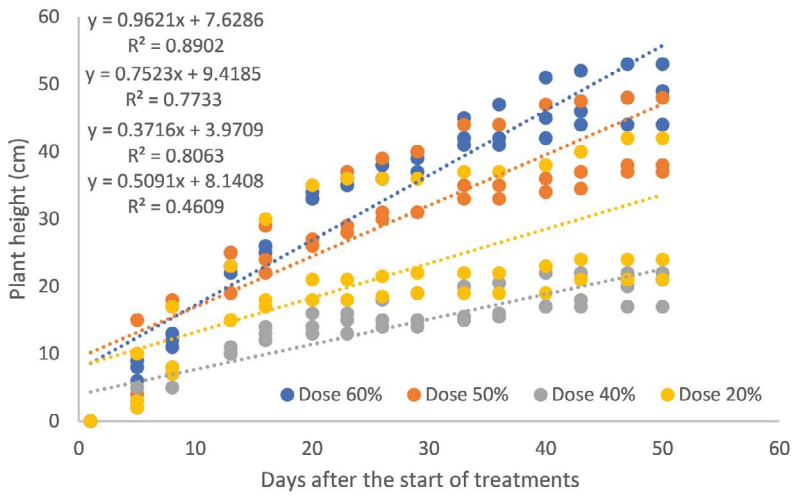

Figure 2. Growth dynamics with the application of 60%, 50%, 40%, and 30% of the nutrient solution.

Analysis of variance of the linear regression

Analysis of variance of the linear regression was applied for each of the nutrient solution levels, 50 days after the start of the experiment, to determine the increase in height in the fig plants. In the case of the dependent variable (increase in height), the regression model used does describe the behavior of the data for the four levels of the nutrient solution applied (α=0.01) (Table 1).

The use of linear regression as a tool to predict biomass increases is based on the correlation between plant structures (Lai *et al.*, 2013). In the case of alfalfa meadows, the height variable has a strong correlation with dry matter yield. If the correlation coefficients are high (>0.80), this variable can be considered to determine yield. In this sense, the crop growth rate (GR) is an approximation of the accumulation of plant biomass over time (Montes-Cruz *et al.*, 2016).

Orthogonal contrasts analysis

The orthogonal contrast test recorded highly significant differences between the plant height increase rate and plant height variables, with the comparison of the 60% nutrient solution with the 50%, 40%, and 30% solutions. When contrasting the 50% nutrient solution with the 40% and 30% nutrient solutions, significant differences were found for the plant height increase rate and plant height variables (Table 2).

Table 1. Analysis of variance of the linear regression of nutrient solution levels on height increase.

Source of variation	Degrees of freedom	Nutritive solution (60%)	Nutritive solution (50%)	Nutritive solution (40%)	Nutritive solution (30%)
Regression	1	9342.5**	5711.3**	1393.8**	2615.8**
Error	43	26.8	38.9	7.8	71.1
Total	44				
r^2		0.89	0.77	0.81	0.46

Table 2. Orthogonal contrasts analysis.

Contrast	Degrees of freedom	Rate of increase in plant height (cmdia^{-1})	Plant height (cm)	number of fruits per plant
Nutritive solution at 60% versus 50%, 40%, 30%	1	0.391**	784**	40.111^{ns}
Nutritive solution at 50% versus 40%, 30%	1	0.192*	544.5*	117.556^{ns}
Nutritive solution at 40% versus 30%	1	0.027^{ns}	121.5^{ns}	6.000^{ns}
Error	8	0.0213	48.333	55.167
Total	11			
r^2		0.78	0.79	0.27
CV		22.52	20.05	64.59

CONCLUSIONS

The application of a linear model allows the estimation of plant growth (height) during the period under study. Regarding the nutrient levels applied 50 days after establishing the experiment, a linear increasing behavior in height was observed. Finally, highly significant differences were recorded between the 60% dose and the rest of the treatments, regarding the plant height increase rate and plant height variables during the same period.

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Colegio de Postgraduados

The importance of blackberry (*Rubus* spp.) production in Mexico

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ABSTRACT
Objective: To carry out a bibliographic review about the current situation of the commercial production and the generation of blackberry varieties in Mexico.
Approach: Based on the existent databases, a bibliographic review about the current blackberry production, production indicators, and generation of new varieties in Mexico was carried out. Berries have been produced in Mexico since the 19th century. The exportation of this product was a response to the North American market windows and generated new varieties adapted to the Mexican weather conditions. The USA market demands berries mainly in autumn, winter, and spring. During this period, prices are very attractive due to the lack of domestic American production. This situation causes a production increase in the berries sector, where innovative practices have been developed. In Mexico, the first commercial blackberry plantation was established in 1983, in Tetela del Volcán, Morelos. The Boysenberry (a raspberry-blackberry hybrid) was the chosen variety. The Brazos variety was the first blackberry variety that was planted and, in 1998, it was replaced by the Tupi variety, which has an excellent quality and shelf lifespan.
Conclusions: The blackberry productive chain is a source of direct and indirect employment during its production and commercialization. Mexico is one of the main blackberry exporters worldwide. Additionally, blackberry consumption has health benefits, because it prevents the development of several diseases.

Keywords: blackberry, production, varieties.

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INTRODUCTION

Blackberry general characteristics

The species of the genus *Rubus* can adapt to a wide range of environments: from the artic to the tropics, from lower to higher altitudes, from acid to alkaline soils, from dry to humid weather, and from shaded to sunny places (Clark and Finn, 2011; Ibarra-Morales *et al.*, 2013). However, they require low temperatures (below 7 °C) to produce shoots, flowers, and fruits (Warmund and Byers, 2002). Blackberries are characterized by their

high morphologic diversity (Clark *et al.*, 2007) which includes creeping, semi-erect, and erect growth species (Clark and Finn, 2008). Their reproduction varies from apomictic reproduction to sexually fertile reproduction. Blackberry species are mostly infertile and the different species intercross; consequently, their taxonomic classification is very difficult or even impossible to carry out (Evans *et al.*, 2007; Marulanda *et al.*, 2010). This situation is mainly the result of the polyploidy, agamospermy, and the frequent inter-species hybridization (Alice, 2002). The flowers and fruits have a panicle or bunch shape (Hummer and Janick, 2007). The flower receptacle has several ovaries, styles and stigmas, and pink or white petals. Flowers with double petals are not common. From the botanical point of view, the blackberry fruits are made up of several drupelets surrounding the receptacle (Clark & Finn, 2011).

Blackberry taxonomy

Blackberries can be taxonomically classified in genus *Rubus*, subgenus *Eubatus*, and family Rosaceae (Thompson, 1997). This family includes approximately 750 species worldwide. *Rubus* is the genus with the highest number of species within the family Rosaceae (Potter *et al.*, 2007; Juinn-Yih and Jer-Ming, 2009). The genus *Rubus* has 12 subgenuses. The raspberry species (*Rubus idaeus* L.) belong to the subgenus *Idaeobatus* and the blackberries (*Rubus* spp.) to the subgenus *Eubatus* (Jennings, 1998). According to Rzedowsky and Calderón de Rzedowsky (2005), 61 species of the genus *Rubus* are distributed in Mexico. Rodríguez-Bautista *et al.* (2021) studied the distribution of 45 wild species of *Rubus* (subgenus *Eubatus*) in Mexico and they also described their ecoclimatic characterization.

Raspberry and blackberry have different morphologic features. The criteria commonly used to classify them consists of cutting the ripe fruits from the plant during harvest. The raspberry fruit is an aggregate of small drupelets that are separated from the receptacle. Meanwhile, the drupelets of the blackberry are attached to the receptacle, which is an edible part of the fruit (Clark and Finn, 2011).

Polyploidy has been fundamental for the species evolution of the genus *Rubus*. Regarding blackberries, the ploidy varies from diploid (2n=14) to dodecaploid (2n=126), including uneven ploidy and aneuploids (Thompson, 1997; Meng and Finn, 1999). Meanwhile, most of the raspberry species are diploid plants (Moore and Skirvin, 1990). The chromosome of blackberries is 1-3 μm long and the DNA content of the diploid species ranges from 0.56 to 0.59 pg (Meng and Finn, 2002).

Origin of the blackberries

Blackberries have been part of the human diet since ancient times (Clark *et al.*, 2007). Already in the 4th century BC, blackberries were eaten as fresh fruit and drank in traditional beverages in Rome. Blackberry leaves were also prepared as tea with medicinal purposes (Patel *et al.*, 2004). Blackberries were domesticated in the 17th century and the first varieties were developed in the 19th and 20th centuries (Galleta and Violette, 1989).

Blackberry varieties grown around the world come from the *Rubus occidentalis* Focke species or from hybrids created from *Rubus idaeus* (Ryabova, 2007). For instance, *Rubus*

parviflorus Nutt. and *Rubus odoratus* L. (Graham and Jennings, 2009) and *Rubus idaeus*, and *Rubus crataegifolius* Bunge (Briggs *et al.*, 1982) are used as source of resistance against pests. *R. crataegifolius* Bunge, *R. palmatus* Thunb, and *R. lambertianus* Hemsley are used to increase the polyphenol content and the antioxidant activity in raspberry varieties (Shigyo *et al.*, 2013). Other significant species for genetic improvement include: *Rubus allegheniensis* Porter, *R. arguti* Weber, *R. caesius* L., *R. canadensis* L., *R. flagellaris* Willd, *R. ursini* Cham, *R. vera-crucis* Rydb, *R. idaeobatus*, and *R. lampobatus* (Clark *et al.*, 2007; Finn, 2008).

Some wild blackberry species are used as commercial crops in different areas of the world: *Rubus glaucus* Benth (South America) (Cancino *et al.*, 2012); *R. armeniacus* Focke (Europe and the Americas); *R. phoenicolasius* Max, *R. coreanus* Miq, and *R. parvifolius* L. (Asia); and *R. chamaemorus* L. and *R. arcticu* L. (North America) (Finn, 2008). In Mexico, the first commercial blackberry plantation was established in 1983, in Tetela del Volcán, Morelos. The Boysenberry (a raspberry-blackberry hybrid) was the chosen variety. The Brazos variety was the first blackberry variety that was planted and, in 1998, it was replaced by the Tupi variety, which has an excellent quality and shelf lifespan (Clark and Finn, 2011).

Nutritional importance of blackberries

Blackberries are eaten fresh and they are also used to prepare fruit juices and concentrates (Siriwoharn *et al.*, 2005). The industrial residues of this products (skin, pulp, and seeds) constitute 4.4-12.2% of the waste resulting from the extraction of the juice. Nevertheless, these residues still have flavonoids, coloring, pectin, and organic acids (Badjakov, 2008; Laroze *et al.*, 2010).

Additionally, blackberries have phenolic compounds (*e.g.*, anthocyanin, flavonols, chlorogenic acid, and procyanidins), which can provide benefits to human health (Moure *et al.*, 2001). Phenols are compounds with potent antioxidant properties and they also remove free radicals protecting major biomolecules against oxidative damage (Who and Consultation, 2003). Several studies have evaluated these compounds in berries (*Rubus*) of different species and varieties (*R.* sp. hyb Marion, *R. laciniatus* Evergreen, *R.* spp. Tupy, and *R. fruticosus*), both as a whole fruit and only its pulp or seeds. These studies have also evaluated several extraction technologies (supercritical carbon oxide extraction, ultrasound-assisted extraction, extraction with pressurized liquids, etc.) in order to retrieve phenols, anthocyanins, fat acids, phytosterols, and tocopherols. These compounds are responsible for antioxidant activity (Siriwoharn and Wrolstad, 2004; Wajs-Bonikowska, 2017). There are less studies about the evaluation of antioxidants in the husks or residues generated by the processing of the berries (Reátegui *et al.*, 2014; Da Fonseca-Machado *et al.*, 2017; Da Fonseca-Machado *et al.*, 2015). Phenolic compounds and dietary fiber are usually studied separately, perhaps as a result of their different chemical structure, physicochemical and biological properties, and metabolic pathways (Saura-Calixto, 2011). However, both are part of vegetable food and are associated with many health benefits. Additionally, they reduce the risk of developing cancer and some chronic diseases (Hooper and Cassidy, 2006; Jaganathan *et al.*, 2014). Dietary fiber plays an essential role in intestinal health and

it also seems to have a significative association with cholesterolemia and the modification of the glycemic response (Anderson *et al.*, 2009).

Genetic improvement of blackberries in Mexico

Currently, there are 99 varieties registered in the Official Gazette of Plant Breeder's Rights of the Servicio Nacional de Inspección y Certificación de Semillas (2023) in Mexico. Out of this total, 48 varieties come from American companies, followed by 40 varieties from Mexico, 6 from Spain, 2 from Chile, 2 from the UK, and 1 from the Netherlands (SNICS, 2022) (Figure 1).

Importance of blackberry cultivation

The profitability and the interest in consuming food with nutraceutical properties, as well as the potential exportation, have been important factors for the fast growth of the worldwide production and commercialization of berries, including blueberry 8 (*Vaccinum corybosum*), strawberry (*Fragaria×anannasa* Duch), raspberry (*Rubus idaeus* L.), and blackberry (*Rubus* spp.) (Carvalho *et al.*, 2010).

Mexico and Papua New Guinea were the two main producers of 2019. The former produced 298,024 t in a 12,900-ha sowing area, obtaining an average yield of 23.1 t per ha; the latter produced 107,642 t in a 21,429-ha sowing area, obtaining an average yield of 5 t per ha. Meanwhile, the USA ranked 8th, with a 28,164-t production sown in 3,439 ha (FAOSTAT, 2022). Guatemala, Colombia, and Mexico produce blackberries from September to December. Therefore, this is an opportunity to supply the American demand for this product, because its domestic production decreases during those months as a result of weather conditions. This situation means that the production could be profitable (Muñoz and Juárez, 1997). The great advantage of Mexico is its production window from November to June. This is a very important period during which most of the producer countries cannot meet the demand. Guatemala is the exception, because its productive window lasts from November to August (Ibarra-Morales *et al.*, 2013).

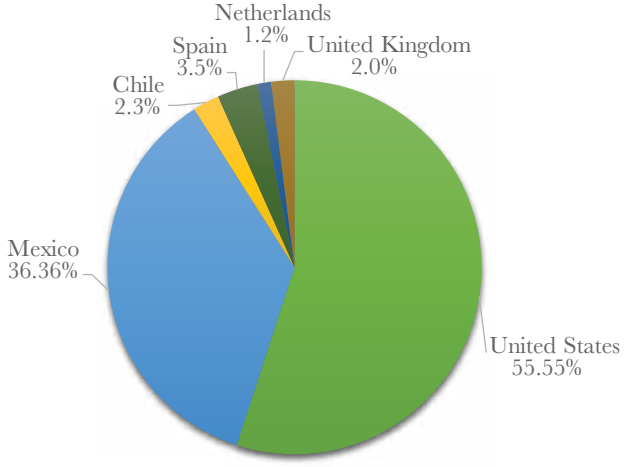

Figure 1. Percentage of participation of the blackberry producer countries in the Official Gazette of Plant Breeder's Right of the Servicio Nacional de Inspección y Certificación de Semillas (2022).

In the first sixteen years of the 21st-century, blackberry production in Mexico had an important and continuous growth: from 1,911.90 ha sowing area in 2000 to 13,081.89 ha in 2016. However, the sowing area decreased to 9,585.16 ha in 2021, obtaining a 212,316-t production and a yield of 22.94 t per ha (Figure 1). Subsequently, the sowing area increased again in 2022 to 222,623.05 t, obtaining an average yield of 23.1 t per ha (SIAP, 2023). The increase in the blackberry Mexican production is mainly the result of the modification of production practices and the introduction of new varieties, whose development does not require cold temperatures (Clark and Finn, 2011). Figure 2 shows an increase in the development of new varieties in Mexico. The domestic production is mainly distributed in the following states: Michoacán, (205,066 t), Jalisco (12,456.11 t), Colima (1,532.58 t), and Baja California (2,462.59 t) (SIAP, 2023).

Exportation and importation

Blackberry cultivation is an important economic source, as a result of the direct and indirect employment generated by its production and commercialization. During the last decade, Mexico was classified as the main worldwide exporter of berries (Sánchez, 2008). In 2014, Mexico exported 123 t of berries, with a value of US$659 million. The main destinations of Mexican berries include: USA, the Netherlands, United Kingdom, Italy, Belgium, France, Canada, Germany, and Chile. Mexico mainly imports frozen fruits (<1,000 t), with an average value of US$3 million (FND, 2015). In 2020, Mexico held the second place (19%) in the blackberry exportation market, only surpassed by Spain (22.54%). Meanwhile, the USA, Canada, and Germany imported 45.85%, 9.66%, and 9.33%, respectively, of the Mexican production (FAOSTAT, 2022).

On the one hand, these two markets (USA and Canada) are potential sale markets for Mexican producers. Their proximity to Mexico and the already established commercial relationship with both countries will facilitate the transportation of the product. On the other hand, factors such as perishable fruit with a short shelf lifespan and the geographic

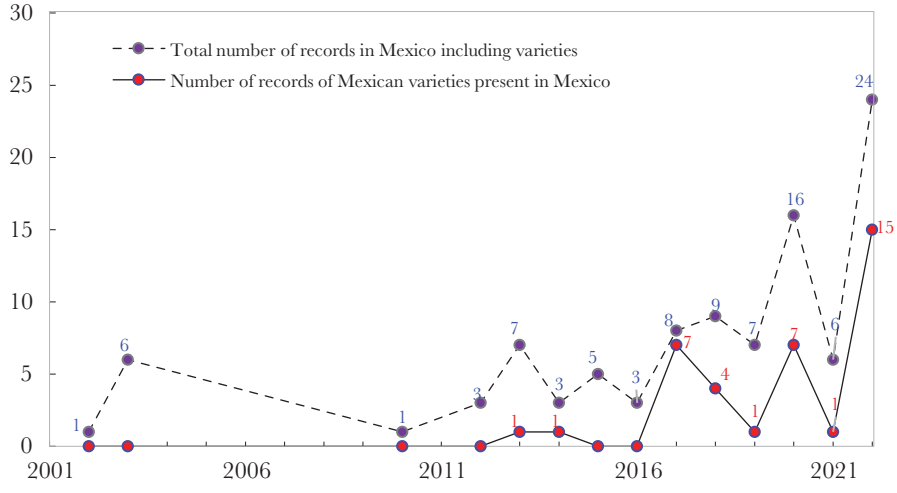

Figure 2. Number of registrations of blackberry varieties in the SNICS per year.

and weather conditions means that Mexican producers play a significant role in the commercialization for these two markets (Ibarra-Morales *et al.*, 2013).

CONCLUSIONS

Blackberry cultivation is an important economic source, as a result of the direct and indirect employment generated by its production and commercialization. Additionally, Mexico is one of the main exporters of blackberry worldwide. Finally, blackberry consumption provides health benefits and prevents the development of metabolic diseases.

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Colegio de Postgraduados

Potential zones for the cultivation of *Actinidia chinensis* var. deliciosa in temperate regions of Veracruz, Mexico

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ABSTRACT

Objective: to identify potential zones with soil and climate characteristics in municipalities of the state of Veracruz, Mexico for kiwifruit cultivation through modeling.

Design/Methodology/Approach: with the zoning methodology of soil and climate variables and the Kriging projection algorithm of the ArcMap GIS®, the municipalities of the state of Veracruz with soil and climate potential for the cultivation of kiwi adapted to tropical conditions were determined. The Kruskal-Wallis test was used to validate the zoning and determine the similarity of municipalities with soil and climate potential. A cluster analysis was applied to assess the similarity between the variables studied.

Results: the municipalities of Hueyapan de Ocampo, Ixhuatlán del Café, Jalacingo, Magdalena, Mariano Escobedo, Tehuipango and Texhuacán present average soil and climate characteristics for the establishment of kiwi cultivation. Chumatlán and Huatusco presented the greatest soil and climate similarity for the cultivation of this fruit shrub.

Limitations of the study/Implications: this information contributes to the decision-making to establish kiwi by increasing the knowledge of the species. As, up to date, the almost non-existent information has limited the establishment of kiwi cultivation.

Findings/Conclusions: of the total territory of Veracruz 29% shows soil and climate characteristics to introduce kiwi cultivation. Its establishment would represent support for food and socio-economic sovereignty for producers. According to this study, the establishment of kiwi as a crop is viable in various geographical points of Veracruz.

Keywords: *Actinidia chinensis*, Veracruz, introducing alternative crop, new fruit plants.

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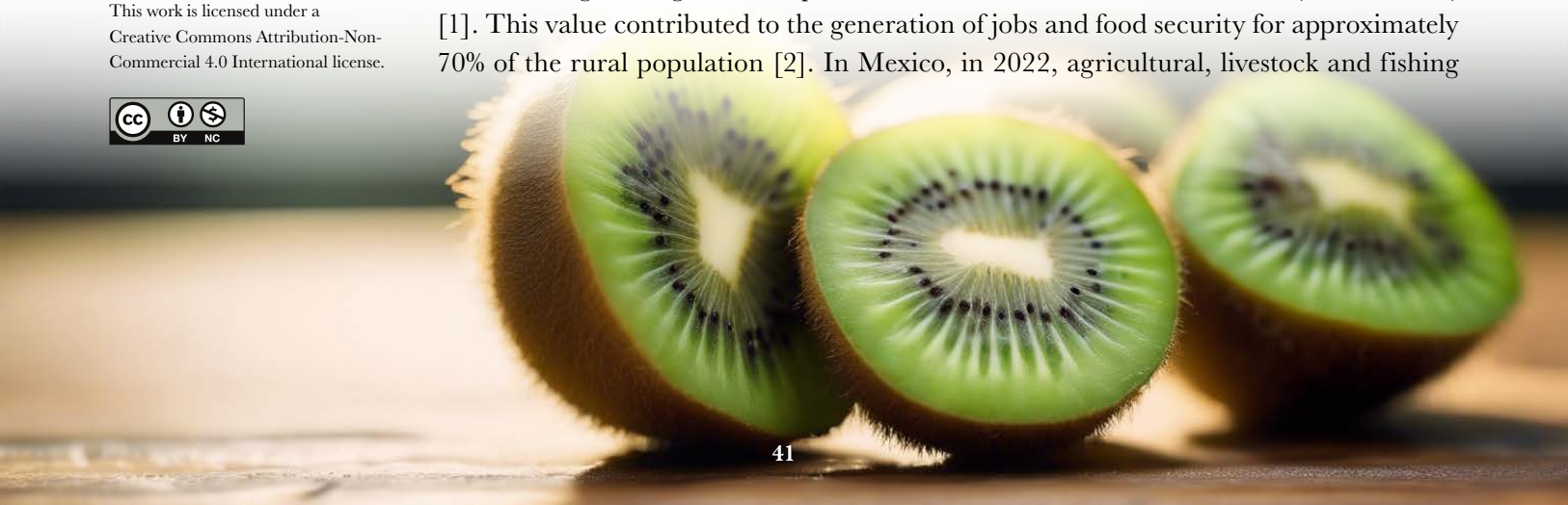
INTRODUCTION

In 2021, global agricultural production was estimated at 4,154,467.01 (million USD) [1]. This value contributed to the generation of jobs and food security for approximately 70% of the rural population [2]. In Mexico, in 2022, agricultural, livestock and fishing



production was 297,626,608 tons, of which the state of Veracruz contributed 31,804,750 tons. With this figure, the state of Veracruz occupied the second place among those with the highest production [3]. However, such value is mostly due to intensive crops such as sugarcane (*Saccharum officinarum*), coffee (*Coffea arabica*), lemon (*Citrus×limon*), orange (*Citrus×sinensis*) and chayote (*Sechium edule*). Intensive crops generate a high rate of deforestation and environmental problems [4]. In this context, it is important to introduce alternative crops that allow the diversification of traditional agricultural systems, promoting land use, profitability, food production, jobs, and economic gains, while focusing on sustainability [5].

Kiwifruit (*Actinidia chinensis* Planch; Ericales: Actinidiaceae), is a crop with wide acceptance in the market due to flavor, high content of ascorbic acid (vitamin C), antioxidants and fiber content [6]. In Mexico, the surface area of kiwifruit cultivation is almost zero, so to supply the domestic demand, this fruit is imported from Chile, USA, Italy, and New Zealand. This causes raising in the cost of kiwifruit to 3-6.5 USD ($60 and $130 MXN pesos) per kg in the national market [7].

Due to the fact that there is already an experimental orchard producing green kiwi (*Actinidia chinensis* var. deliciosa) adapted to tropical high-altitude conditions in Huatusco, Veracruz [8]; it is possible that, based on the soil and climate conditions of that geographical point of the kiwi orchard, the environmental coincidence in other municipalities of the state can be modeled, which would allow the identification of potential areas for the implementation of its cultivation [9].

Soil and climate zoning is a technique for mapping the environmental capacity of a geographical space, which a species can take advantage of [10]. In order to obtain soil and climate zoning, data sources are required on soil and climate characteristics needed for the development of the crop [11]. This makes it possible to identify, among a wide range of climate variables, those with similarity in other regions and to make decisions about the viability of areas to establish a given crop [12].

Therefore, the objective of this study was to identify potential zones with soil and climate characteristics in municipalities of the state of Veracruz, Mexico apt for kiwi cultivation (*A. c.* var. deliciosa).

MATERIALS AND METHODS

The state of Veracruz was selected to identify municipalities with geographic areas with environmental potential for kiwi cultivation. Soil and climate parameters of 112 municipalities in the state of Veracruz were evaluated. To this end, the characteristics of the soil and climate conditions of temperature, dominant soil, land use, mean annual precipitation, altitude and type of climate were collected. These variables were obtained from the INEGI database [13], as well as from climate-environmental reports [08].

In the experimental orchard with kiwi cultivation [Project: Cultivo de kiwi (*Actinidia chinensis*) alternativa para las montañas del estado de Veracruz-México. 23170-C-87, Universidad Autónoma Chapingo], the variables were monitored and recorded daily during the year 2021 and 2022, with an electronic device that has temperature and relative humidity sensors, integrated into a USB device (EXTECH brand datalogger

model RHT 10). To determine the soil characteristics of the orchard, a profile analysis was carried out in accordance with NOM-021-RECNAT-2000, AS-01 and AS 09 [14] (Table 1).

With the information on the soil and climate characteristics obtained from the area of the experimental kiwi orchard, the Agroecological Zoning (AEZ) methodology proposed by the FAO [15] was implemented and adjusted, which aims to locate areas with optimal conditions for the cultivation of green kiwifruit. Additionally, the methodological proposal to analyze the agroecological potential of *Moringa oleifera* Lam. for the state of Veracruz [16] was followed; for this, the soil and climate factors that favor the development of kiwi cultivation were used.

In order to elaborate the potential zoning, databases and layers of soil and climate content were developed, and through the plugin Point Sampling Tool in Q-GIS©, the information of each environmental layer was collected for each of the municipalities of the state of Veracruz. Once collected, data were analyzed to select those municipalities that were within the environmental range (ecological valence) that the kiwi plant requires for its establishment. ArcMap® 10.3.1 was used to generate models to identify optimal areas for kiwifruit development [17]. Where the soil and climate layers were subjected to the spatial interpolation procedure by the Kriging method, because the algorithm is related to the best unbiased linear predictor (BULP o MPLI). This reduces the variance of errors in the prediction, making this zoning method more reliable [18].

The criteria for classifying the municipalities were set at high (100-80%), medium (40-79%) and low (1-39%) coincidence within the range of optimal soil and climate conditions in the municipalities of Veracruz, which should be statistically similar to those of the experimental kiwi orchard. To prove that the soil and climate conditions present in the experimental orchard were statistically similar to those predicted by the zoning models, a non-parametric Kruskal-Wallis's analysis of variance [19] and a rank comparison test were performed for each soil and climate variable. Additionally, a principal component analysis was applied to identify the influence of soil and climate variables in the municipalities for kiwi plantation. These analyses were performed at $P<0.05$ of confidence level with InfoStat [20].

Table 1. Soil and climate characteristics of the experimental kiwi orchard in the town of Elotepec, municipality of Huatusco, Veracruz, Mexico.

Coordinates	Latitude 19.187 N; Longitude -97.187 W.
Temperature (°C)	10.75
Climate	Warm-wet
Mean altitude (msnm)	1950
Average annual precipitation (mm)	1800
Agricultural land use (%)	52.18
Dominant soil (%)	Andosol (44.28)
	Luvisol (42.88)
	Leptosol (9.87)

RESULTS AND DISCUSSION

The total area of the state of Veracruz is 71,820 km^2, of which 17,651 ha are dedicated to the agriculture of various crops according to the soil, climate, and environmental conditions. In this context, the zones for the adaptation of kiwifruit to tropical high-altitude conditions was located in 60 municipalities which present soil and climate characteristics similar to those of the experimental orchard (Figure 1).

Soil and climate factors limit or promote the development and production of kiwifruit. Temperature, precipitation, altitude, and soil type are characteristics of physiological importance for kiwi plant development development [21]. Since kiwi cultivation requires short winters with moderately low temperatures in spring; without risk of frost and mild summers, a rainfall of between 1300 and 1500 mm, deep soils, and good drainage capacity. All of those coincide with the data reported in Table 2, which describes the average and within range values of soil and climate conditions of the municipalities with characteristics for kiwi cultivation.

The Kruskal-Wallis non-parametric analysis of variance (Table 3) indicated that the areas identified in the zoning models for the establishment of kiwi cultivation were significantly equivalent to those recorded in the experimental orchard in the town of Elotepec, Huatusco, Veracruz. For this reason, it is considered feasible to establish small

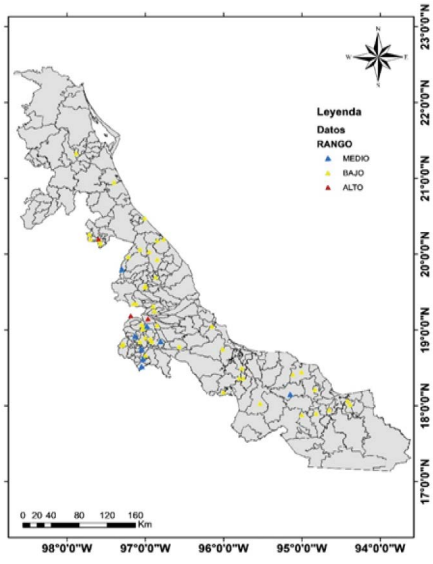


Figure 1. Municipalities with soil and climate conditions for kiwi cultivation.

Table 2. Soil and climate parameters for municipalities with a high percentage of coincidence for the establishment of a kiwifruit crop.

Statisticians	Temperature (°C)	Altitude masl	Precipitation (mm)	Soil type%		
				Andosol	Luvisol	Leptosol
Average	18	1223	1813	29	28	10
Standard deviation	7	768	1047	25	25	0.1
Maximum value	24	1950	1825	44	43	10
Minimum value	11	420	1800	0	0	10

Table 3. Kruskall-Wallis non-parametric analysis of variance to identify the similarity in soil and climate conditions in the selected municipalities with the agroecological zoning, compared to those of the experimental kiwi orchard.

Kruskai wallis in pairs		
Variable	**H**	**P**
Temperature	59.75	0.4757
Altitude	58.87	0.4755
Precipitation	58.54	0.4755
Suitable for agriculture	60.00	0.4757
luvisol soil	51.34	0.4757
Leptosol soil	47.67	0.4757
andosol soil	49.62	0.4757

or backyard plantations in the municipalities indicated, to evaluate the adaptability of the kiwi plant and subsequently evaluate its productivity.

The principal components analysis (Figure 2) indicated that the municipalities with the least variation and higher similarity in soil and climate factors to those of the experimental orchard were Magdalena, Texhuacán, Tehuipango, Huatusco, Ixtaczoquitlán, Sochiapa and Aquila.

Soil and climate conditions have an impact on agricultural productivity. The relationship between a crop and the soil is influenced by various interactions among soil physical and

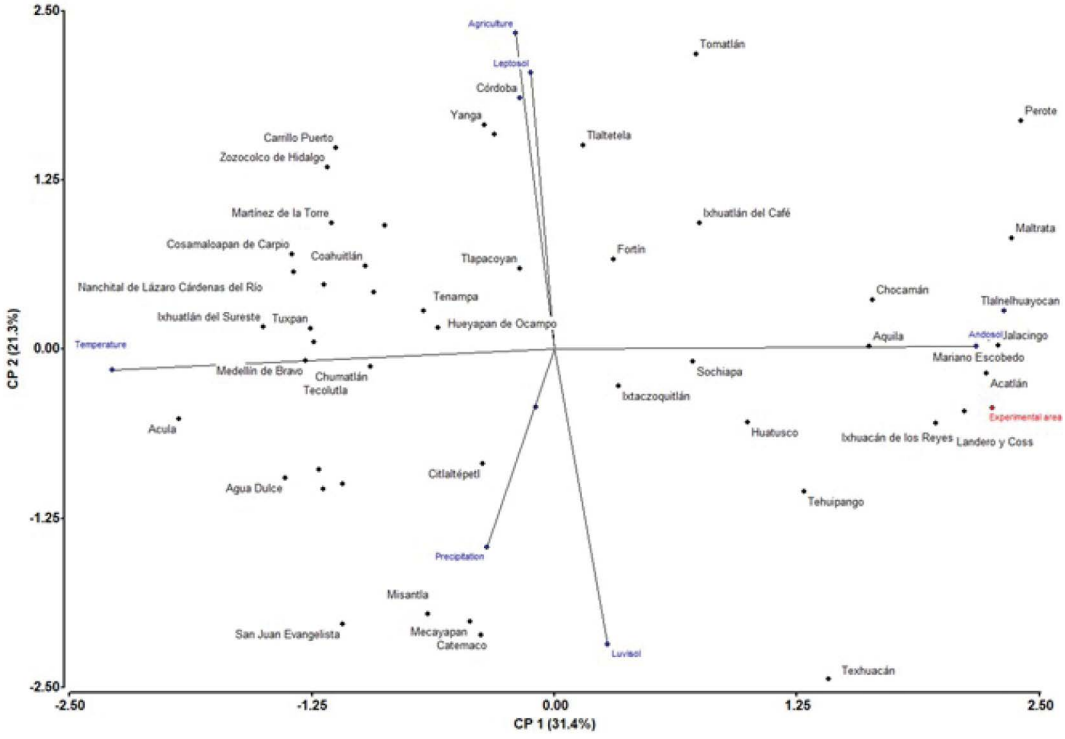


Figure 2. Soil and climate parameters for municipalities with three or more coincidences for the establishment of kiwifruit in Veracruz.

chemical conditions, and external environmental factors [22]. When compared to the soil and climate conditions of the kiwi introduction orchard, 60 municipalities of the state of Veracruz showed soil and climate coincidences. Out of which, 49 municipalities showed a low coincidence percentage; seven a medium percentage (Hueyapan de Ocampo, Ixhuatlán del café, Jalacingo, Magdalena, Mariano Escobedo, Tehuipango and Texhuacán); and two, a high coincidence percentage (Huatusco and Chumatlán) (Figure 3). However, municipalities with less coincidence in conditions can adapt them through agricultural management, such as irrigation, substrates at different concentrations, considering the requirements of the kiwi crop.

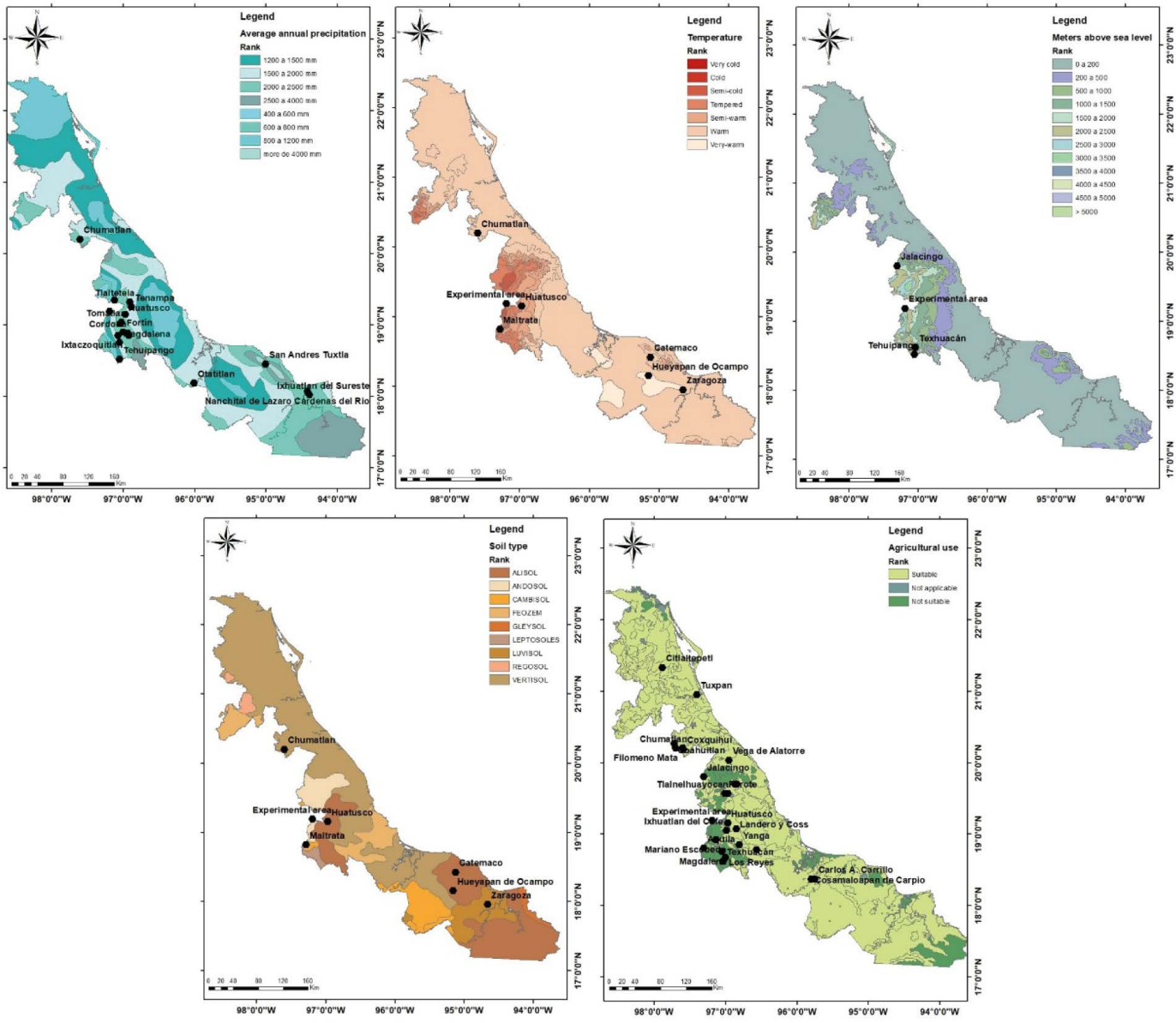


Figure 3. Municipalities of Veracruz (Mexico) with soil and climate aptitude for kiwifruit.

CONCLUSIONS

The municipalities of Veracruz with the greater soil and climate conditions similarity to the experimental orchard were Huatusco and Chumatlán. These municipalities have an average annual temperature of 18 °C, 1813 mm of precipitation and are located at an altitude of 1223 m, with Andosol (29%), Luvisol (28%) and Leptosol (10%) soils available for kiwifruit cultivation.

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Colegio de Postgraduados

Mass propagation of tobala mezcal maguey (*Agave potatorum* Zucc.) in a temporary immersion system compared with a solid medium

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ABSTRACT

Objective: To assess a temporary immersion system for the *in vitro* propagation of *Agave potatorum* Zucc., compared with the traditional micropropagation technique that uses a solid medium.

Design/Methodology/Approach: The effect of treatments in a solid medium with low and high doses of the BA (Benzylaminopurine) growth regulator (0.5 mg L^{-1} and 2 mg L^{-1}) on the number of sprouts per explant was assessed in a first phase. Since the best treatment was 2 mg L^{-1} of BA, three forms of propagation were considered: solid medium, liquid medium in a paper bridge, and liquid medium in a temporary immersion system.

Results: From the initial test, an average of 6.6 shoots per explant were obtained with 2 mg L^{-1} of BA. Regarding the different systems, the solid medium, the paper bridge, and the temporary immersion system recorded 6.4, 7.2, and 14.4 shoots per explant, respectively.

Findings/Conclusions: Mass sprout production is higher in the temporary immersion system, as a consequence of the use of a liquid medium that increases the absorption of nutrients and regulators, combined with the injection of air with oxygen that can accelerate cellular processes.

Keywords: Micropropagation, RITA®, liquid medium.

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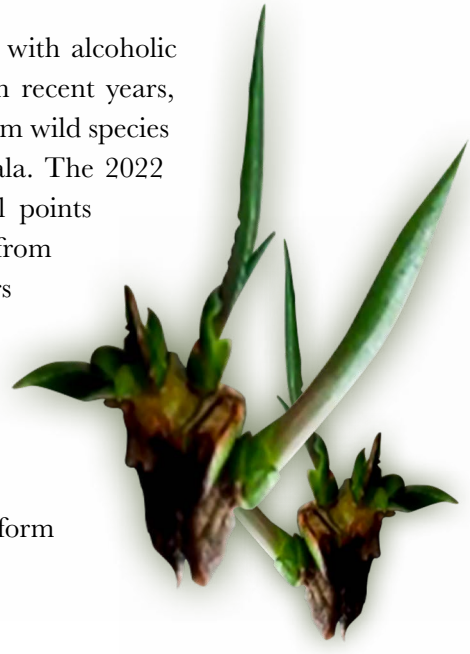
INTRODUCTION

Maguey or agave (*Agave* L.) production is associated with alcoholic beverages such as pulque, sotol, tequila, and mezcal. In recent years, mezcal demand has grown exponentially, particularly from wild species such as *Agave potatorum* Zucc., popularly known as tobala. The 2022 statistical summary of the Mezcal Regulatory Council points out that the production of certified mezcal increased from 1,044,696 million liters (2012) to 8,099,591 million liters (2022), an indirect indication of the plant demand required to satisfy mezcal production. In the case of *A. potatorum*, it does not generate shoots (adventitious sprouts) and its propagation is therefore asexual (Gentry, 2004). Consequently, several authors have explored propagation per tissue culture as an alternative form



of multiplication which does not require waiting for seed production. Bello *et al*. (2023) recently reported the use of temporary immersion systems for the *in vitro* propagation of this species. Previous research has raised interest on the assessment of the difference in the multiplication rate of the traditional micropropagation technique in a solid medium versus a temporary immersion system.

METHODOLOGY

Establishment under aseptic conditions

To set up the initial material of *A. potatorum* under aseptic conditions, basal buds were taken from young 8-month-old plants. The collection was carried out in 2 locations (Table 1). All specimens were disinfected as follows: 4 washes with liquid soap and rinsing with running water, immersion in a 70% alcohol solution for 2 min, followed by immersion in a 30% sodium hypochlorite solution for 18 min, and finally 3 rinses with sterile water (in a laminar flow hood). At the end of this process, the explants in test tubes were randomly assigned to the media under evaluation.

Culture media

The study was carried out in 2 sequential tests. Three treatments were assessed in the first test, using the Murashigue and Skoog (1962) "MS" solid medium (with vitamins) as basis. Treatments consisted of the application of different doses of the BA (Benzylaminopurine) growth regulator: treatment 1, 0 mg L^{-1} (control); treatment 2, 0.5 mg L^{-1} (low dose); and treatment 3, 2.0 mg L^{-1} (high dose). The growth regulator had three levels, which matched the doses in a solid medium.

In the second test, the high dose (2.0 mg L^{-1}) of growth regulators in solid medium —which had the best biostatistics performance in the first test— was considered as a reference value; two immersion systems were also analyzed as part of this test. Afterwards, the immersion systems factor —keeping 2.0 mg L^{-1} of BA as a constant— included 3 treatments: a) Treatment 1, solid medium as control; b) Treatment 2, liquid medium in paper bridge (filter paper as bridge and 7 mL of medium per tube); and c) Treatment 3, liquid medium in a RITA® immersion system (50 mL of medium in the container, immersion every 4 h, and 3-min immersion periods).

In both the preliminary test and the immersion system comparison, the final measurement of the number of shoots variable and the length per explant variable was made at 60 days. In both tests, the experimental units (test tubes) were randomly assigned to the treatments. In the conditions under which the study was carried out, a completely randomized design was considered, in which the growth regulator dosage and the immersion system were the factors in the first and second tests, respectively.

Table 1. Origin of shoots used as initial explants.

Accession	Sprout (Number)	Common name	Scuentific Name	State	Location
A-O-01	20	Agave Tobalá	*Agave potatorum*	Oaxaca	Miahuatlán Cerro Metate
A-O-02	30	Agave Tobalá	*Agave potatorum*	Oaxaca	San Pedro Teozacoalco San José Rio Minas

Table 2. Treatments in solid medium to determine sprout formation response (Phase 1).

Treatment	Media	Growth regulator and concetration
1	Murashigue y Skoog	Sin regulador (testigo)
2	Murashigue y Skoog	BA 0.5 mg L^{-1} (dosis baja)
3	Murashigue y Skoog	BA 2.0 mg L^{-1} (dosis alta)

Table 3. Treatments of the comparison of *in vitro* propagation systems (Phase 2).

Treatmet	Sistem	Media	Growth regulator
MSBALFS	Medio sólido	MS	BA 2 mg L^{-1}
MSBALFP	Puente de papel	MS	BA 2 mg L^{-1}
MSBALSI	RITA®	MS	BA 2 mg L^{-1}

Plant acclimatization

As suggested by Monja-Mio *et al.* (2020), plant acclimatization was carried out in two stages: laboratory and greenhouse. In both cases, the same substrate was used: 3 equal parts of Peat Moss®, vermiculite, and perlite sterilized in an autoclave at 120 °C for 30 min. For laboratory acclimatization, the plants remained in plastic containers with lids for 6 weeks. Once they were taken to the greenhouse, the plants were transplanted to 1-L pots with the same substrate for 60 days, before they were transferred directly to soil of the field. After 60 days, their development was assessed.

Statistical analysis

Growth regulator dosage test

Based on the experimental design, the data were analyzed considering a Generalized Linear Model which, according to Stroup (2014), has the following linear predictor:

$$\eta_j = \eta + \tau_j \text{ y } \eta_j = \log\left(\mu_j\right), \text{ then, } \mu_j = \exp\left(\eta + \tau_j\right).$$

Where: $y_{ij} \sim Negative\ Binomial\left(\mu_j, \mu_j + \psi\mu_j^2\right)$ is the number of sprouts in the *i-th* explant of the *j-th* treatment. In this case, the variance contains an overdispersion term $\psi\mu_j^2$: the larger ψ, the larger the overdispersion. η represents the mean value for the reference level. τ_j represents the log-mean difference for the level of the treatment with the reference level.Additionally, $y_{ij} \sim Gamma\left(\mu_j, \Phi\mu_j^2\right)$ is the length of sprouts in the *i-th* explant of the *j-th* treatment. Since the experimental units did not form clusters, neither intensively, nor naturally, the were considered independent, within and between treatments.

Immersion systems test

In this case, the number of sprouts variable took into consideration the negative binomial distribution, while the length of sprouts variable was modeled using the inverse Gaussian distribution. Therefore, $y_{ij} \sim IGAUSS\left(\mu_j, \Phi\mu_j^3\right)$.

Given the nature of the statistical model —which identifies the stochastic process of data—, the analysis was performed using PROC GLMMIX (SAS, 2018), specifying the negative binomial distribution (for the number of sprouts) or the range of the Gaussian inverse (for their length).

RESULTS AND DISCUSSION

Growth regulator dosage test, Phase 1

Number of sprouts

The basic statistical values for this variable were: minimum=1, mean=3.63, maximum=11, and variance=9.4. The variance registered an almost triple value. Figure 1 shows that the number of sprouts of treatment 1 recorded the lowest value, while treatment 3 was slightly higher than the mean of 6 (empty circle). The variability of the observations in treatment 3 was also significantly higher than in treatments 1 and 2. The interquartile range of treatment 3 was 6.

The statistical analysis that considered the negative binomial distribution helped to identify the differential effect on the number of sprouts variable of the treatments assessed. Therefore, F had a value of 17.31 (p-value <0.0001), based on 2 and 27 degrees of freedom in the numerator and in the denominator, respectively. Figure 2 shows the means predicted for each treatment, as well as the 95% confidence intervals. Once again, these results confirm that treatment 3 had a better mean value (6.6) than treatments 2 and 3. The analysis of the multiple comparison of pairs of treatments using Tukey's test reported that 3 perfectly identifiable independent groups are formed (Table 3).

The statistical analysis based on the negative binomial distribution was considered appropriate, because the Shapiro-Wilks test for normality of the residuals showed a value of 0.94 and a p-value of 0.1176. In fact, compared with those predicted under normal conditions, the observed percentiles of the Pearson residuals practically fall in a straight line that passes through the y-intercept (Figure 3).

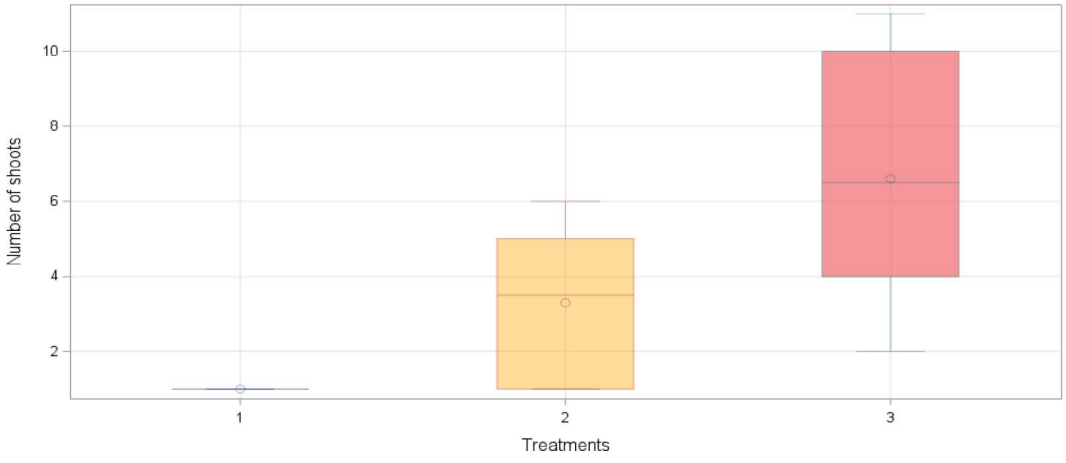


Figure 1. Distribution of the number of shoots per treatment.

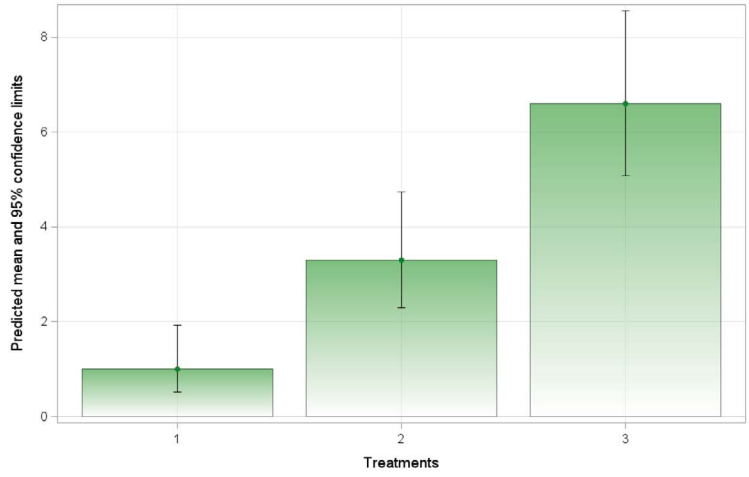


Figure 2. Mean number of shoots (prediction) and 95% confidence intervals.

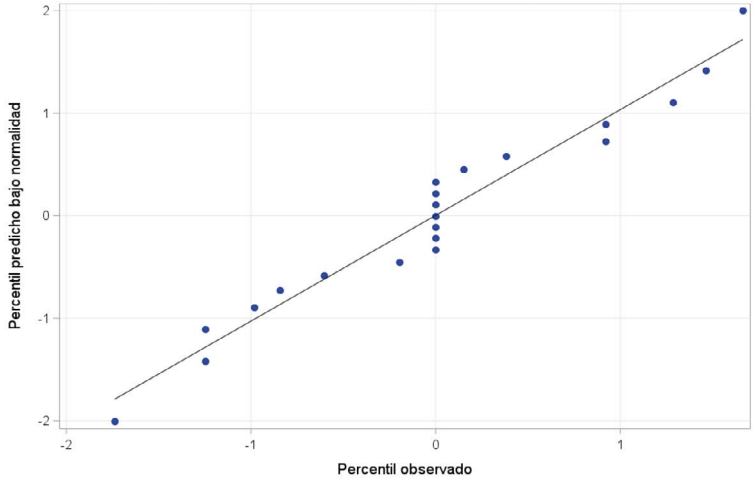


Figure 3. Percentiles (observed and modeled under normality) of the residuals resulting from the analysis of the negative binomial distribution.

Table 4. Tukey's grouping considering differences in predicted means.

Treatment	Media	Cluster
3	6.6	A
2	3.3	B
1	1.0	C

Shoots length (cm)

Regarding sprout length, treatment 3 had the highest average, slightly exceeding 4.5 (empty circle). Treatment 1 was the most variable with an interquartile range of 1 (Figure 4).

The statistical analysis that considered the sprout length variable with gamma distribution detected that this variable behaves differently in each of the treatments. F had a value of 7.85 (p-value=0.0021), calculated starting from 2 degrees of freedom in

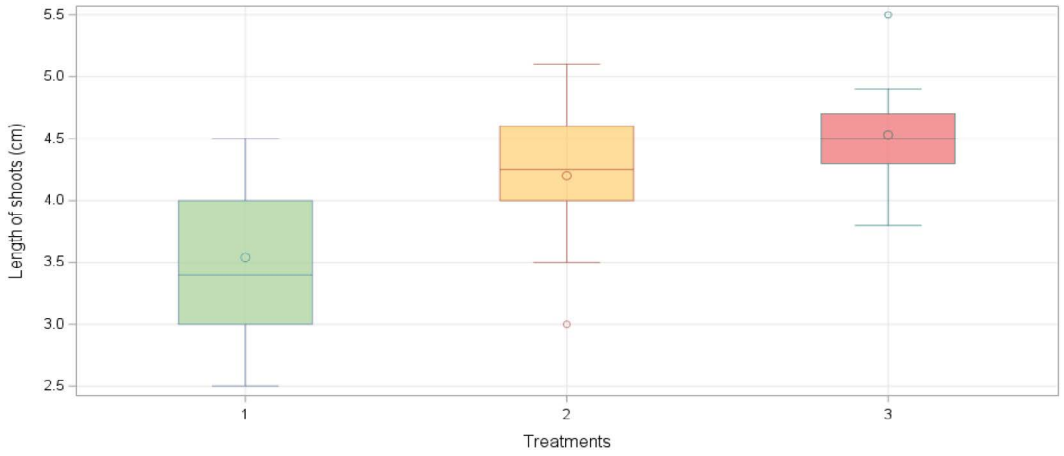


Figure 4. Sprout length per treatment.

the numerator and 27 in the denominator. In light of these results, at least 2 means are different. Figure 5 shows the predicted means and 95% confidence intervals for the sprout length variable for each treatment. Treatments 1 and 2 were surpassed by treatment 3. According to Table 5, two groups were analyzed through the multiple comparison of pairs of treatments using Tukey's test (0.05 significance level); treatments 2 and 3 were included in the same group —a logical conclusion of the observation of an overlap in a large percentage of their respective confidence intervals.

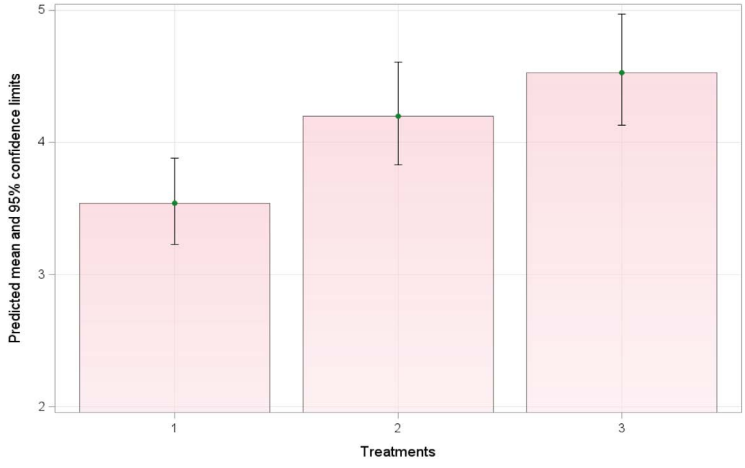


Figure 5. Predicted mean of sprout length and 95% confidence intervals.

Table 5. Tukey grouping considering differences of the predicted means (alpha=0.05).

Treatment	Media	Cluster
3	4.53	A
2	4.20	A
1	3.54	B

The Shapiro-Wilks test for normality of the residuals obtained a value of 0.97 and a p-value of 0.6620. The percentiles observed for the Pearson residuals versus those predicted under normality generally fall on a straight line crossing the y-intercept. Consequently, a gamma distribution-based statistical analysis is considered appropriate.

Propagation systems test, Phase 2

Number of shoots

For this variable, the minimum, mean, maximum, and variance values were 3, 9.4, 18, and 16.73, respectively. Variance was almost two times higher than the mean. Therefore; this result (as in the previous test) justified the use of the negative binomial distribution. Results showed that treatment 3 (RITA® temporary immersion system) generated 14.4 sprouts per explant, while treatment 1 (traditional system with a solid medium) barely reached 6.4 sprouts per explant and treatment 2 (immersion in paper bridge) recorded just 7.4. It should be noted that treatment 1 had the greatest variability, leading to a variance of 5.3 and an interquartile range of 4 (Figure 7).

Considering the F-test value —equal to 19.30, based on 2 and 27 degrees of freedom in the numerator and the denominator, respectively—, as well as a significance level of 0.05, the treatments have a significant differential effect on the discrete number of the sprouts variable. Consequently, the means predicted for treatments 1, 2, and 3 (6.4, 7.4, and 14.34, respectively), as well as the 95% confidence intervals, show that treatment 3 significantly exceeded the other two treatments under study (Figure 8). In fact, pairwise multiple comparisons of observations made with Tukey's test identified 2 groups (Table 6). It should be noted that treatments 1 and 2 were put in a single group (Table 7).

Statistical analysis based on negative binomial distribution is considered appropriate, because the Shapiro-Wilks test for normality of Pearson residuals yielded a statistic of 0.97 and a p-value of 0.7122. Furthermore, the graph of the percentiles observed in Pearson

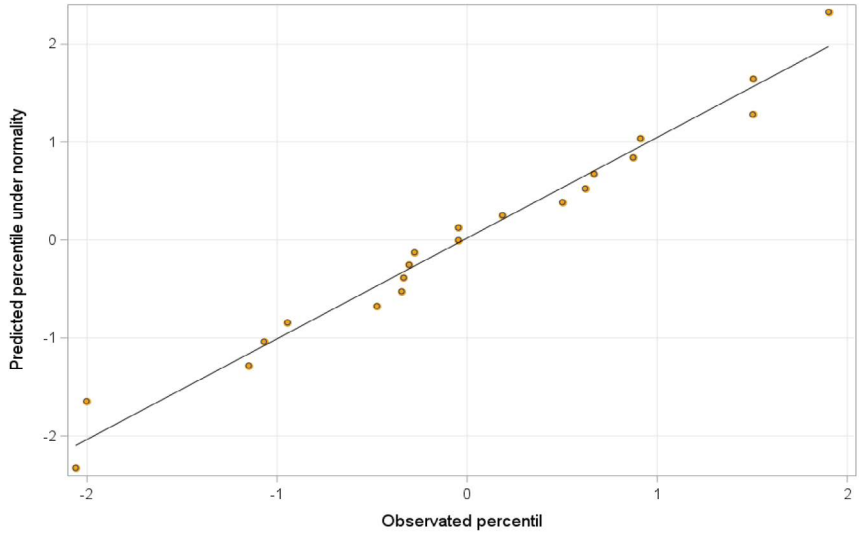


Figure 6. Percentiles (observed and modeled under normality) of Pearson residuals obtained through gamma distribution.

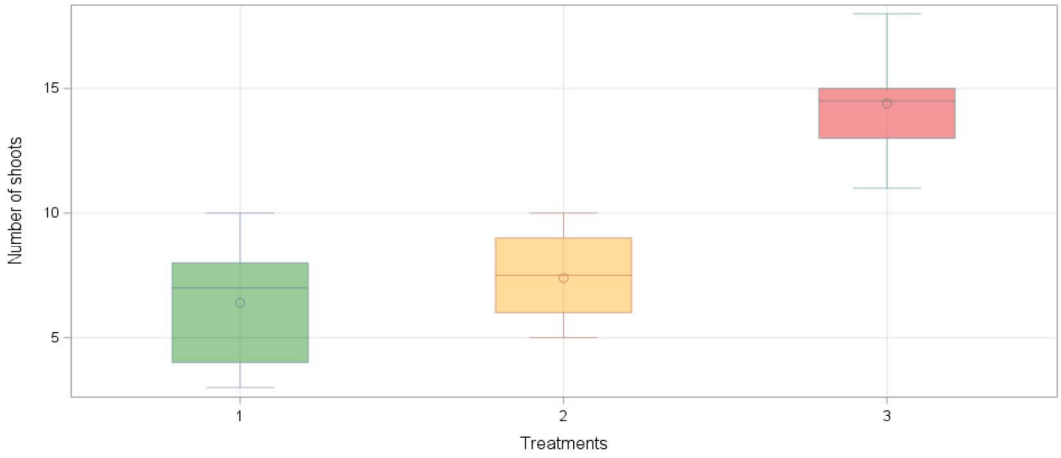


Figure 7. Number of sprouts per treatment.

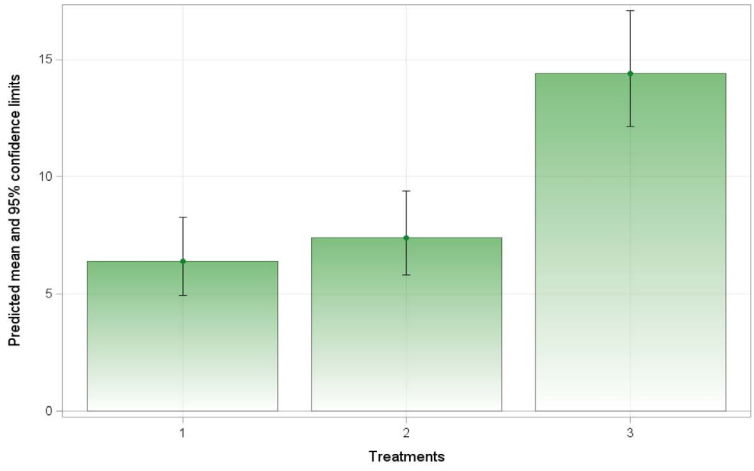


Figure 8. Predicted mean and 95% confidence limits of number shoots of diferents treatments.

Table 6. Tukey grouping considering differences between predicted means (alpha=0.05).

Treatment	Media	Cluster
3	14.40	A
2	7.40	B
1	6.40	B

residuals and those modeled considering normality indicates that they are distributed along a straight line that passes through the y-intercept (Figure 9).

Shoots length

This variable recorded values of 2, 3.42, 6.90, and 2.45, for the minimum, mean, maximum and variance, respectively. Treatment 3 reached a mean of 5.49 cm; this result

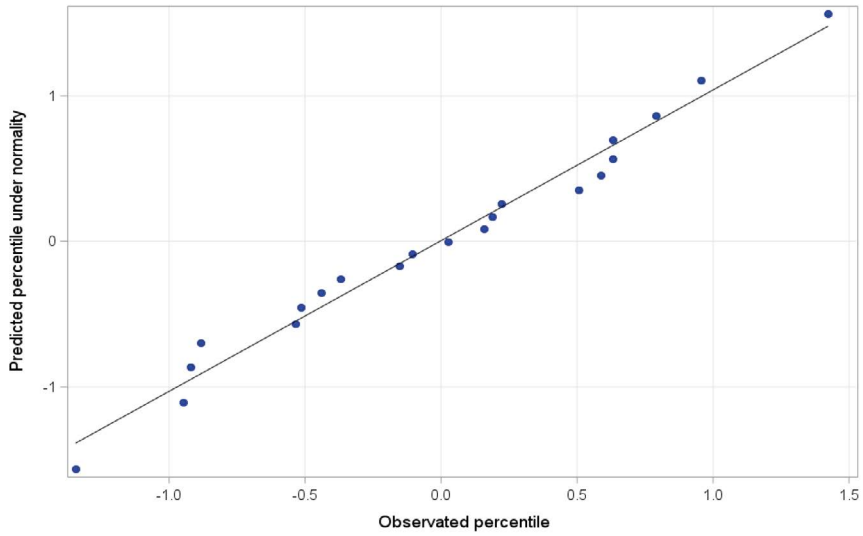


Figure 9. Percentiles (observed and modeled under normality) of Pearson residuals obtained through negative binomial distribution.

was significantly higher than treatments 1 and 2, which showed similar mean values (≤2.5 cm). Treatment 3 also showed a slightly larger interquartile range than treatment 2 (Figure 10).

The value of the F-test was 87.61, with a p-value significantly lower than 0.05, suggesting that the immersion systems have a different influence on the behavior of the sprout length variable. The mean of treatment 3, as expected, exceeded the means of treatments 1 and 2 by a little more than twice. In this sense, the 95% confidence interval of treatment 3 had noticeably different values than the other 2 treatments (Figure 11). Therefore, the analysis of the multiple pairwise comparisons of the means made with Tukey's test generated one group made of two treatments (1 and 2) and a group with a single treatment (3) (Table 7).

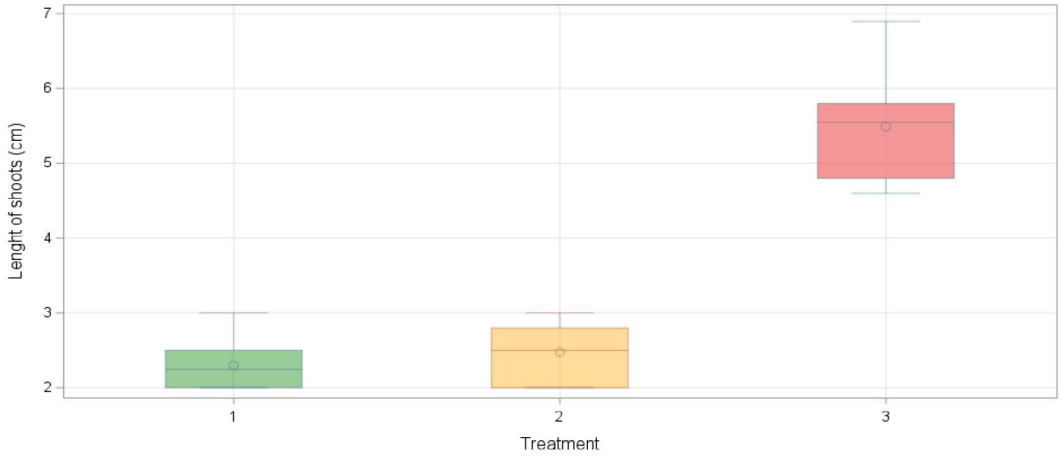


Figure 10. Shoots length per treatment.

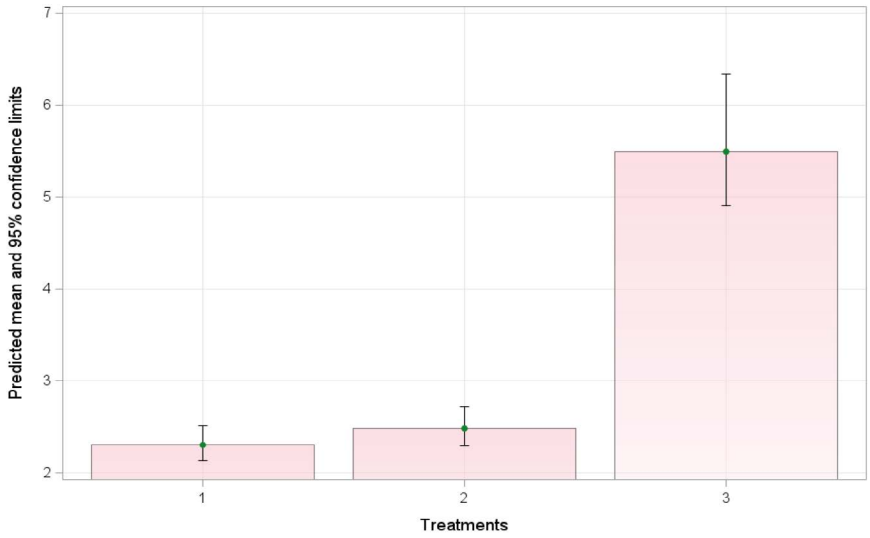


Figure 11. Predicted mean and confidence intervals of sprout length from different treatments of the immersion system.

Table 7. Tukey grouping considering differences between the predicted means (alpha=0.05) for the sprout length variable.

Treatment	Media	Cluster
1	2.30	A
2	2.48	A
3	5.49	B

Even though dispersion of the percentiles of residuals observed and modeled under normality deviate slightly from the y-intercept, modeling of the sprout length variable in the immersion system can be considered acceptable, given the value (W=0.95) and p-value (0.2190) yielded by the Shapiro-Wilks test of normality (Figure 12).

Meanwhile, the number of sprouts was significantly higher with the temporary immersion system (14.4) than with the solid medium (6.4). Authors such as Ríos-Ramírez *et al.* (2017) recorded a proliferation of 32 sprouts per explant in *A. agustifolia*, on a solid medium with a 4 mg L^{-1} concentration. Monja-Mio *et al.* (2021) found similar results and reported higher sprout production in *A. angustifolia*, when they compared a solid medium with the RITA® temporary immersion system. Authors such as Ramírez-Mozqueda *et al.* (2022) and Correa-Hernandez *et al.* (2022) reported a substantial increase in sprout production using the Ebb-and-Flow temporary immersion system with *Agave potatorum*. Ontaneda *et al.* (2020) mentioned the cost-wise efficiency of the temporary immersion systems, resulting from the increase and obtaining of *in vitro* plants compared with the conventional solid medium system, although they failed to compare it with other temporary immersion systems.

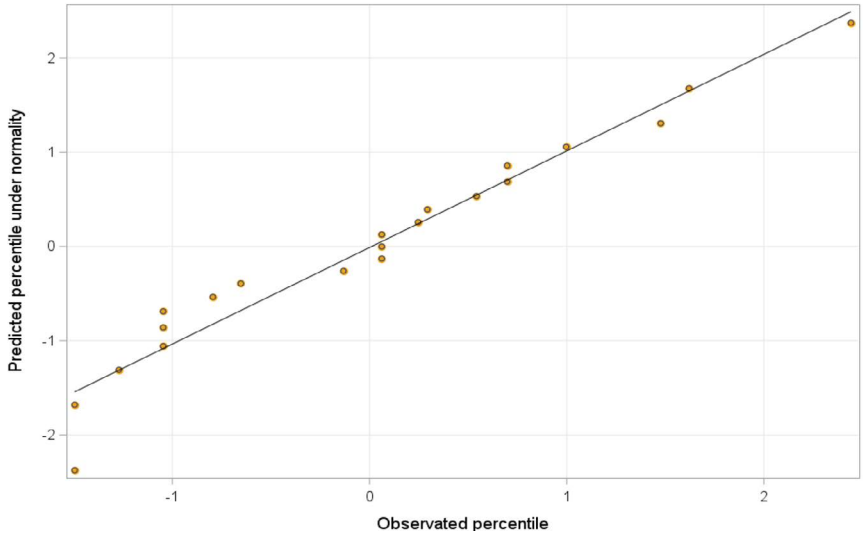


Figure 12. Percentiles (observed and modeled under normality) of Pearson residuals obtained by inverse Gaussian distribution.

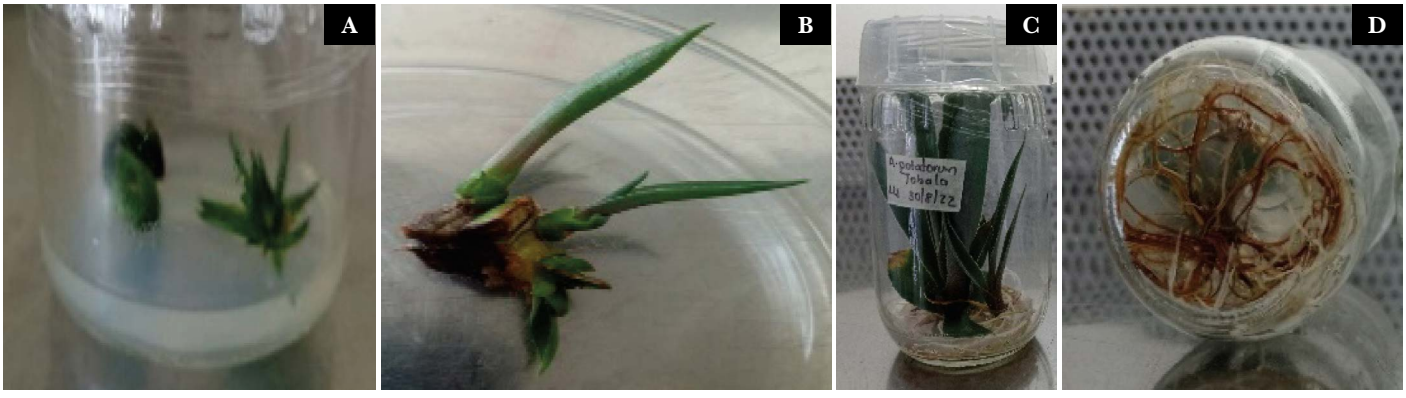


Figure 13. Explant responses. A: solid medium B: sprouts grown in an immersion system, C: *in vitro* seedling growth, D: developed roots.

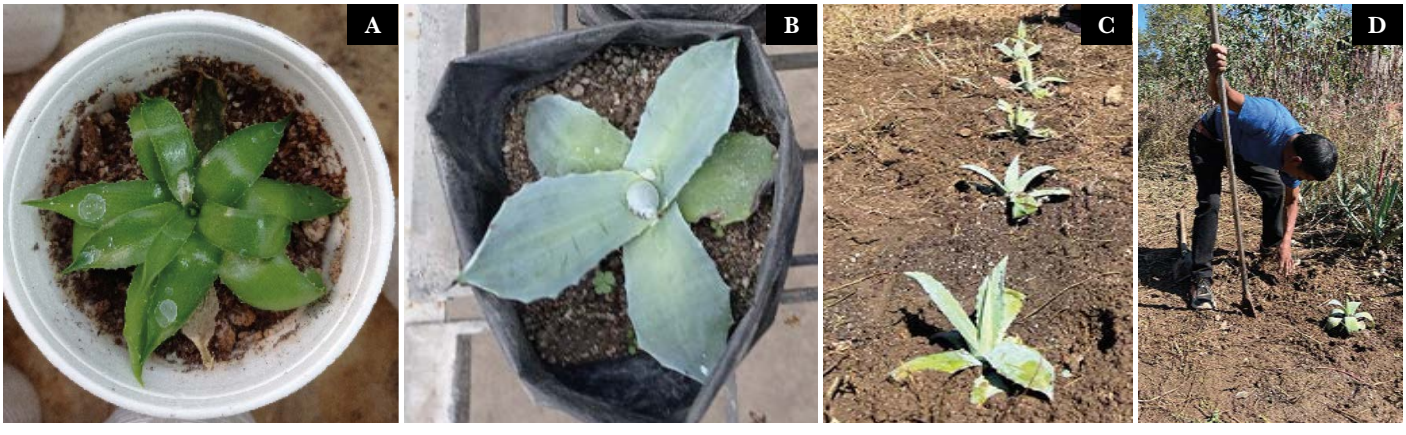


Figure 14. A: acclimatization in laboratory. B: hardening off in greenhouse. C and D: field establishment of *Agave potatorum* propagated under *in vitro* conditions.

Plant acclimatization

The study specimens were initially acclimatized in the growth laboratory chamber (93% survival) and plant hardening off was carried out in the greenhouse. Then, plants were taken to the field in Oaxaca where they were received by a cooperating farmer. One-hundred percent of the plants were finally established after a 60-day assessment.

CONCLUSIONS

Propagating Tobala agave (*Agave potatorum*) under *in vitro* conditions by means of a temporary immersion system (RITA®) is a more efficient mass propagation technique, both for shoots formation and for growth and development (length), than the traditional solid medium, achieving a higher growth rate of the sprouts per explant which satisfactorily reaches the acclimatization and field establishment phases.

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Colegio de Postgraduados

Dosimetry and radio-stimulation in mesquite (*Neltuma laevigata* W.) seeds

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ABSTRACT

Objective: To carry out research focused on the germination response of mesquite (*Neltuma laevigata*) to different doses of gamma radiation (Cobalt 60), in order to obtain a higher germination response than with a non-irradiated seed.

Design/Methodology/Approach: Seeds had different collection times and identities. One set was collected in Durango (10 years) and another in Hidalgo (2 months). Both sets were exposed to sixteen different doses of gamma radiation and a control (non-irradiated); they were subsequently subjected to *in vitro* conditions using a Murashige and Skoog basal medium. They were monitored daily for two weeks in order to develop an accurate record of their germination.

Results: The best treatment for the radio-stimulation of germination in the Durango set was observed at 30 gray (12% higher than the control). Meanwhile, the Hidalgo set received 6 gray radiation (56% higher than the control).

Study Limitations/Implications: Only two different populations were evaluated for this study. Given the differences found between them, working with material from other origins would be ideal.

Findings/Conclusions: Low doses of gamma radiation cause an increase in the germination rate of seeds.

Keywords: Gamma radiation, germination, hormesis, *Neltuma laevigata*.

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INTRODUCTION

Mesquite (*Neltuma laevigata*) (Fabaceae) plays an outstanding ecological role and, according to Rodriguez *et al.* (2014); it is an excellent soil fixer, improves soil fertility, controls erosion, and prevents desertification. It is considered a valuable resource, because all its parts are put to a good use. In the Mezquital Valley (State of Hidalgo, Mexico), it is mainly used for firewood, although it is also used as a source of honey, flour, and cattle

Image by Michael at Pixabay

feed. The term *Prosopis laevigata* was proposed by Bentham (1842, 1875) and confirmed by Burkart in his 1976 monograph, but Catalano *et al.* (2008) recently confirmed that molecular phylogenies show that *Prosopis* is a polyphyletic genus and proposed the scientific name *Neltuma laevigata*. According to Gómez *et al.* (1970), mesquite trees are distributed mainly in Sonora, Chihuahua, Coahuila, Nuevo León, Durango, Zacatecas, Guanajuato, and Querétaro. The seeds of this species retain their viability for up to ten years after their collection (with the endocarp) and three years (without the endocarp). Their germination rate without the endocarp was of 80 to 90%. *In vitro* seed cultures provide nutrients to the seed that would be very difficult to obtain under normal conditions (Fay, 1992; Pierik, 1993). In this regard, hormesis is the result of the low-dose stimulation and high-dose inhibition of a physical or chemical agent. High doses result in a beneficial adaptability in the cell (Calabrese & Baldwin, 2007). According to Guerrero *et al.* (2019); radiation, heat, heavy metals, and antibiotics are the most relevant hormetic agents. Gamma radiation has been used to eliminate insects from the grains of *Coffea arabica* L. and to improve *Abies religiosa* with ≤300 Gy doses. There does not seem to be any documented antecedents of seeds of *Neltuma laevigata* treated with gamma radiation to improve their germination; therefore, the objective of this study was to evaluate the germination response of *Neltuma laevigata* seeds to different radiation doses, in order to determine the effect of radio-stimulation.

MATERIALS AND METHODS

After the seeds of *Neltuma laevigata* were collected (Table 1), the endocarp was removed with tweezers. They were then irradiated with Cobalt 60 (Co^{60}) using the Gammacell 220 irradiator (Figure 1) at the Instituto Nacional de Investigaciones Nucleares (ININ). The dose ratio was 18.360642 Gy/h (Table 2). The different irradiation doses were: 0, 2, 4, 6, 8, 10, 12, 15, 30, 50, 100, 150, 200, 250, 300, and 400 Gray (Gy); using 25 seeds per treatment.

Table 1. Records of *Neltuma laevigata* seeds.

Provenance	Municipality	Year of collection	Coordinates
Durango	Valle del Guadiana	2012	23.9908979° N, 104.5236664° N
Hidalgo	Ixmiquilpan	2022	20.41543° N, 99.21716° N

Table 2. Dose ratio (Gy) per treatment applied to the seeds.

Dose (Gy)	Time (min)	Dose (Gy)	Time (min)
2	6.5	50	163.2
4	13.1	100	326.5
6	19.6	150	489.7
8	26.1	200	652.9
10	32.6	250	816.9
12	39.2	300	979.4
15	49.0	400	1307.1
30	97.9		

In order to evaluate the germination percentage of the seeds at different doses, an *in vitro* seeding was carried out at the Biotechnology laboratory of CENID-COMEF of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). A total of 5.1 L of MS medium (Murashige and Skoog, 1962) was prepared. Each liter contained 4.43 g of medium, 30 g of sucrose, and 8 g of agar, all with a 5.7 pH. In each (previously sterilized) test tube, 6 mL of the prepared medium were added. During the disinfection process, the seeds were washed and rinsed (with soap and water) four times; they were then immersed in 70% alcohol for 5 min; rinsed with sterile water, and left in 30% commercial chlorine for 20 min; and, finally, rinsed three times with sterile water in a laminar flow hood (Figure 1c). Petri dishes with (previously sterilized) paper were placed under the hood to remove excess water and ultimately to reduce the likelihood of contamination (Figure 1d). One seed was sown per test tube with medium and sealed hermetically with plastic wrap. In order to have a better control over each experimental unit, the tubes were labeled with the seed number and its corresponding treatment. To finalize this procedure, the seeds were kept in a conservation room at 35 °C ± with white light 24/7 (Figure 1f).

The seeds from two different origins were subjected to different doses of radiation. They were evaluated during a 2-week period (starting from their sowing), in order to identify their germination. Under the conditions of the study and following Agresti *et al.* (2015), the following statistical model was used for the analysis:

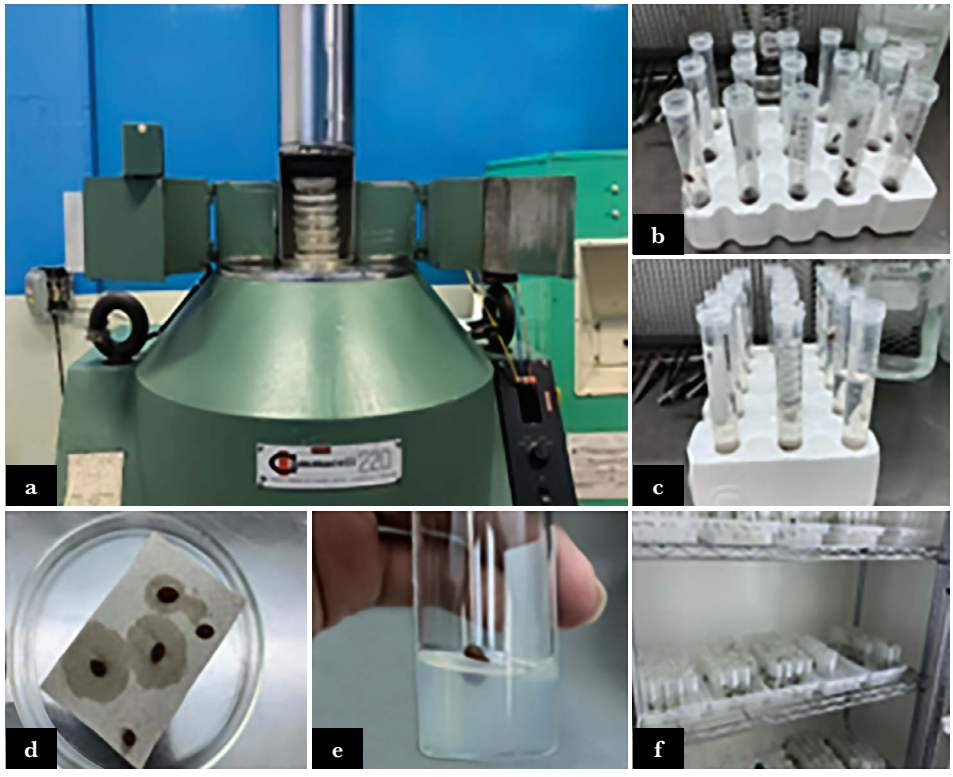

Figure 1. Radiation and *in vitro* seed establishment process. a: Gammacell 220 irradiator; b: washing with soap and water; c: rinsing with chlorine; d: seed drying; e: seed sowing; f: storage of sets.

$$\log\left(\frac{\pi_{ij}}{1-\pi_{ij}}\right)=\mu+P_i+\tau_i+P\tau_{ij}$$
$$Y_{ijk}\sim Binomial\left(25,\pi_{ij}\right)$$

Where: Y_{ijk}=1: germinated; Y_{ijk}=0 non-germinated; k=seed number (1, 2, 3...25); i=provenance (1 and 2); j=treatment (1, 2, 3...16).

The whole statistical analysis was carried out with PROC GLIMMIX (generalized linear mixed model) in the Statistical Analysis System statistical software (SAS 15.1).

RESULTS AND DISCUSSION

No significant differences were identified at the treatment level; however, there were significant differences in the provenance-locality interaction, considering an alpha of 0.57 (Table 3). The treatments responded differently in each of the localities evaluated (Durango and Hidalgo).

Comparison of seed germination with the different localities

According to the model used, the treatment with the best germination response for the seeds from Durango was 30 Gy, while the least effective treatments were 6 and 150 Gy. For the set from Hidalgo, the best treatment was 6 Gy and the least effective was the control (Figure 2).

Germination response in seeds from Durango

In total, 317 seeds from Durango germinated. The treatment with the greatest hormetic effect for this set was reported at 30 Gy, with a 96% germination rate, while the control treatment (0 Gy) recorded a 48% germination. Starting from the control treatment, the second-best treatments were 0 and 2 Gy with 82% germination, while 50, 250, 300, and 400 Gy only recorded a 72% germination (Figure 3). The treatments with the lowest germination rate were 6, 10, and 150 Gy, with a 36.3% average germination rate.

Germination response in seeds from Hidalgo

In total, 117 seeds from Hidalgo germinated. The treatment with the greatest hormetic effect for this set was 6 Gy, with a 64% germination rate. Compared with the 8% germination

Table 3. Type III fixed effects test.

Effect	DF N	DF D	Value F	Pr>F
Origin	1	768	81.65	<.0001
Treatment	15	768	1.65	0.0570
Provenance * Treatment	15	768	4.39	<.0001

DF N: degrees of freedom of the numerator.
DF D: degrees of freedom of denominator.

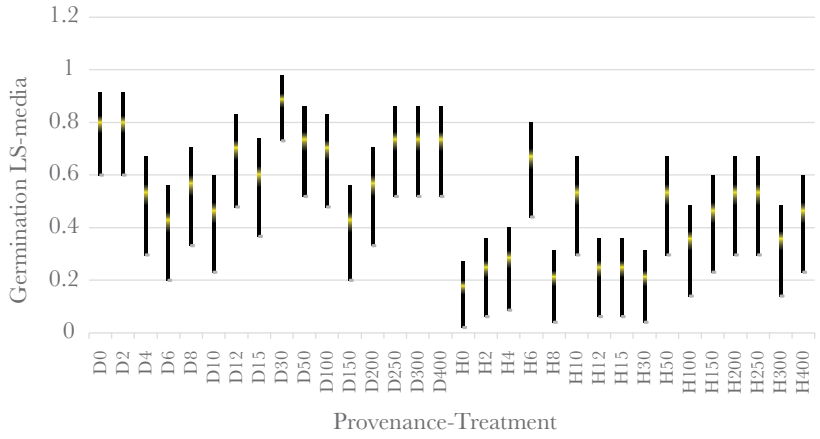

Figure 2. Comparison of germination response. Behavior of the different treatments and different origins (Durango and Hidalgo), where D represents the seeds from Durango and H represents the seeds from Hidalgo.

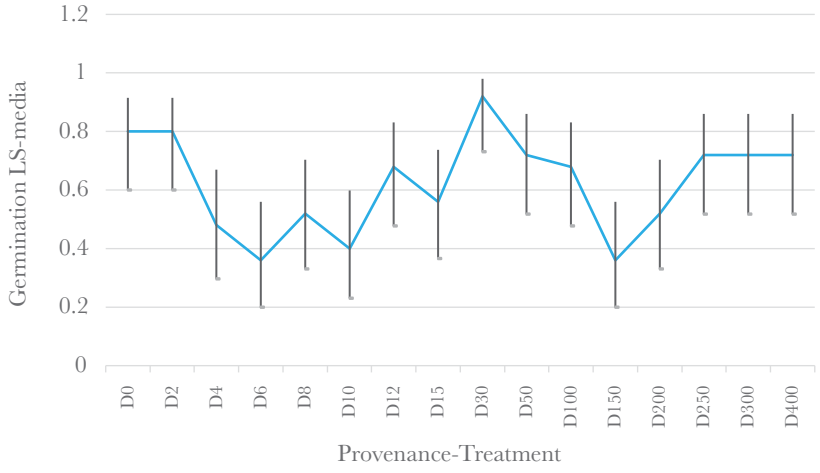

Figure 3. Comparison of germination response in seeds from Durango.

reported for the control treatment (0 Gy), the second-best treatments were 10, 200, and 250 Gy (48% average germination rate) and the third-best treatment was 400 Gy (39% germination) (Figure 4). The treatments with the lowest germination rate were 8 and 30 Gy, which reported a similar behaviour, with a 12% germination.

Some of the treatments behave similarly, which helps to decide which dose to use on the seeds, since a higher the dose increases the time required in the irradiator and consequently the economic cost (Figure 5).

The different treatments react differently in each locality

Regarding radio-stimulation, the treatment with a 30-Gy dose was evidently the most effective in the Durango set (germination percentage: 96%). These results match the findings of different studies, including: Chaomei and Yanlin (1993), who found that high doses of gamma radiation decrease the probability of germination; Salomón Díaz *et al.* (2017), who discovered that 20 Gy was the best dose to stimulate germination in potato

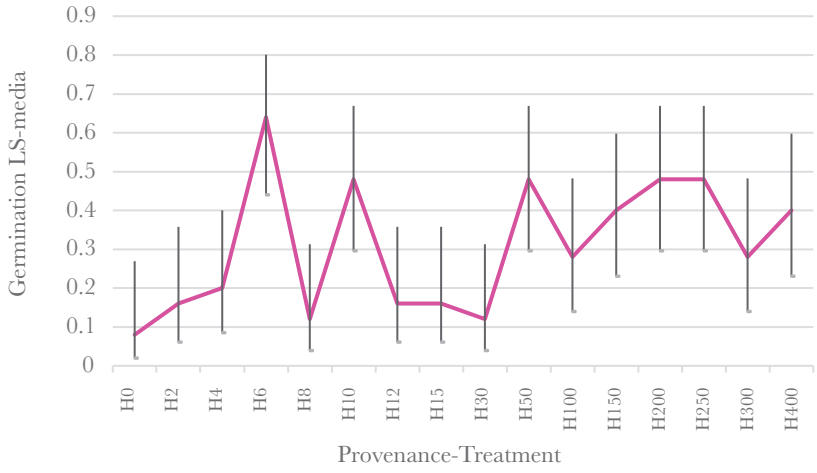

Figure 4. Comparison of germination response in seeds from Hidalgo with the different treatments.

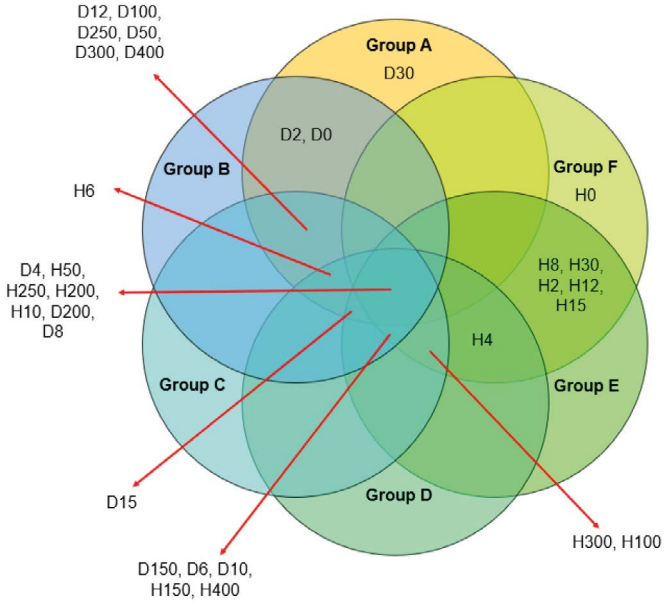

Figure 5. Grouping diagram. Treatments from different localities have similar behavior (D: Durango; H: Hidalgo).

(*Solanum tuberosum* L.); Melki *et al.* (2010) who reported that 20 Gy is the ideal dose to stimulate germination in durum wheat (*Triticum durum*) seeds; Gutierrez *et al.* (2021), who found evidence of germination in eucalyptus (*Eucalyptus nitens*) with lower doses of gamma rays; and finally Ferreira *et al.* (1980), who reported positive effects on the germination of black pine (*Pinus nigra*) seeds with a 10-Gy dose. In another study, Beyaz *et al.* (s/f) reported that, under *in vitro* conditions, 150 Gy is a dose with good germination response in Araguaney (*Lathyrus chrysanthus*) seeds, but that it decreased significantly with higher doses. Avendaño *et al.* (2021) reported that, under field conditions, coffee (*Coffea arabica* L.) seeds recorded a lower germination response with doses of 10 and 50 Gy than their

control and that, at a dose of 300 Gy, seed germination was almost completely disabled. Ramirez *et al.* (2006) mentioned that radiation is a precursor of seed germination. Low radiation doses are a precursor of germination in seeds, since, as suggested by Akshatha and Chandrashekar, (2014), they activate metabolic processes. This would explain the effect of germination stimulation. Another possible cause is the composition of the seed coat. García *et al.* (2022) reported that the mesquite seed has a four-layered coat made of cuticle, epidermis, hypodermis, and parenchyma. Together, these four layers have an approximately 300-μ thickness.

CONCLUSIONS

Low doses of gamma irradiation induce radio-stimulation, increasing the germination rate in *Neltuma laevigata* with different responses, depending on their origin (6 Gy for Hidalgo, and 30 Gy for Durango), increasing germination in seeds from Durango and from Hidalgo by 12% and 56%, respectively, compared with the control treatment of each origin.

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In vitro multiplication of lulo (*Solanum quitoense* Lamarck) for preservation purposes

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ABSTRACT
Objective: To evaluate the effect of treatment, temperature, and time on 2-mm long stems of *Solanum quitoense*, using the minimal growth technique, under in vitro conditions.
Design/Methodology/Approach: Eight treatments with different concentrations of mannitol, sucrose, and Murashige and Skoog (1962) (0 5g L^{-1}, 10 g L^{-1}, 15 g L^{-1}, 20 g L^{-1}, 25 g L^{-1}, 30 g L^{-1}, and 30 g L^{-1}) were analyzed. The experiments were placed in two rooms at 25 °C and 21 °C. Stem growth was recorded every fifteen days.
Results: The Generalized Linear Model showed that the treatments with the best results were 20 g L^{-1} and 30 g L^{-1} mannitol, which reduced the *in vitro* growth of *S. quitoense* to a remarkable degree, preserving the subsistence and vigor characteristics, at a temperature of 21 °C. meanwhile the applied concentrations of sucrose promoted a rapid growth of both the stem and shoots.
Findings/Conclusions: *S. quitoense* recorded resistance to 30 g L^{-1} mannitol, enabling a 3-month preservation of seedlings; however, *S. quitoense* could potentially be preserved for longer periods.

Keywords: minimal growth, mannitol, temperature, *Solanum quitoense*.

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INTRODUCTION

Lulo (*Solanum quitoense* Lam.) (Solanaceae) is native to the Andes and it is mainly grown in Colombia and Ecuador (Gallo *et al.*, 2018). The interior of this round-oval fruit is divided into four parts, each one of which is filled with a green pulp and many small seeds. Its pulp is very fragrant and has a sweet taste; likewise, it has a high vitamin A, C, B1, and B2 content (INIAP, 2010; Silva, 2015). The consumption of lulo has increased in the American, Canadian, German, French, Japan, and Chinese markets, as a result of its nutraceutical value (Gomez-Merino *et al.*, 2014; Cámara de Comercio Bogotá *et al.*, 2015; Alvarez-Duque *et al.*, 2021).

Image by David Hernandez at Pixabay

Nevertheless, the low transportation resistance of lulo limits its exportation as fresh fruit. This situation forces exporters to replace fresh fruit with products made with processed pulp, juices, and preserves (Lago-Burgos, 2011). Arias *et al.* (2014) recorded a deficit in the production of lulo which could be an opportunity for Mexico to develop its own technologies to grow and sale this fruit, both in the domestic and export markets. As a whole, research have shown the need to develop propagation protocols, including grafting, rooting, and the minimal growth technique under *in vitro* conditions. The main objective of the third technique is the preservation and the exchange of multiple phytogenic resources, promoting the potential storage of vegetal germplasm in a limited area and facilitating access to plant material (García-Águila *et al.*, 2007). Likewise, minimal growth extends the time between cultures and subcultures, compared with the 3-5 week regular intervals. The length of the intervals depends on the species. Additionally, minimal growth enables the micropropagation of the plant material in limited spaces and reduces cost. Several studies about minimal growth have been carried out with species such as *Swietenia macrophylla* King and *Tectona grandis* L. (Montiel-Castelán *et al.*, 2016), *Stevia rebaudiana* (Zayova *et al.*, 2017), *Ipomoea batatas* L. (Rayas *et al.*, 2019), *Solanum chilotanum* (Muñoz *et al.*, 2019), and *Arnica montana* L. (Petrova *et al.*, 2021). Since *S. quitoense* is not native to Mexico, no biological variation can be used to develop a selection and genetic improvement program. Consequently, determining *in vitro* propagation and multiplication protocols is important to generate plants that can be subjected to irradiation processes. The purpose of these processes is to induce variation and mutagenesis as soon as possible, as well as to obtain clones that can be exploited as a commercial crop. Therefore, the stems of *Solanum quitoense* were evaluated and multiplied under *in vitro* conditions, with different minimal growth treatments, modifying the culture medium and the temperature to preserve and obtain plants suitable for cultivation.

MATERIALS AND METHODS

Plant material

The experiments were carried out in the Laboratorio de Biotecnología y Germoplasma, CENID-COMEF, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). The lulo fruits were collected in Huatusco, Veracruz, Mexico (19° 08' 56" N, 96° 57' 58" W, at 1,344). The local climate is warm humid, with summer rains, and a mean temperature of 19.4 °C (maximum temperature: 26.3 °C; minimum temperature: 12.4 °C) (CONAGUA, 2022). The vegetation is typical of the cloud forest category, with 85% relative humidity and an annual precipitation of 2,250 mm. The soils have abundant nutrients. They are moderately fertile, with a thick texture, volcanic glass fragments, and a slightly acid pH (4.3-6.5); they also have abundant organic matter, with low Ca content and high Fe, Mn, and Zn content (Cadena-Iñiguez *et al.*, 2011).

The initial propagation used seeds of a variety acclimatized to the area since 2014 as a self-fertilization pure line. The seeds were extracted by hand and washed with running water five times to remove mucilage. Afterwards, they were immersed in a 70% alcohol solution for 3 min and then washed again with running water. Finally, they were placed in a 30% chlorine solution for 20 min. Once this process concluded, the seeds were

washed with sterile water in a laminar flow hood; the excess water was removed with a sterile paper.

Establishment of the *in vitro* cultivation

The seeds were placed in test tubes with 6.0 mL of a MS medium (Murashige and Skoog, 1962). The tubes were kept in a room at 25 °C, during a 24/0 (light:dark) photoperiod. The seeds germinated after four weeks (Gutiérrez *et al*., 2019). Subsequently, they were kept for 60 additional days in the culture medium, in order to obtain the tallest plants possible and the said medium was used for the minimal growth technique.

Minimal growth

Table 1 shows the treatments used to evaluate the lulo (*S. quitoense*) stems grown in a MS medium, with different sucrose and mannitol ratios. Stem width and length were measured at 2.0 cm to reduce growth differences. The incubation conditions for the culture were two rooms with different temperatures (25 °C and 21 °C) and a 24/0 photoperiod. Evaluations were carried out every 15 days for three months.

Variables

n=210 experimental units (test tubes) were subjected to eight treatments which, in their turn, were divided into two blocks with different temperatures (25 °C and 21 °C). Each experimental unit recorded stem height seven times, resulting in a total of n=1,470 observations.

Statistical model

Considering the experimental design, the data were analyzed with a Generalized Linear Model. According to Stroup (2014), the model has the following predictor:

$$\log\left(\mu_{jkl}\right) = \mu + \alpha_j + \tau_k + (\alpha\tau)_{jk} + \beta_l$$

Then, $\mu_{jkl} = \exp\left(\mu + \alpha_j + \tau_k + (\alpha\tau)_{jk} + \beta_l\right)$

Table 1. Treatments of the evaluated mediums of *Solanum quitoense* Lamarck.

Treatment	MS (g $L^{-1)}$	Agar (g L^{-1})	Sucrose (g L^{-1})	Mannitol (g L^{-1})
1	4.4	8	30	0
2	4.4	8	0	30
3	4.4	8	0	0
4	4.4	8	25	5
5	4.4	8	20	10
6	4.4	8	15	15
7	4.4	8	10	20
8	4.4	8	5	25

MS=Murashige and Skoog (1962).

Where: $y_{ijkl} \sim Gamma\left(\mu_{jkl}, \phi\mu^2_{jkl}\right)$ is the height of the stem in the *i*-th experimental unit (tube) of the *j*-th treatment in the *k*-th time or the moment when the measurement took place in the *l*-th block (temperature level). μ is the mean value for the reference level. α_j is the log-mean difference for the *j*-th treatment level with the reference level. τ_k is the log-mean difference for the *k*-th time level with the reference level. β_l is the log-mean difference for *j*-th the block level with the reference level.

The y_{ijkl} are considered independent regarding block treatments. Likewise, they have a first-order autoregressive covariance regarding time within the same experimental unit.

RESULTS AND DISCUSSION

A differentiated effect was observed in the treatment and time for the stem height variable. Overall, a higher variation was recorded among the three observations at the end of the evaluation period. In that respect, although treatments 2 and 3 were similar at the start of the period, the latter almost doubled the height of the former by the end of the evaluation (Figure 1). Figure 2 shows a growing linear trend in all the treatments. Treatments 1 and 4 recorded a higher growth rate, while treatment 2 and 7 grew at a slow rate. Treatment 4 achieved an almost 80 mm height; meanwhile, the stems of treatment 2 grew almost 25 mm at the end of the evaluation period.

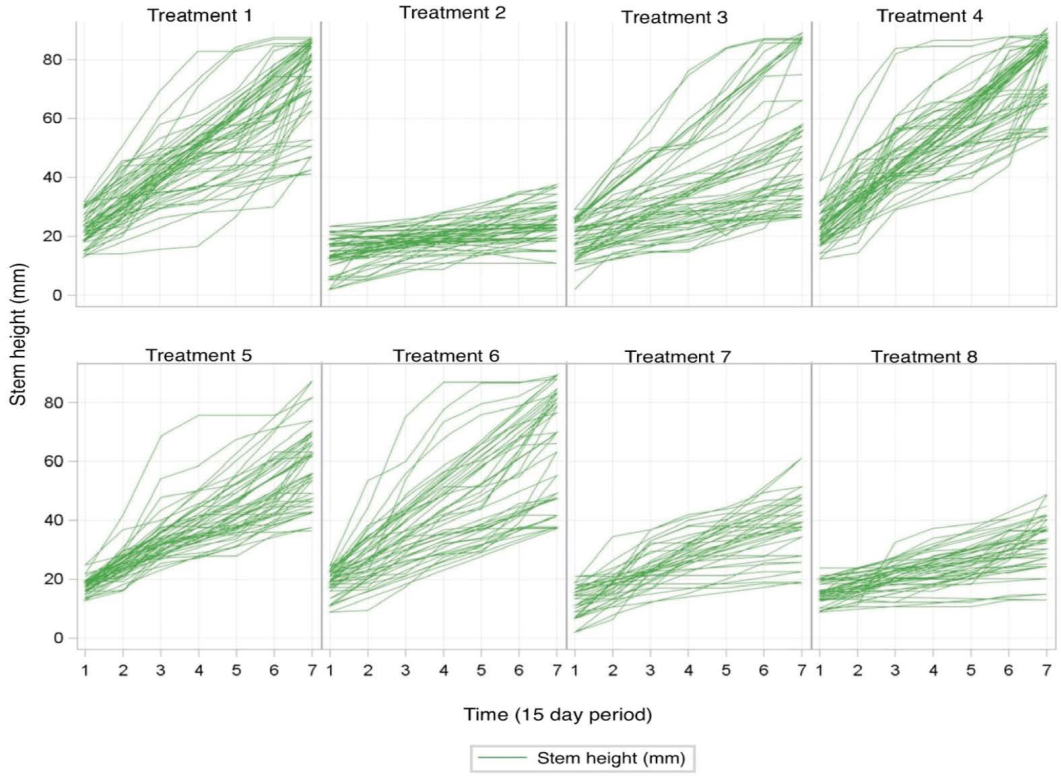

Figure 1. Stem height of *Solanum quitoense* Lamarck per treatment, as a function of time. T1: 30 gL^{-1}, T2: 30 gL^{-1}, T3: 0 gL^{-1}, T4: 25 gL^{-1}, T5: 20 gL^{-1}, T6: 15 gL^{-1}, T7: 10 gL^{-1}, and T8: 5 gL^{-1}. Average values of 1470 observations.

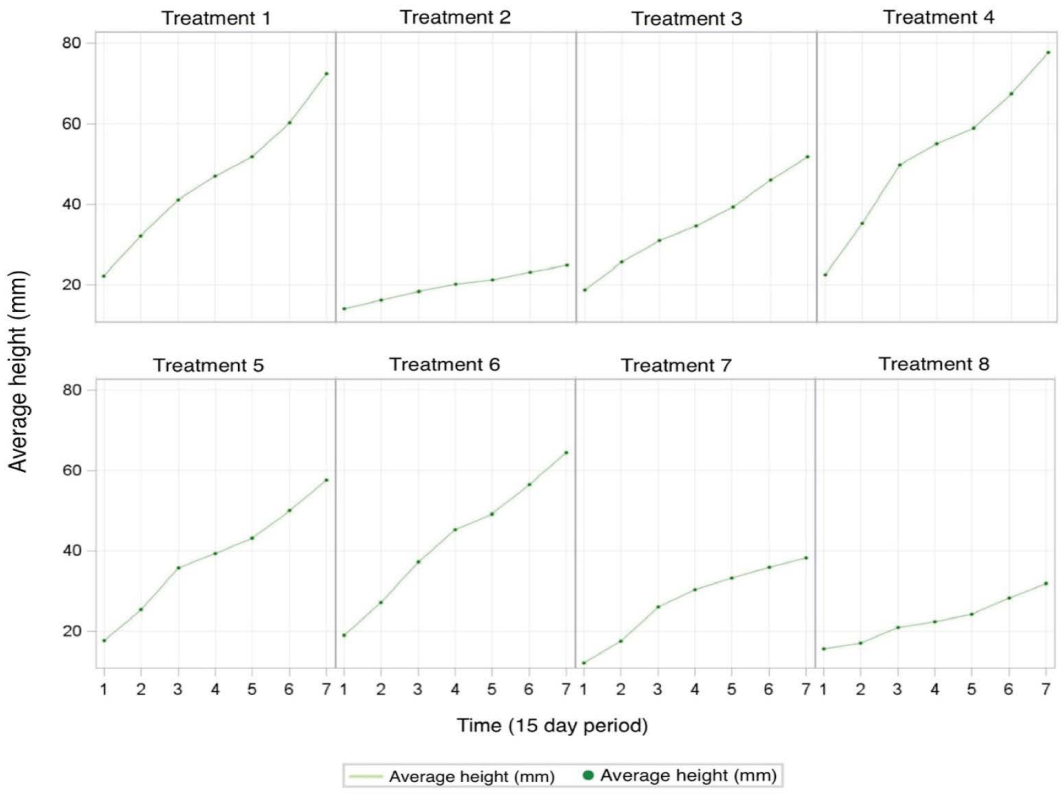

Figure 2. Mean stem height of *Solanum quitoense* Lamarck per treatment, as a function of time. T1:30 gL^{-1}, T2: 30 gL^{-1}, T3: 0 gL^{-1}, T4: 25 gL^{-1}, T5: 20 gL^{-1}, T6: 15 gL^{-1}, T7: 10 gL^{-1}, and T8: 5 gL^{-1}. Average values of 1,470 observations.

Evaluation of the effect of temperature and time evaluation on stem height

Time observations within the same experimental unit were highly correlated in closer measurements; however, as they separated, the temporal dependency notably decreased. In conclusion, the correlation between times 1 and 7 reached 50% regarding the correlation between the two first times (Table 2).

The statistical analysis took into consideration the gamma distribution and the first-order autoregressive covariance. Consequently, it identified the main effects of the treatments and time, as well as their interaction, both of which have a significant

Table 2. Correlation matrix estimated between measurement times for the stem height of *Solanum quitoense* Lamarck, under *in vitro* conditions.

Time	1	2	3	4	5	6	7
1	1.0000	0.8644	0.7472	0.6458	0.5583	0.4826	0.4171
2	0.8644	1.0000	0.8644	0.7472	0.6458	0.5583	0.4826
3	0.7472	0.8644	1.0000	0.8644	0.7472	0.6458	0.5583
4	0.6458	0.7472	0.8644	1.0000	0.8644	0.7472	0.6458
5	0.5583	0.6458	0.7472	0.8644	1.0000	0.8644	0.7472
6	0.4826	0.5583	0.6458	0.7472	0.8644	1.0000	0.8644
7	0.4171	0.4826	0.5583	0.6458	0.7472	0.8644	1.0000

effect on stem length. Therefore, the F value of the interaction —based on the degree of freedom in the numerator (42) and the dominator (1,212)— was 4.27, with a <0.0001 p-value (Table 3).

This situation confirmed that treatments had a significantly different effect on stem length throughout the study. The interaction-based multiple comparison tests showed that the observations of time 7 (treatments 4 and 1) were the highest, while the observations of time 1 (treatments 7, 2, and 8) were the lowest (Figure 3). Overall, the observations of the different times of treatment 2 held the lowest positions among the Tukey groups. Figure 4 shows the remarkable differences in growth between treatment 1 (control) and treatments 2 and 7.

Pineda-Lázaro *et al.* (2021) observed that 0.8% mannitol reduced the stem growth (height) and the number of nodes of the explants of *Solanum tuberosum*, showing that

Table 3. Gamma distribution and first-order autoregressive covariance for temperature, time, and *Solanum quitoense* Lamarck stem treatments, under *in vitro* conditions.

Efect	Numbered degrees of freedom	Denominator of degrees of freedom	Value F	Pr>F
Temperature	1	201	12.90	0.0004
Treatment	7	201	44.02	<.0001
Time	6	1212	218.13	<.0001
Treatment *Time	42	1212	4.27	<.0001

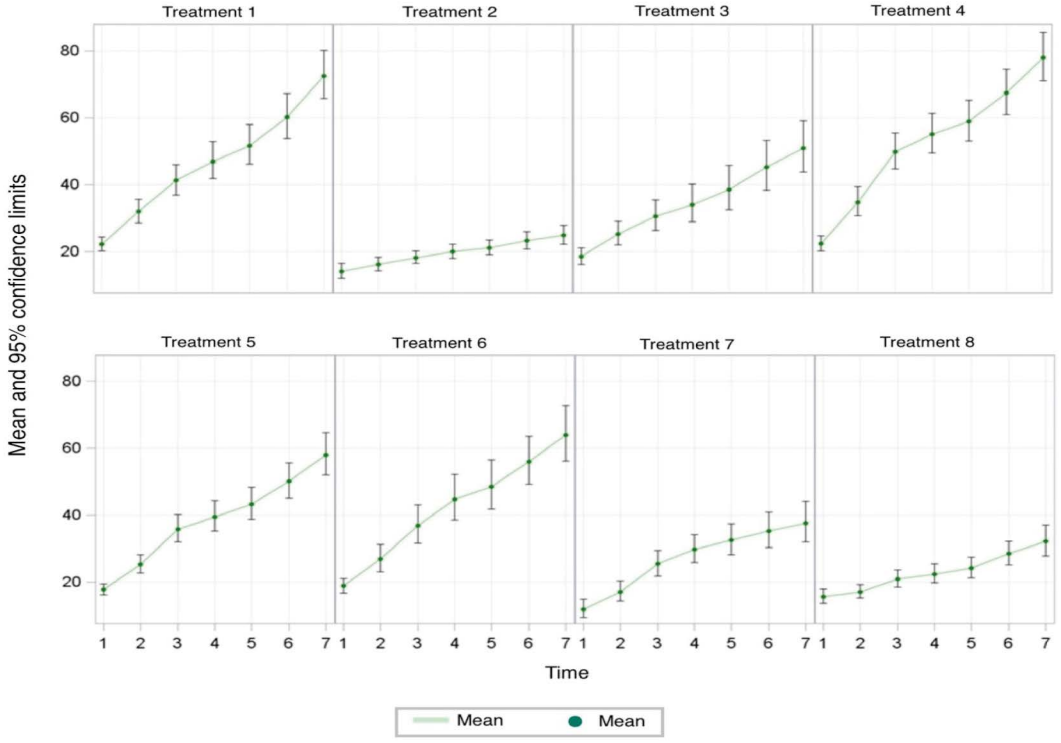

Figure 3. Mean and least-square confidence intervals of *Solanum quitoense* Lamarck stem height, under *in vitro* conditions.

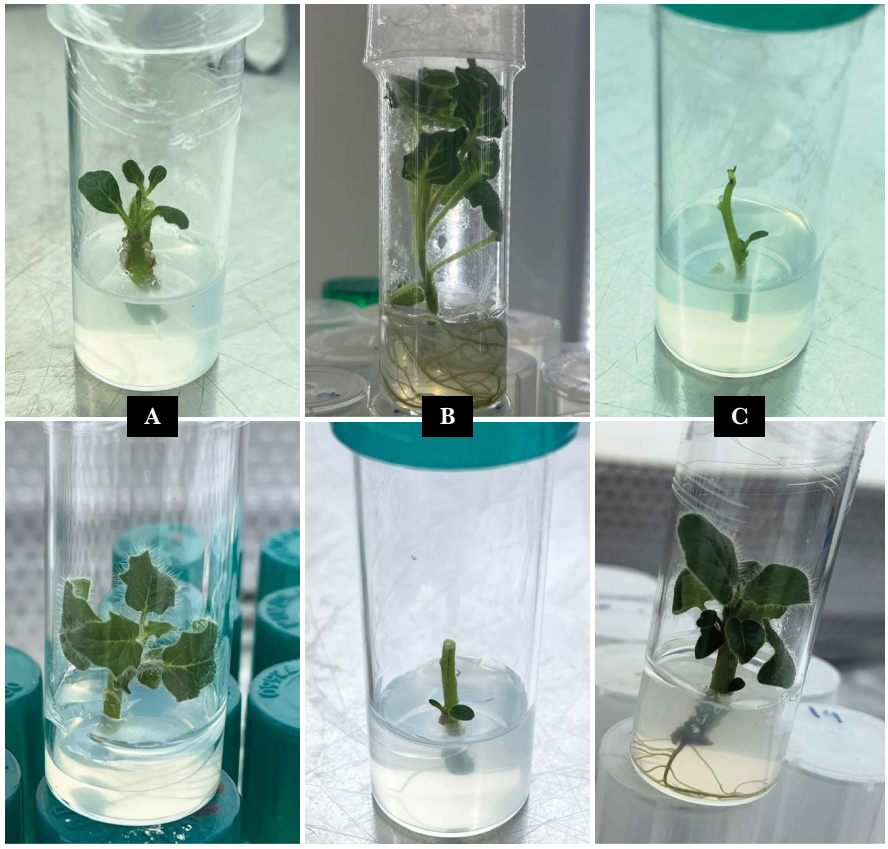

Figura 4. *Solanum quitoense* Lamarck. 1: first measurement. 2: last measurement. Temperature: 21 °C. A (treatment 1), B (treatment 2), and C (treatment 7).

mannitol efficiently preserves a long-term or a minimal growth. The final plant height results were similar in other studies: Pineda-Lázaro *et al.* (2021) recorded 39.1 mm resulting for their treatment C5, while this study recorded 39.8 for treatment 7 (Figure 2). Other preservation studies —which focused on different plant species— were analyzed for comparative purposes. Ventura (2019) studied the use of mannitol and sorbitol for the in vitro preservation of *Physalis peruviana* and concluded that a 20 g L^{-1} mannitol treatment was more efficient than a 20 g L^{-1} sorbitol treatment.

According to Carmona *et al.* (2015), the minimal growth response is linked to a reduction of the water collection required for the growth of the sprouts or the seedlings. The resulting lack of water causes a reduction of turgor pressure, preventing the expansion of cells. For their part, Pérez-Molphe *et al.* (2012) pointed out that a lower nutrient absorption will reduce growth without altering the biochemical balance of the vegetable cells.

According to Rayas Cabrera *et al.* (2019), the in vitro preservation of *Ipomoea batatas* L. should be carried out using 10 g L^{-1} mannitol, because >1.0 g L^{-1} can impact preservation. However, no impacts were recorded in this experiment when that amount of mannitol was used, except for a reduction of the growth. The final height recorded during this experiment was 25 mm (treatment 2). Nevertheless, Rayas Cabrera *et al.* (2019) carried out their last measurement at eight months, while the plants of this experiment

were measured at 3 months. The resulting time difference between the measurements suggest that several results can match the results of this study. However, the amount of mannitol can vary depending on the species studied. Loureiro da Silva *et al.* (2011) and Bello-Bello *et al.* (2015) pointed out that, in some cases, high concentrations of mannitol can be toxic or even deadly for some species. Although a lethal concentration depends on the species, the Solanaceae family can resist an amount of mannitol similar to the one used for the minimal growth technique.

Temperature is an important factor for minimal growth because it can reduce the metabolic activity and, consequently, the growth of the explants (Engelmann, 1991; Sánchez-Chiang *et al.*, 2010; Vásquez *et al.*, 2011; Tandazo, 2015). Jaime-Guerreo (2021) indicated that the Solanaceae family (lulo) are plants from cold weather regions. However, they develop faster in higher temperatures, which encourage earlier harvests, unlike under cold weather conditions. Montiel-Castelán *et al.* (2016) studied the *in vitro* preservation of *Tectona grandis* L. and *Swietenia macrophylla* King, with temperatures of 18 °C, 24 °C, and 28 °C. They proved that a lower temperature, combined with mannitol, is the best preservation alternative, as a result of its direct effect on metabolism.

Meanwhile, Cioloca *et al.* (2021) evaluated temperatures of 16 °C, 20 °C, and 24 °C. Their last evaluation was carried out at day 140, under different conditions. They observed that the most feasible temperatures were 16 °C and 20 °C. These results match the findings of this research. For their part, Espinosa Reyes (2003) recorded null survival under *in vitro* conditions of sweet potato (*I. batata*) plant material at 25 °C; meanwhile, 60% of all clones survived 17 °C. These results confirm that lower temperatures promote minimal growth.

Lima-Brito *et al.* (2011) studied the effect of temperature (18 °C and 25 °C) on the survival and preservation of *Syngonanthus mucugensis* plants. They proved that 18 °C promoted the feasibility of the material for 180 days and recorded a significatively higher plant survival percentage than the plants subjected to a temperature of 25 °C, regardless of the medium (sucrose, mannitol, and sorbitol).

CONCLUSIONS

Unlike other species, *S. quitoense* recorded resistance to 30 g L^{-1} mannitol, enabling a 3-month preservation of seedlings; however, *S. quitoense* could potentially be preserved for longer periods.

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Colegio de Postgraduados

Molecular docking and dynamics of *Annona muricata* L. megastigmanes and metabolites on enzyme markers of breast cancer and their effect on heterodimer formation

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ABSTRACT

Objective: To analyze the molecular docking of secondary metabolites of soursop on the enzyme markers of breast cancer.

Design/Methodology/Approach: Crystals of PARP2 and PRMT5 enzymes were obtained from RCSB-PDB. Both crystals were processed using bioinformatic tools (*e.g.*, SWISS-MODEL, UCSF-Chimera, and ScanProsite), prior to molecular docking and dynamics. The *Annona muricata* L. metabolites were obtained from Pubchem for their use in several *in silico* analysis. The Autodock algorithm was used to obtain the molecular docking. Once the most stable conformations were obtained for the ligands of each enzyme, their complexes were subjected to 10 ns of molecular dynamics using GROMACS. Meanwhile, the HPF1-PARP2 and the MEP50-PRMT5 heterodimeric interactions were carried out using the HDOCK server. Finally, the possible biotransformation reactions were studied using QSAR models.

Results: The kaempferol-3-O-rutinoside metabolite showed potential biopharmaceutical use as an inhibitor of the PARP2 enzyme. The coreximin ligand showed potential biopharmaceutical use as an inhibitor of the PRMT5 enzyme. The inhibitor impacted the PRMT5-MEP50 interaction. The QSAR models indicated that methylation, O-glucuronidation, and O-dealkylation were the most likely biotransformation reactions among the metabolites with the highest degree of inhibition.

Study Limitation/Implications: *in silico* analysis on inhibition of key proteins.

Findings/Conclusions: The kaempferol-3-O-rutinoside chemical compound showed potential as a PARP2 inhibitor. The coreximin chemical compound showed potential as a PRMT5 inhibitor. The protein-protein interaction between PRMT5 and MEP50 was impacted by the inhibitor; however, this was not the case with the PARP2 enzyme.

Keywords: soursop, proteins, inhibition.

Image by Rattakarn at Pixabay

INTRODUCTION

Breast cancer is currently one of the main health problems worldwide. According to the World Health Organization (WHO) and the Pan American Health Organization (PAHO), it is the most common cause of death by cancer among women. Around a million different cases of breast cancer are detected each year and about 400,000 women die every year as a result of this disease (Ramos *et al.*, 2015). Women in countries with high socio-economic levels have a higher risk of getting breast cancer, while women in low economic levels countries face a higher risk of dying of cancer, because they cannot easily have access to healthcare services, which allows an early detection, treatment, and cure of this disease (Cárdenas *et al.*, 2015). The comprehensive treatment of breast cancer requires a multidisciplinary approach, including local-regional and systemic treatments. Surgery and radiotherapy are the chosen local-regional treatments and they include three types: neoadjuvant, adjuvant, and palliative. Meanwhile, the systemic treatment includes chemotherapy, endocrine therapy, and molecular target therapy (Arce *et al.*, 2011). Currently, the inhibition of the catalytic activity of poly (ADP-ribose), polymerase 2 (PARP2), and PRMT5 (argine protein n-methyltransferase 5) —as well as other specific enzymes that are important for survival and the behavior of cancer cells— has been suggested as an study objective (Murai *et al.*, 2012; Wang *et al.*, 2018). The PARP 2 enzyme belongs to the PARP family. These enzymes bind themselves to the damaged DNA through their zinc finger protein motif in the aminus terminal, activating their catalytic region near the carboxyl terminus and hydrolyzing the NAD+, which produces lineal and branched PAR chains. These chains can expand through hundreds of ADP-ribose units. The action produced by this enzyme in the damaged DNA plays an important role, activating the repair pathways in a single chain or breaking both DNA chains (Murai *et al.*, 2012). In response to the damage caused in the DNA, the PAR post-translational modification mainly takes place in the serine amino acids, involving the participation of the HPF1 factor, which changes the specificity of the glutamate residue left by the serine in PARP2 (Bilokapic *et al.*, 2020). Different PARP2 inhibitors (such as olaparib, veliparib, and MK-4827) are found in the advanced stage of clinical trials. The aim is to use them to treat several types of cancer (Murai *et al.*, 2012). Human beings have a total of 9 proteins of the PRMT family and they are divided into three different types. These enzymes transfer a methyl group of the S-Adenosyl methionine (SAM) to the arginine residues found in the histones, releasing an equivalent of S-Adenosyl-L-homocysteine (SAH) (Wang *et al.*, 2018). The Type II PRMTs include PRMT5 and PRMT9, which can catalyze the ω-NG-monomethylarginine (MMA) and ω-NG, NG–Symmetric dimethylarginine (sDMA) (Kim *et al.*, 2020). PRMT5 is the main enzyme in change of the symmetric dimethylation in the arginine residues of the histones, controlling and consequently regulating several biological processes that take place in the cells of mammals (Wang *et al.*, 2018). Owing to the WD repeat domain, the PRMT5 develops stable complexes, using the MEP50 factor. The *in vitro* formation of this complex favors the symmetric monomethylation and dimethylation of the histones, as a result of the high affinity that both enzymes have for the SAM substrate (Wang *et al.*, 2018; Kim *et al.*, 2020). Methylation caused by the PRMT proteins has an essential function in the development of cancer cells, including the regulatory function of PRMT5 in the gene expression involved in

tumor formation (Kim *et al.*, 2020). Two types of inhibitors can mainly inhibit the PRMT5: SAM uncompetitive inhibitors and SAM competitive inhibitors (Richters, 2017). The latter include drugs such as sinefungin, which is of natural origin and is a great inhibitor of PRMT5; however, as a result of its similarity to the SAM compound, it usually inhibits other methyltransferase proteins (Wang *et al.*, 2018). Currently, some patients have been known to develop resistance to cancer medicines, most of which have cytotoxic effects that hinder cell proliferation, regardless of the healthy or malignant characteristics of the said cells. Natural products have always been a focus of interest in the fight against cancer; some of these products include alkaloids (vinca), terpene (paclitaxel), or etoposides (Efferth *et al.*, 2017). The National Cancer Institute of Sudan has proved that 69% of the anti-cancer medicines developed from 1980 to 2002 have a natural origin (Newman *et al.*, 2007).

MATERIALS AND METHODS

Obtaining crystals and ligands

The crystals of the PARP2 and PRMT5 enzymes came from a *Homo sapiens* organism and were obtained from the RCSB PDB (Protein Data Bank) data base (https://www.rcsb.org/). The crystal with ID 4ZZX was chosen as model for PARP2, while the ID 6V0P was used for the *in silico* studies of PRMT5. In the case of the PARP2 crystals, the missing amino acids were completed in the 3D model. The task was carried out with Expasy's SWISS-MODEL (https://swissmodel.expasy.org/). Finally, the structures were visualized using the UCSF Chimera v.1.16 software, while the Expasy's ScanProsite (https://prosite.expasy.org/) was used to obtain their conserved domain. The ligands from *Annona muricata* L. used in the in silico studies were obtained from a list developed by Moghadamtousi *et al.* (2015). The focus was put on those compounds with alkaloid-type structures. Afterwards, those compounds were searched in the NCBI Pubchem data base (https://pubchem.ncbi.nlm.nih.gov/).

Ligand-Protein Molecular docking

The same structural procedure was used for both enzymes before the molecular docking. The crystals were cleaned using the Chimera v.1.16 software, adding polar hydrogens and Kollman charges with AutoDock Tools v.1.5.6. In the case of the ligands, their structure was minimized and protonated under a 7.4 pH, using the Avogadro v.1.2.0 software and a MMFF94 force field. Subsequently, the Gaisteiger charges were calculated using the AutoDock Tools v.1.5.6. The molecular docking of each enzyme and their ligands was carried out using the algorithm of the AutoDock v.4.2 software. A 68×68×68 box was developed for the PARP2 enzyme; this box contained the determining area of its catalytic action (136-363 aa). Meanwhile, a 76×76×76 box was generated for the PRMT5 enzyme, covering amino acids 308 to 463. The structure with the best docking energy of each ligand and its respective enzyme were kept as a complex. Afterwards, they were analyzed using the Protein Plus tool (https://proteins.plus/) to obtain 2D images of the ligand-protein interactions. Another molecular docking was carried out between PARP2 and the isoindolinone compound to validate the molecular dockings of PARP2, following the already mentioned parameters. Subsequently, the RMSD was calculated,

using the USCF Chimera v.1.16 software. This calculation was carried out with the best isoindolinone compound obtained from the molecular docking (PARP2-isoindolinone) and the isoindolinone from the crystal. The same validation procedure was used for PRMT5, using isoindolinone instead of sinefungin.

Molecular dynamics

A total of two molecular dynamics were carried out. The models were the best structure of the Kaempferol-3-O-rutinoside-PARP2 complex and the best structure of the Coreximin-PRMT5 complex. The GROMACS v.2022.2 software was used to develop the molecular dynamics. The conditions were the same for both models: a simulation was carried out inside a water box, adding Na+ and Cl− ions, using the Monte-Carlo method, to neutralize the charges of the complexes. The distance between the edge of the boxes and the models was 1.5 nm. The energies were minimized following the steepest descent method (615 steps), while the system was balanced at 125 ps, keeping the NVT values constant. The models obtained after the minimization and the balance were used to start the production stage. The simulation included temperature conditions of 310.15 K and a pressure of 1 bar. The whole simulation lasted 10 ns. The full process was carried out using a CHARMM36m force field. In order to determine RMSD and RMSF, the results were analyzed using the functions of the GROMACS v.2022.2 software: the formation of the hydrogen bonding between the ligand and the protein was carried out using the gmx_hbond function; the distance between the ligand and the protein was measured using the gmx_pairdist function; and the total energy of the complexes was obtained using the gmx_energy function. A structural alignment between the models was performed, using the MatchMaker tool of the UCSF Chimera v.1.16 software. This process was used to analyze the structural differences between the PARP2 (PDB) and PARP2-kaempferol-3-O-rutinoside models and the PRMT5 (PDB) and PRMT5-coreximin models, after the molecular dynamics were carried out. Additionally, the four models were analyzed using the PROCHECK tool to obtain the Ramachandran plots and to compare the amino acid torsion.

Protein-protein molecular docking

Given the importance of the HPF1-PARP2 and MEP50-PRMT5 complexes formation for the appropriate functioning of these enzymes (Suskiewicz *et al.*, 2020; Antonysamy, 2017), the potential differences of the interactions between the HPF1-PARP2 and HPF1-PARP2-kaempferol-3-O-rutinoside and the MEP50-PRMT5 and MEP50-PRMT5-coreximin were analyzed. The PARP2 and PRMT5 models obtained from the molecular dynamic were used for this purpose. Additionally, the PARP2 and PRMT5 crystals were used to carry out the dockings with HPF1 and MEP50, respectively. The crystal of the human HPF1 (ID 6M3G) was obtained from the RCSB PDB (Protein Data Bank) data base. The missing amino acids were added to the crystal, after they had been reconstructed by homology using Expasy's SWISS-MODEL. The MEP50 structure (ID 7U30) was also obtained from the same data base (Table 1). All the dockings between proteins were carried out using the HDOCK online server (http://hdock.phys.hust.edu.cn/). The result

of the HPF1-PARP2-kaempferol-3-O-rutinoside docking was compared with the crystal of the 6TX3 complex (obtained from the same data base); meanwhile, the MEP50-PRMT5-coreximin docking was compared with the 7U30 crystal. Both comparisons were carried out using the UCSF Chimera v.1.16 software.

In silico analysis of the biotransformation

A study of the potential biotransformations of the kaempferol-3-O-rutinoside, coreximin, and coclaurine compounds inside the human body was carried out. These changes could have an impact on the ability of the compound to inhibit the target enzyme. The Way2Drug (http://www.way2drug.com/) online tools were used to carry out this analysis. The potential chemical reactions within the human body and the potential metabolic area were determined using the Reactive Atom tool. The PASS and SOMP tools were used to establish which enzymes could generate first and second phase metabolism in the compounds.

RESULTS AND DISCUSSION

Obtaining the crystals and ligands

The human PARP2 crystal used during the experiment was 363-amino acids long; it had a molecular weight of 82.70 kDa for the complex and a 1.65 Å resolution. It was obtained using the x-ray diffraction technique, with overall good quality metrics. Two domains were found between the amino acids 11 to 128 (PARP alpha helical domain) and 136 to 363 (catalytic domain). For its part, the human PRMT5 crystal was 637-amino acids long; it had a molecular weight of 110.48 kDa for the complex and a 1.88 Å resolution. It was also obtained from an x-ray diffraction, with overall good metrics (Table 1): Its domain was found between residues 308 to 615 (PRMT type domain, SAM-dependent methyltransferase). A total of 21 ligands were tested, 18 of which were evaluated in both enzymes (Table 2). Ten of the 18 ligands were megastigmanes, while 8 were alkaline.

Ligand-Protein molecular docking

In the case of PARP2, the best compound was Kaempferol-3-O-rutinoside, given its high binding capacity (−11.46 kcal/mol binding energy). This compound derived from megastigmanes. Regarding PRMT5, the alkaloid coreximin compound recorded the best binding capacity (−10.33 kcal/−mol binding energy). The coclarine compound had

Table 1. Crystals of the enzymes used during the research (obtained from the RCSB PDB).

Crystal (PDB ID)	No. Amino acids	Crystal molecular weight (kD)	Resolution (Å)	Technique
PARP2 (4ZZX)	363	82.70	1.65	X-ray diffraction
PRMT5 (6V0P)	637	110.48	1.88	X-ray diffraction
HPF1 (6M3G)	356	39.50	1.57	X-ray diffraction
HPF1-PARP2* (6TX3)	598	68.97	2.96	X-ray diffraction
MEP50-PRMT5 (7U30)	325	109.46	2.60	X-ray diffraction

*Catalytic fragment of the PARP2 protein.

the best binding capacity for both enzymes, obtaining a −9.48 kcal/mol and −9.79 kcal/mol binding union for PARP2 and PRMT5, respectively. This compound is an alkaloid (Table 3).

Table 2. Ligands used for the molecular docking of the PARP2 and PRMT5.

Ligand	Pubchem ID	Type	Enzyme used	Binding energy (kcal/mol)
Annoionol A	101564134	Megastigmane	PARP2 PRMT5	−7.03 −7.20
Annoionol B	101564135	Megastigmane	PARP2 PRMT5	−6.83 −6.55
Annoionoside	101564136	Megastigmane	PARP2 PRMT5	−7.01 −7.59
Roseoside	9930064	Megastigmane	PARP2 PRMT5	−8.13 −6.57
Citroside A	14312560	Megastigmane	PARP2 PRMT5	−9.18 −5.81
Blumenol C	118284	Megastigmane	PARP2 PRMT5	−7.55 −6.98
Rutin	5280805	Megastigmane	PARP2 PRMT5	−10.47 −3.87
Kaempferol-3-O-rutinoside	5318767	Megastigmane	PARP2 PRMT5	−11.46 −4.33
Kaempferol-3-O-robinobioside	15944778	Megastigmane	PARP2 PRMT5	−10.69 −5.56
Kaempferol-3-O-glucoside	10095180	Megastigmane	PARP2 PRMT5	−8.92 −9.71
Annomuricine A	157682	Alkaloid	PARP2 PRMT5	−5.99 −0.14
Reticuline	439653	Alkaloid	PARP2 PRMT5	−8.72 −9.76
Coclaurine	160487	Alkaloid	PARP2 PRMT5	−9.48 −9.79
Olaparib*	23725625	Drug	PARP2	−11.75
Coreximine	7037179	Alkaloid	PARP2 PRMT5	−8.27 −10.33
Atherosperminine	96918	Alkaloid	PARP2 PRMT5	−8.83 −9.67
Stepharine	98455	Alkaloid	PARP2 PRMT5	−8.67 −9.51
Anomurine	157218	Alkaloid	PARP2 PRMT5	−9.75 −9.45
Annomuricine	157209	Alkaloid	PARP2 PRMT5	−8.62 −9.17
Sinefungin**	65482	Nucleoside	PRMT5	−10.16

*Compound used as inhibitor of the PARP2 (commercial use).
**Compound used as inhibitor of the PRMT5 (experimental use).

Table 3. Results of the best molecular dockings for each enzyme, individually and together.

Ligand	Enzyme	Binding energy (kcal/mol)	Inhibition constant (nM)	Reference RMSD	Atoms in hydrogen bond	Amino acids
Kaempferol-3-O-rutinoside	PARP2	−11.46	4.01	58.47	5	GLY209, SER210, SER250.
Coclaurine	PARP2	−9.48	111.48	57.44	4	GLY209, ASN214, GLN112, SER250.
Olaparib*	PARP2	−11.75	2.46	59.04	3	TYR242, GLY209.
Coclaurine	PRMT5	−9.79	66.54	100.8	3	MET420, GLU392, GLU444.
Coreximine	PRMT5	−10.33	26.87	95.86	1	LEU437.
Sinefungin**	PRMT5	−10.16	35.62	99.66	5	LYS333, LEU437, GLU392, GLU444.

***Compound used as inhibitor of the PARP2 (commercial use).
****Compound used as inhibitor of the PRMT5 (experimental use).

The interactions between the Kaempferol-3-O-rutinoside-PARP2 compound obtained with Protein Plus show a hydrogen bonding formation with the Ser250, Gly209, Glu338, Ser210, Glu115, and Arg224 residues, while hydrophobic interactions were recorded with the Tyr253, His208, and Tyr242 amino acids (Figure 1A). Meanwhile, the hydrophobic interactions of the coreximin-PRMT5 complex took place with the Glu435, Phe327, Pro314, and Leu436 amino acids, while the hydrogen bonding formation took place with the Glu444, Cys449, Leu437, and Lys333 amino acids (Figure 1B). For its part, the coclaurine-PARP2 complex had hydrophobic interactions with the Tyr253 and Ser210 residues and hydrogen bonding formations with Ser250, Gly209, Gln112, and Asn214 amino acids (Figure 1C). In the case of the coclaurine-PRMT5 interactions, the hydrogen bonding formation took place with the Met420, Glu392, Tyr324, and Glu444 residues, while hydrophobic interactions were observed with the Pro314, Gly365, and Lys393 amino acids (Figure 1D). The RMSD between the best isoindolinone formation —resulting from the second molecular docking— and the isoindolinone in the PARP2 crystal —used for the in silico trials— recorded a result of 5.573 Å. Regarding the PRMT5, the RMSD between the best formation of the sinefungin obtained after the second molecular docking and the sinefugin in the crystal was 6.652 Å, which validates de molecular dockings carried out with both enzymes.

Molecular dynamics

The results of the molecular dynamic for the PARP2 complex and its ligand showed an acceptable stability between PARP2 and the kaempferol-3-O-rutinoside. The RMSD value was <3 Å, while the RMSD of the protein and the ligand did not exceed 2 Å and 1 Å, respectively. These values were constant during the 10ns that the simulation lasted (Figure 2A). The RMSF graph for the complex shows low energy levels for most of the amino acids with which the kaempferol-3-O-rutinoside interacts, which favors the bonding and the stability of the compound and the enzyme. However, some of the enzyme's zones are not favorable to this interaction, such as the >300-residues area (Figure

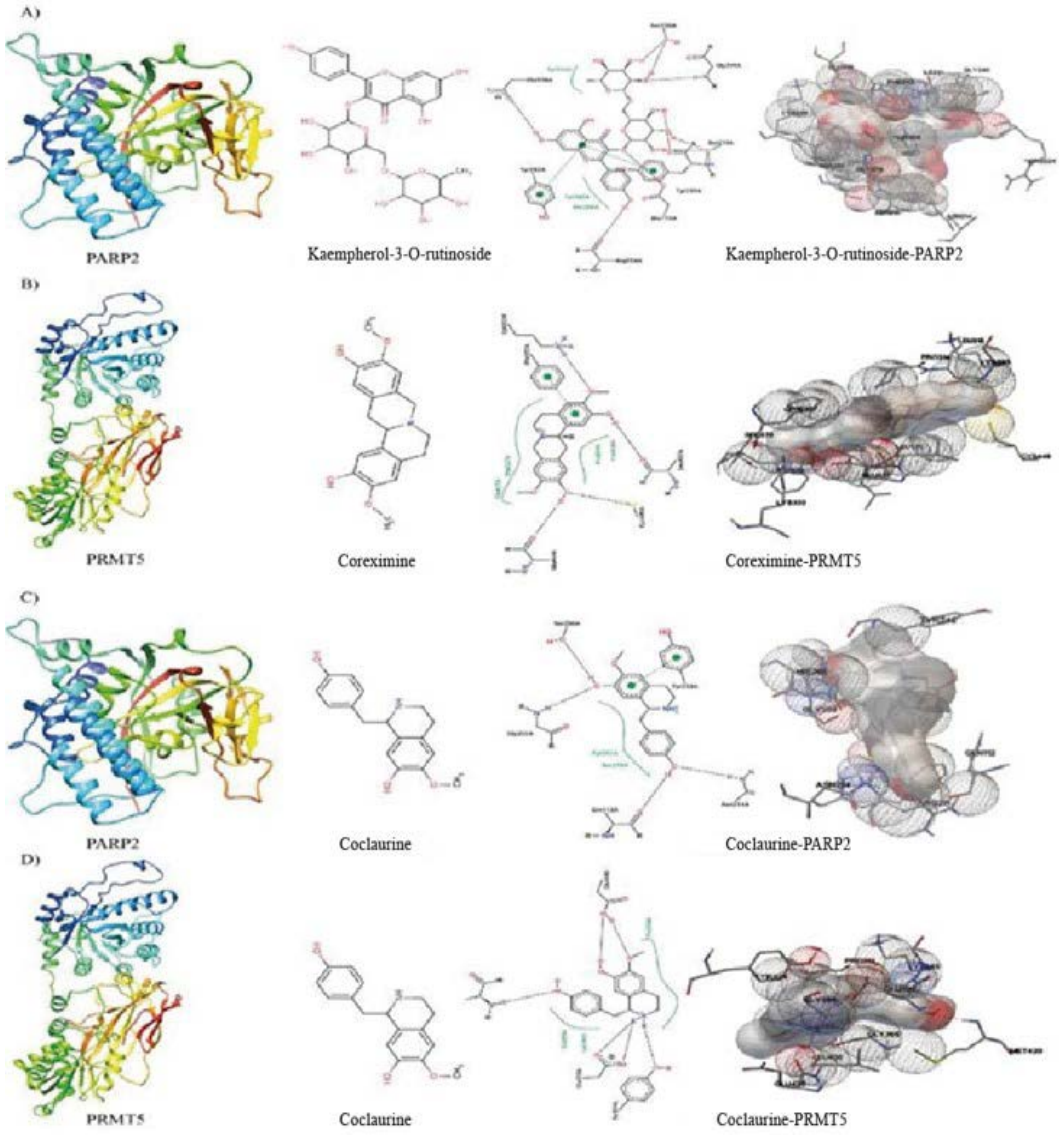

Figure 1. Best results obtained from the molecular docking between the soursop metabolites and the PARP2 and PRMT5 enzymes. The interactions between the molecules are shown as 2D diagrams (ProteinPlus; center) and 3D (Autodock Tools; right). A) Molecular docking between PARP2 and kaempferol-3-O-rutinoside; B) molecular docking between PRMT5 and coreximine; C) molecular docking between PARP2 and coclaurine; and D) molecular docking between PRMT5 and coclaurine.

2B). Regarding the formation of hydrogen bondings (HB) between the kaempferol-3-O-rutinoside and the PARP2, the simulation showed that, during the 10 ns of the dynamic, the maximum number of HB was 10 —a figure recorded before the simulation reached 2 ns. The minimum HB was reached before 1ns. However, a good number of HB links were recorded in the complex throughout the simulation (Figure 2C). The distance between the PARP2 residues and their ligand varied constantly during the whole 10 ns, with a minimum and maximum distances of 0.155 nm and 0.2 nm, respectively (Figure 2D). After the dynamics, the total energy of the system reached approximately −3.8e+05 KJ/mol

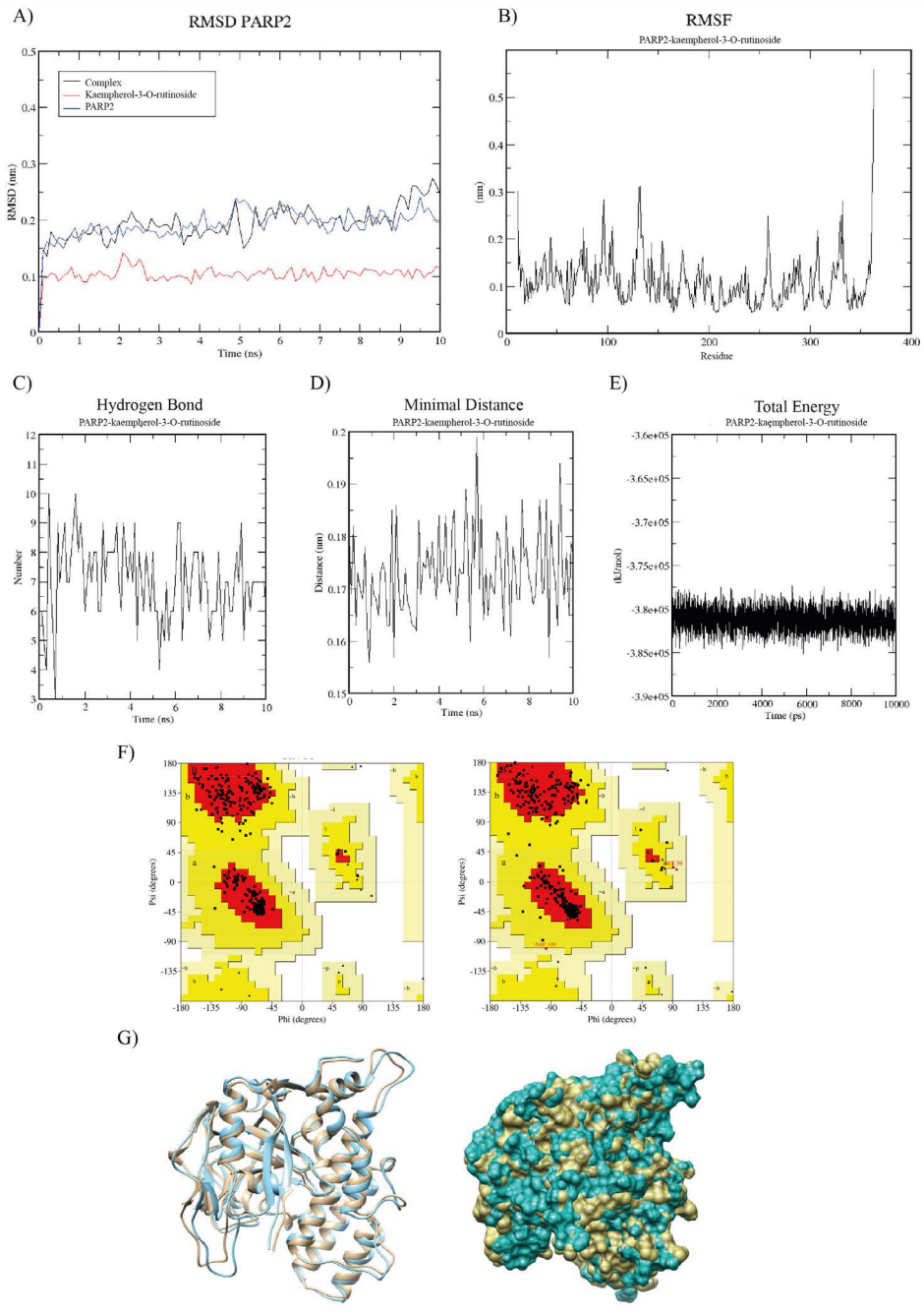

Figure 2. Results obtained after 10 ns of molecular dynamics, using GROMACS for the PARP2 and kaempferol-3-O-rutinoside complex. A) Quadratic median deviation in the structure of the PARP2, of the kaempferol-3-O-rutinoside, and of the complex resulting from both; B) quadratic median deviation of the total energy flows resulting from the PARP2 and kaempferol-3-O-rutinoside complex; C) formation of the hydrogen bondings between the ligand and the enzyme, developed during the 10 ns of molecular simulation; D) variation of the distance between the ligand and the enzyme during the 10 ns of the simulation; E) total energy of the system during the whole simulation, after the minimization, the balance, and the production; F) Ramachandran plots before the detection of kaempferol-3-O-rutinoside (left) and after the detection, during the molecular dynamic (right); G) structural changes caused by kaempferol-3-O-rutinoside (cream) and the PARP2, after the detection of kaempferol-3-O-rutinoside (blue); G) treadmill model (left) and sphere model (right) of the overlay proteins.

(Figure 2E). The structural comparison between the PARP2-kaempferol-3-O-rutinoside and the PARP2 crystal showed a clear torsion of the angles of the residues; the change was more significant for the Lys39 and ASP330 amino acids (Figure 2F). Overall, this change in the angles of the protein modified some hydrophobic areas, therefore altering the surface of the protein (Figure 2G). The PRMT5- coreximin and the protein by itself kept a <3 Å total RMSD value, throughout the 10 ns of the simulation, while the RMSD value for the coreximin was <1 Å during the whole simulation (Figure 3A). The RMSF analysis showed an unstable binding between the 150 and 200 residues of the PRMT5, while the residues where the ligand-protein interaction is more likely to take place are found between amino acids 300 and 500 (Figure 3B). The distance between the coreximin and the PRMT5 ranged between 0.17 nm and 0.19 nm during the 10 ns of the simulation, reaching a minimum and maximum distances of 0.155 nm and >0.21 nm, respectively (Figure 3C).

The formation of hydrogen bondings between the PRMT5 and the coreximin resulted in a 1-2 variant, during most of the simulation (Figure 3D). The total energy of the system during the 10 ns of the simulation remained close to −1.483+06 KJ/mol (Figure 3E). The comparison between the structures of the PRMT5-coreximin model and the crystal of the PRMT5 showed small changes in the rotation of some amino acids and in the surface of the protein (Figures 3F and 3G).

The molecular docking between HPF1 and PARP2-kaempferol-3-O-rutinoside (recorded after the molecular dynamic) was carried out in the HDOCK server and recorded a −423.79 docking score and a 0.69 RMSD ligand. Meanwhile, the HPF1 and PARP2 complex (obtained from the PDB) had a 0.40 RMSD and a −363.73 docking score. The analysis of the docking between the PARP2-kaempferol-3-O-rutinoside, the PARP2 (obtained from the PDB), and the HPF1 (obtained with UCSF Chimera v.1.16) showed that the His381 residue of the PARP2-kaempferol-3-O-rutinoside moved away from the ASP283 residue of the HPF1, increasing from 2.10 Å to 2.78 Å (Figure 4A and Table 4). In the case of the docking between the MEP50 and the PRMT5-coreximin, the PRMT5 lost its binding capacity with the MEP50 in the expected area, while the ARG63 and ARG67 of the PRMT5 did not interact with the Trp44 and Phe289 amino acids of the MEP50. In this case, the docking score was −236.47, while the RMDS was 174.40. Finally, the docking between MEP50 and PRMT5 (obtained from PDB) kept the interaction between the said amino acids, reaching a docking score of −678.96 and a RMSD of 71.16 (Figure 4B and Table 4).

Biotransformation *in silico* analysis

The potential reactions to the kaempferol-3-O-rutinoside compound within a human body include: methylation, O-glucuronidation, hydrolysis, and O-sulfation; the most common reactions are methylation (76.3%) and O-glucuronidation (61.3%). Regarding coreximin, the potential reactions include O-glucuronidation, O-dealkylation, O-sulfation, methylation, and aliphatic hydroxylation; the results indicate that the most common reactions are O-glucuronidation (55.8%) and O-dealkylation (50.2%). Meanwhile, the reactions caused by coclaurine included O-dealkylation, O-sulfation,

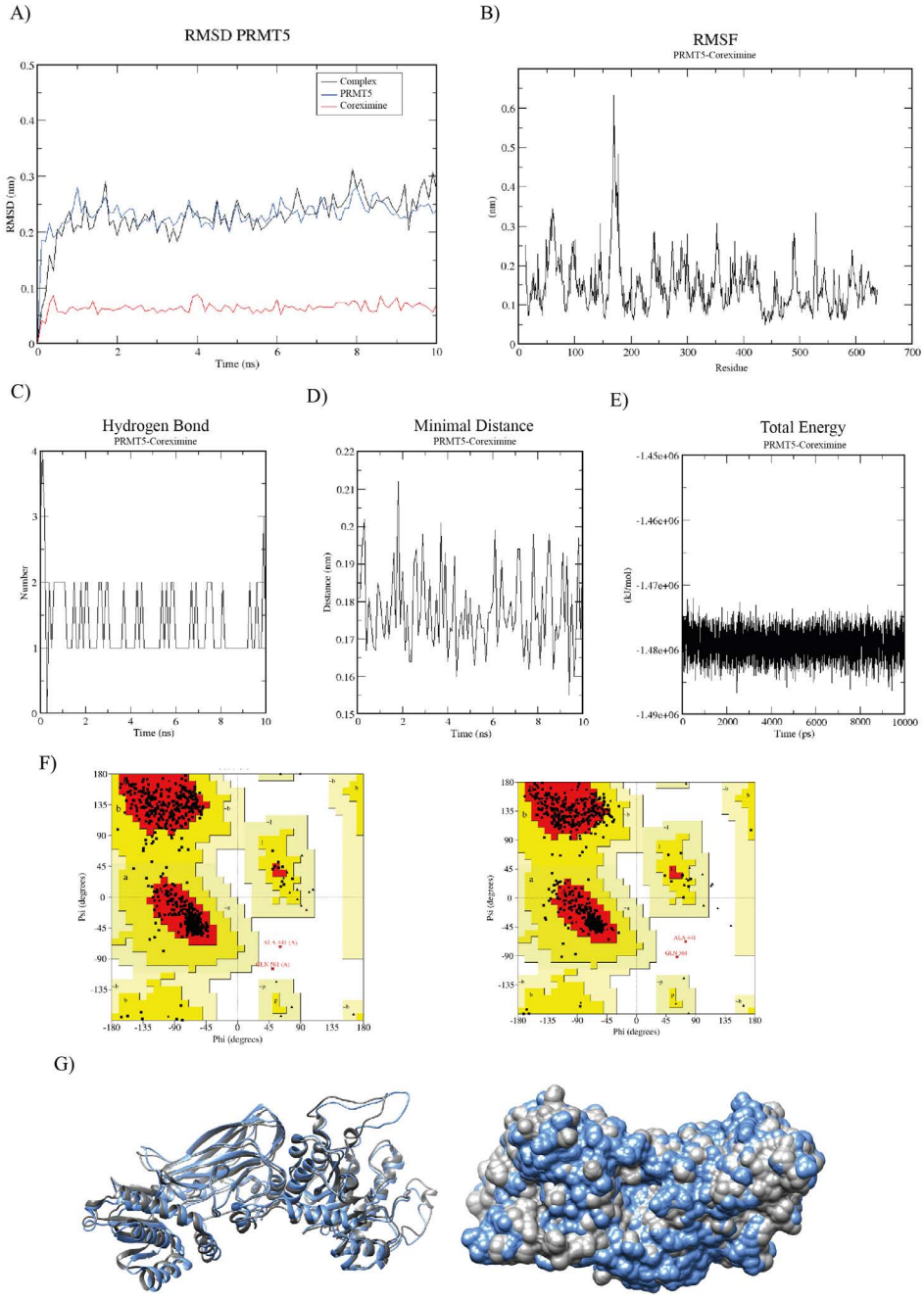

Figure 3. Results obtained after 10 ns of molecular dynamics, using GROMACS for the PRMT5 and coclaurine complex. A) Quadratic median deviation in the structure of the PRMT5, of the coclaurine, and of the complex resulting from both; B) quadratic median deviation of the total energy flows resulting from the PRMT5 and coclaurine complex; C) formation of the hydrogen bondings between the ligand and the enzyme, developed during the 10 ns of molecular simulation; D) variation of the distance between the ligand and the enzyme, during the 10 ns of the simulation; E) total energy of the system during the whole simulation, after the minimization, the balance, and the production; F) Ramachandran plots before the detection of coclaurine (left) and after the detection, during the molecular dynamics (right); G) structural changes caused after the molecular dynamics and the detection of the ligand, the PRMT5 before the presence of the coclaurine (gray) and the PRMT5 after the presence of the coclaurine (blue). G) treadmill model (left) and sphere model (right) of the overlay proteins.

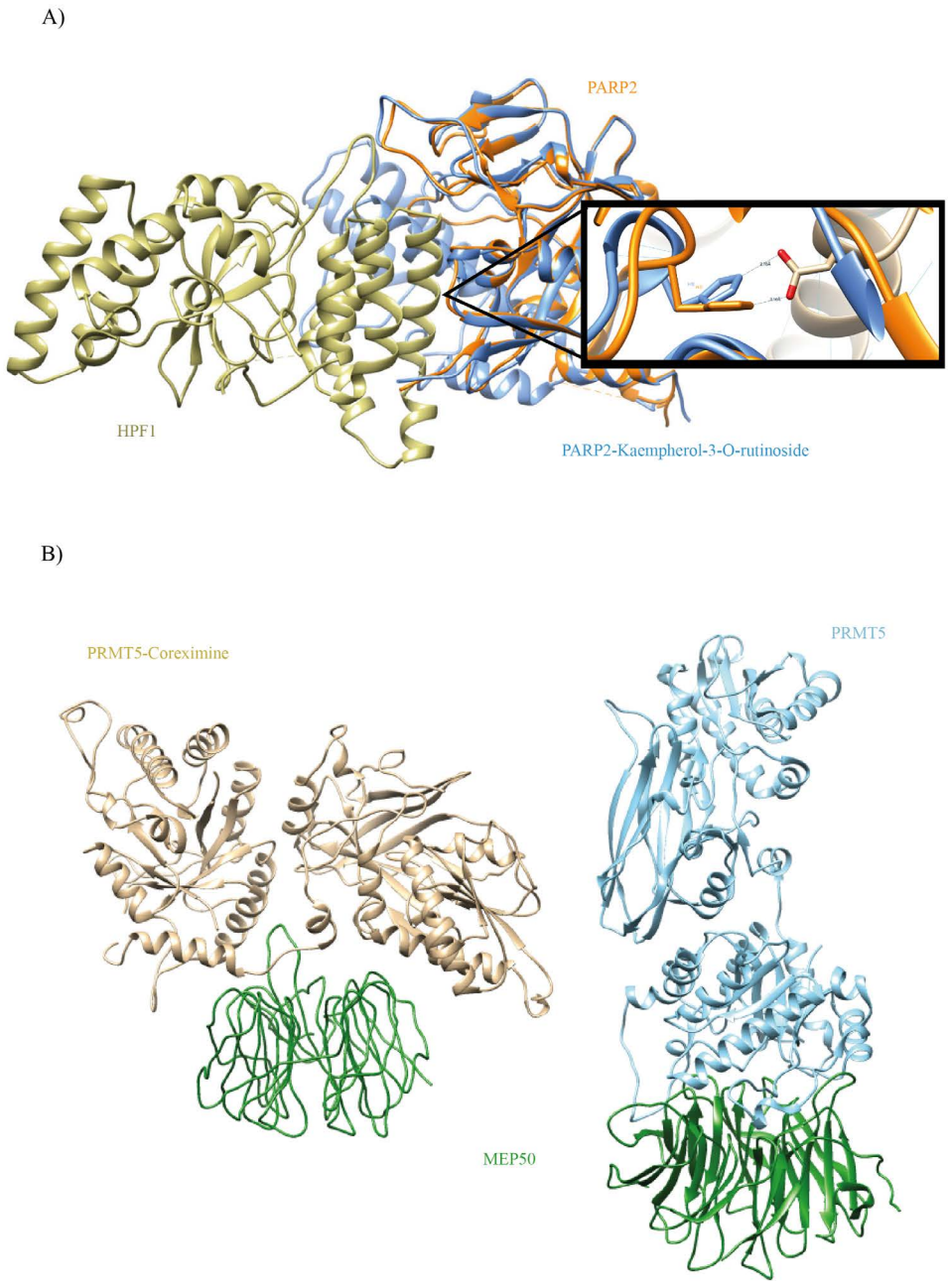

Figure 4. Comparison between the HPF1 and PARP2 interactions. Golden: HPF1; orange: PARP2 without the changes produced by the kaempferol-3-O-rutinoside; blue: PARP2 with the structural changes caused by the molecular dynamics in the presence of the kaempferol-3-O-rutinoside. A) A 0.68 Å change in the distance between the His381 residue of the PARP2 and the Asp283 of the HPF1; B) comparison between the MEP50 and PRMT5 interactions. Green: MEP50; blue: PRMT5 without coreximin; brown: PRMT5 with the structural changes caused by the molecular dynamics in the presence of the coreximin. Coreximin had a significant impact on the MEP50 and PRMT5 interactions.

O-glucuronidation, and methylation; the most common reaction was O-dealkylation (60.8%) (Table 5).

Table 4. Predicted reactions for the reactive atom tool of Way2Drug. ΔP value (Activity probability [AP] - Inactivity probability [IP]) shows the probability that the said reaction takes place.

Compound	SMILES of the compound	W2D/RA (ΔP)
Kaempferol-3-O-rutinoside	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC=C(C=C5)O)O)O)O)O)O)O	Methylation (0.763) O-Glucuronidation (0.613) Hydrolysis (0.186) O-Sulfation (0.220)
Coreximine	COC1=C(C=C2C3CC4=CC(=C(C=C4C-N3CCC2=C1)OC)O)O	O-Glucuronidation (0.558) O-Dealkylation (0.502) O-Sulfation (0.453) Methylation (0.420) Aliphatic hydroxylation (0.279) Aromatic hydroxylation (0.228) Dehydrogenation (0.154) N-Glucuronidation (0.039)
Coclaurine	COC1=C(C=C2C(NCCC2=C1)CC3=CC=C(C=C3)O)O	O-Dealkylation (0.608) O-Sulfation (0.288) O-Glucuronidation (0.246) Methylation (0.231)

The PASS and SOMP results showed that the enzymes that might cause deteriorations to the kaempferol-3-O-rutinoside, through first phase metabolic reactions, are the CYP3A4 (50.3%), CYP2C9 (28.3%), and CYP1A2 (36.9%) cytochromes. Meanwhile, the O-glucuronaidation (which belongs to the second phase metabolism) could be caused by the UDP-Glucuronyl Transferase (UGT) enzyme (94.8%). In the case of coreximin, the first phase metabolism would be caused by the CYP3A4 (34.8%), CYP2D6 (56.5%), CYP2C19 (39.4%), CYP2C9 (3%), and CYP1A2 (11.8%) cytochromes. The UGT has a low involvement in the second phase metabolism (19.4%). The CYP2D6 (16.7%) cytochrome is the only cytochrome involved in the first phase metabolism of coclaurine. It has a low affinity with the UGT enzyme, during the second phase metabolism (28%) (Table 6).

Molecular docking is currently one of the most effective and used methods for the *in silico* analysis of the potential interactions between molecules and their biological objectives. This process is usually focused in the prediction of the formation of the ligand based on the receptor molecule. Its affinity is subsequently estimated using a scoring function. These tools enable the understanding of how the chemical compounds interact with their molecular objective, leading to the development of innovative drugs (Pinzi *et al.*, 2019).

Table 5. Probability that the Kaempferol-3-O-rutinoside, coreximin and coclaurine compounds become substrates of the CYP (first phase metabolism) and UGT (second phase metabolism) isoforms. The recorded values are the ΔP (PA-Pi), obtained from the PASS and SOMP tools, both in the Way2Drug server.

	First phase metabolism					Second phase metabolism
Compound	**CYP3A4**	**CYP2D6**	**CYP2C19**	**CYP2C9**	**CYP1A2**	**UDP-Glucuronyl Transferase**
Kaempferol-3-O-rutinoside	0.503	-	-	0.283	0.369	0.948
Coreximine	0.348	0.565	0.394	0.030	0.118	0.194
Coclaurine	-	0.167	-	-	-	0.280

Table 6. Results of the protein-protein docking obtained in the HDOCK server.

Model	Docking score	RMSD Ligand
HPF1-PARP2-Kaempferol-3-O-rutinoside	−423.79	0.69
HPF1-PARP2 (PDB)	−363.73	0.40
MEP50-PRMT5-Coreximine	−236.47	174.40
MEP50-PRMT5 (PDB)	−678.96	71.16

In addition to the molecular docking, tools such as simulations or molecular dynamics are very important for the accurate prediction of the molecule-receptor interaction. This process has drastically expanded during the last few years (Pinzi *et al.*, 2019; Hollingsworth and Dror, 2018).

The molecular docking between the PARP2 protein and the kaempferol-3-O-rutinoside compound has a similar binding capacity to the one shown by olaparib, a residue used to bind the substrate of the PARP2. The presence of kaempferol-3-O-rutinoside in PARP2 caused changes in the rotation of the amino acids of the protein; however, they did not affect the formation of the HPF1-PARP2 heterodimer. Likewise, the binding capacity of the molecular docking between the PRMT5 enzyme and the coreximin compound was very similar to the one shown by sinefungin. This compound is used as inhibitor of the PRMT5. Just like in the case of PARP2, the PRMT5-coreximin complex shows stability after a 10-ns long simulation. Therefore, coreximin has the potential to be used as an inhibitor of the PRMT5. The presence of coreximin in the PRMT5 gave rise to structural changes in the protein, causing an important impact on the MEP50-PRMT5 interaction.

The Quantitative Structure Activity Relationship (QSAR) models are computational methods based on mathematical models, used to search for statistically important relationships between the structures and the functioning of chemical compounds. They are currently used as a major prediction tool during the creation of innovative medicines (Verma *et al.* 2010). Applying these tools to the kaempferol-3-O-rutinoside, coreximin, and coclaurine compounds showed that they are susceptible to such reactions as methylation, O-glucuronidation, and O-dealkylation. These reactions can be caused by the UDP-Glucuronyl Transferase enzymes or by the CYP3A4, CYP2D6, CYP2C19, CYP2C9, and CYP1A2 cytochromes. These first and second phase metabolisms are not uncommon, because they are some of the most common reactions that take place when drugs are introduced into the human body.

CONCLUSIONS

The kaempferol-3-O-rutinoside compound has the potential to become an inhibitor of the PARP2 enzyme; however, according to the QSAR models, it undergoes methylation and O-glucuronidation within the human body. The presence of this compound had no impact on the HPF1-PARP2 heterodimer formation. The coreximin compound showed the potential to become an inhibitor of the PRMT5 enzyme; however, according to the QSAR model, it experiences O-dealkylation and O-glucuronization within the human body. The presence of this compound did not have an impact on the formation of the

MEP50-PRMT5 heterodimer. Performing in vitro experiments might corroborate the inhibitory capacity of these soursop compounds on growth-relevant enzymes and in the development of cancer cells.

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Response of different sowing densities on agronomic parameters in the cultivation of *mejen* corn in Tabasco, Mexico

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ABSTRACT

Objective: To evaluate the response of sowing densities on agronomic parameters in the native *mejen* corn.

Design/Methodology/Approach: A randomized complete block design with four repetitions was used for the treatments: T1 (0.25 m between plants and two seeds per hole (80,000 plants ha^{-1})), T2 (0.50 m between plants and three seeds per hole (60,000 plants ha^{-1})), T3 (0.75 m between plants and four seeds per hole (53,333 plants ha^{-1})), and T4 (1 m between plants and five seeds per hole (50,000 plants ha^{-1})). The following variables were determined: plant height without male flower (PHWMF, cm), ear size (ES, cm), plant, bracts, and rachis dry biomass (t ha^{-1}); number of bracts, rows per ear, grain per row, grains per ear, and grain yield (GY, t ha^{-1}).

Results: Sowing densities influence the morphological response of plants, ears, and GY. The treatment with 80,000 plants ha^{-1} recorded a GY of 4.75 t ha^{-1} in traditional systems in Tabasco —greater than the regional average of 1.94 t ha^{-1}.

Study Limitations/Implications: The architecture of native corn allows an increase in productivity, as a result of the use of high densities.

Findings/Conclusions: Although treatments with greater sowing distances obtained a lower number of grains per ear, this phenomenon is compensated by the greater number of plants per row that leads to higher grain yields.

Keywords: ear, bracts, rachis, grain yield, rows.

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INTRODUCTION

Corn is the most studied crop in Mexico. It is grown in 475,339.43 ha, with an average yield of 3.87 t ha^{-1}. In the case of Tabasco, grain production reaches 149,737.65 t, with a yield of 1.92 t ha^{-1}. Approximately 11,667 ha are cultivated in Huimanguillo, one of Tabasco's municipalities, obtaining a yield of 1.94 t ha^{-1}, which accounts for 14.36% of the state production (SIAP, 2020).

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In these agricultural areas, farmers prefer to use native corn genotypes and implement systems such as the *milpa* (corn associated with horticultural and forestry crops) and low sowing densities, which causes variability in grain yield (Macías *et al.*, 2023).

Mejen is one of the most cultivated native corns in different regions of Tabasco, especially as part of the agroecosystem known as *marceño*, which is based on the traditional agriculture of the floodplains of Tabasco (Salgado-Velázquez *et al.*, 2021). In the *marceño* system, the *mejen* is grown during the dry season, taking advantage of the residual soil moisture. At its physiological maturity, *mejen* is tolerant to flooding, germinates in humid soils, matures between 2.5 to 3.5 months, and is accepted for human consumption (Narez-Jiménez *et al.*, 2015; Peraza-Villarreal *et al.*, 2019). However, the current worldwide trend is to implement agricultural practices that increase grain yields, mainly based on genetic improvement and high sowing densities, regardless of the different edaphoclimatic conditions (Aguilar, 2014).

Sowing density affects corn grain production. A wrong decision regarding this factor causes competition for resources within the plot, resulting in small plants, ear malformation, lack of grain filling, and low weight. It also causes an inadequate use of the crop area, as a consequence of greater evaporation, greater lodging, less leaf area, and less light capture. In this respect, it is important to choose an optimal sowing density, especially in native corn, since the increase in leaf decline generates a more homogeneous distribution of solar radiation in the growing area; meanwhile, the time it takes for corn to be covered by the canopy is reduced (Sánchez-Mendoza *et al.*, 2017; Salgado-García *et al.*, 2021). A recent work about native corn in Tabasco showed that the use of high sowing densities doubles local yields (Ramírez-Gómez *et al.*, 2020). Therefore, a greater or lesser regularity in the spatial distribution of plants can generate differences in yield between plots with the same type and population of corn (Chérrez, 2015). Therefore, the spatial arrangement of *mejen* corn sowing should be improved through the evaluation of different sowing densities and their effect on agronomic parameters, with the objective of evaluating which sowing distances can increase the productivity of *mejen* corn.

MATERIALS AND METHODS

Study area

During the spring-summer 2021 cycle, a *mejen* corn experiment was established in the Ranchería de Rio Seco y Montaña 2da section, in Huimanguillo, Tabasco, located at the UTM geographic coordinates X-439240.5 and Y-1985195.9. Corn has been planted in this area for more than 70 years. For this study, the native mejen corn, characterized by thin rachis and semi-toothed grain, was used.

Experimental design and treatment generation

A randomized complete block design was used, with the following treatments under study: T1 (0.25 m between plants and two seeds per hole (80,000 plants ha^{-1})), T2 (0.50 m between plants and three seeds per hole (60,000 plants ha^{-1})), T3 (0.75 m between plants and four seeds per hole (53,333 plants ha^{-1})), and T4 (1 m between plants and five seeds per hole (50,000 plants ha^{-1})).

The experimental plot consisted of three 2-m long furrows. Each treatment had four repetitions, generating 16 experimental plots. The central furrow of each experimental plot was taken as the useful section for data collection. Three plants were chosen at random from each central furrow to collect data, generating a total of 12 repetitions per treatment. The furrows of all experimental units were separated by one meter.

The seeds were placed by hand in each sowing hole, according to the corresponding treatment. Twenty-five days after the plant emerged, the 120N-80P-40K fertilization dose —with the commercial presentations of urea (46% N), triple superphosphate (46% P), and potassium chloride (60% K)— recommended for corn in the tropics (Barrón, 1998) was applied. The agronomic management was based on the technological package developed by INIFAP for corn cultivation (INIFAP, 2015). Hand weeding and insect pest controls were carried out. Pest control was first carried out 25 days after emergence, using 3 mL L^{-1} of cypermethrin; subsequently, it was carried out every eight days. Once the grain began to emerge, common traps (bird scarers) were set up. Finally, relief irrigation was carried out after 45 days of emergence.

Variables

Plant height without male flower (PHWMF, cm) and ear size (ES, cm)

The PHWMF was measured prior to the VT stage (ear emergence) and the ES during the R1 stage (female flowering). Three plants were selected at random from the central furrow of each experimental unit and were measured with a flexometer (Ali *et al.*, 2017).

Male flower length (MFL, cm) and number of leaves below the ear (NLBE)

The MFL and NLBE were determined in three plants of the central furrow of each experimental unit.

Yield

Plant, bracts, and rachis dry biomass (t ha-1) and grain yield (GY, t ha^{-1})

The plant, bracts, and rachis fresh weight was determined in the field during stage R6 (physiological maturity), using a grain scale. Three ears of three plants were selected from the central furrow of each experimental unit for their measurement. The samples were placed in manila envelopes and labeled for their identification. The samples were dried in an oven with air flow at 65 °C for 72 h to determine their humidity percentage; subsequently, the plant, bracts, and rachis dry weight was calculated in the Petroleum Chemistry laboratory of the Universidad Popular de la Chontalpa (UPCh).

To determine the grain yield, the total grains per ear were weighed and multiplied by the number of plants in each of the sowing densities.

Number of leaves (bracts), rows, grain per row, and grain per ear

Three ears were chosen at random from the central part of each experimental unit. The number of bracts, the number of rows, and the grains per row were counted for each physiologically mature ear. Finally, the grains per row were multiplied by the number of rows to obtain the total number of grains per ear.

The randomized complete blocks were subjected to an analysis of variance. The variables with statistically significant differences were subjected to a multiple-comparison test of means, using the Tukey method (P≤0.05). All analysis were performed with SAS 9.3 software.

RESULTS AND DISCUSSION

Plant height without male flower (PHWMF, cm) and ear size (ES, cm)

Based on the analysis of variance (Table 1), the PHWMF and ES did not present statistically significant differences between sowing density treatments. The coefficients of variation (19.53 for PHWMF and 19.51 for ES) are considered admissible.

The results of this study are similar to those reported by Sánchez-Mendoza et al. (2017) in Valles Altos de México and Ramírez-Gómez *et al.* (2020) in Tabasco, both of whom evaluated native corn with different sowing densities. The low PHWMF and ES values were lower than those established by Ramírez-Gómez *et al.* (2020); this low height could be caused by the low soil moisture contents and the scarce precipitation in the period (date) of the field experiment. However, lower height and size values for PHWMF and ES are desirable, since other reports have related these variables to acame (lodging) in native corn (Del Carmen-Bravo *et al.*, 2022). Likewise, the reported ES facilitates the manual harvest of *mejen* corn, especially when it has been established in the lowlands of Tabasco, which face problems from excess humidity (Ramírez-Gómez *et al.*, 2020).

Male flower length (MFL, cm) and number of leaves below the ear (NLBE)

MFL and NLBE did not present statistical differences between sowing densities, according to the analysis of variance (Table 1). These results are contrary to the findings of Cao *et al.* (2021), who point out that the sowing density must be taken into account: a high density reduces the transmission of light within a crop and accelerates the senescence of the leaves, which affects the crop's photosynthesis and the distribution of substances, limiting grain development (Chávez *et al.*, 2021).

Table 1. Analysis of variance and Tukey means of the different sowing densities treatments in the study and their effect on the plant height without male flower, ear, inflorescence, and bracts of *mejen* corn in Huimanguillo, Tabasco.

Treatment (Plants ha^{-1})	PHWMF cm	ES cm	MFL cm	NLBE	
80 000	144.69a	74.28a	37.73a	8.25a	
60 000	147.06a	89.27a	44.75a	9.00a	
53 333	141.48a	79.27a	40.33a	7.50a	
50 000	129.11a	69.99a	30.90a	7.50a	
CV	19.53	19.51	18.09	10.59	
Prob. of F.	0.798		0.357	0.086	0.083
MSD	57.62		32.02	14.58	1.79

PHWMF: plant height without male flower; ES: ear size; MFL: male flower length; NLBE: number of leaves below the ear; CV: coefficient of variation; MSD: minimum significant difference. Values in a column with the same letters have no significant difference (NS), according to Tukey's tests (p>0.05).

Performance variables

According to the analysis of variance (Table 2), the dry biomass of the plant and the bracts did not have statistical differences, unlike the dry biomass of rachis. Tukey's test ($P<0.05$) showed that the sowing density treatment with 80,000 plants ha^{-1} was superior to the others.

The 80,000-plants ha^{-1} sowing density treatment had values of 7.87, 1.21, and 0.68 t ha^{-1}, for the highest plant, bracts, and rachis dry biomass, respectively. These yields were higher than those found by Córdova-Sánchez *et al.* (2012), who reported a production of 3.20 t ha^{-1} of plant dry matter, 0.78 t ha^{-1} of bracts, and 0.68 t ha^{-1} of rachis, with the same variety (*mejen*) and with a sowing density of two seeds per hole, 0.25 cm between plants, and one meter between furrows. In fact, the competition for major growth factors is minimal among more evenly spaced plants. The growth factor that is most affected is light, followed by nutrients and water —a phenomenon which is perhaps related to the dry biomass of the plant and bracts, although it does not influence the biomass of the rachis (Testa *et al.*, 2016).

Grain yield (GY, t ha^{-1})

The analysis of variance (Table 2) showed statistically significant differences between the different sowing density treatments. According to Tukey's test ($P<0.05$), the treatments with 80,000 and 53,333 plants ha^{-1} obtained the best results. Likewise, grain yields and rachis dry weight showed a trend as sowing densities increased.

The highest GY was recorded with the sowing density of 80,000 plants per hectare (4.75 t ha^{-1}) and it was higher than the GY reported by Ramírez-Gómez *et al.* (2020), who used native corn from Tabasco and sowing densities of 0.20 and 0.25 between plants (with GYs of 3.86 and 3.80 t ha^{-1}, respectively), 1.0 m between rows, and two seeds per hole.

The results of this study show that it is possible to increase local yields by 1.94 t ha^{-1} of grain for native corn in the state of Tabasco (SIAP, 2021). Like many native corns, the

Table 2. Analysis of variance and Tukey's test for the different sowing densities treatments under study and their effect on the plant, bracts, and rachis dry biomass (t ha^{-1}) and grain yield (t ha^{-1}) per hectare of *mejen* corn, in Huimanguillo, Tabasco.

Treatments Plants ha^{-1}	Dry biomass			
	Plant	Bracts (Joloche)	Rachis (Bacal)	Grain Yield (GY)
	t ha^{-1}			
80 000	7.87a	1.21a	0.68a	4.75a
60 000	6.70a	1.01a	0.46ab	3.56b
53 333	6.63a	0.91a	0.49ab	3.70ab
50 000	5.26a	0.63a	0.36b	2.55b
C.V.	23.02	30.32	24.22	15.49
Prob. of F.	0.174NS	0.081NS	0.01*	0.001**
MSD	3.20	0.60	0.25	1.18

Values in a column with the same letter have no significant difference (NS), *: a highly significant difference.

architecture of the leaf angle is wider than in the hybrids, in which it is more erect and closed. When it is combined with a greater distance between rows, it allows the crop to efficiently take advantage of the solar radiation, assimilating photosynthates, especially in the leaves adjacent to the ear; this phenomenon is reflected in higher grain yields (Sánchez-Mendoza *et al.*, 2017). Table 3 shows that an increase in the sowing density and a shorter distance between plants improves grain yield (Gao *et al.*, 2021), despite its low number of grains per ear (Blanco-Valdés and González-Viera, 2021). Therefore, increasing sowing densities causes a decrease in yield per plant, although the greater number of plants increases grain yield (Getaneh *et al.*, 2016).

Number of bracts, rows per ear, grains per row, and grains per ear

According to the analysis of variance (Table 3), the number of bracts and the number of rows per ear did not have a statistically significant difference, unlike grains per row and grains per ear. According to Tukey's test ($P<0.05$), grains per row and grains per ear had similar values in the treatments with 53,333 plants ha^{-1}.

These results differ from the findings of Jia *et al.* (2018), who reported that the number of rows per ear increased with sowing densities. The highest number of total grains per row and per ear were found with 53,333 plants ha^{-1}, 32.15 total grains per row, and 335.93 total grains per ear. This increase in the number of grains per ear and per plant —when a greater space is established between rows— is attributed to a greater net assimilation rate of corn varieties and to the division and reduction of competition in larger spaces (Shaka *et al.*, 2019). This was the case of the 53,000 and 60,000 plants ha^{-1} sowing densities, which had greater spacing between plants per row.

Consequently, ears with higher grain numbers do not always generate higher grain yields: the treatment with 80,000 plants ha^{-1} recorded the highest grain yield and obtained the lowest number of grains per ear. This phenomenon is attributed to greater competition in the surface of the soil among roots, as a result of shorter distances between plants, which reduces root weight and their correct functioning (Gao *et al.*, 2021).

Table 3. Analysis of variance and Tukey's test for the different densities analyzed and their effect on the number of bracts, rows of grains, grains per row, and grains per ear of *mejen* corn, in Huimanguillo, Tabasco.

Plants ha^{-1}	Number of brachts (Joloche)	Rows per ear	Grains per row	Grains per ear
80,000	12.40a	10.25a	23.65b	242.45ab
60,000	10.50a	9.78a	26.25ab	257.98ab
53,333	12.23a	10.43a	32.15a	335.93a
50,000	12.78a	10.23a	22.93b	233.88b
C.V.	12.99	7.31	14.31	17.34
Prob. of F.	0.22NS	0.65NS	0.01*	0.03*
MSD	3.27	1.56	7.89	97.38

Values in a column with the same letter have no significant difference (NS), according to Tukey's test ($P<0.05$).

CONCLUSIONS

Sowing densities influence plant morphological adaptation, ears, and particularly grain yield response. The increase in sowing densities represented an increase in grain yield for native mejen corn. The treatment with 80,000 plants ha^{-1} had a grain yield of 4.75 t ha^{-1} —a value higher than the 1.92 t ha^{-1} regional average in low-density systems in Tabasco. The results indicate that the lower number of grains per ear of the treatments with greater sowing distances is compensated by the higher number of plants per row, given the higher grain yields obtained.

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Presence of endophytic fungi in cacao plantations (*Theobroma cacao* L.), in the state of Tabasco, Mexico

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ABSTRACT

Objective: The present work was done with the objective of identifying endophytic fungi associated with *Theobroma cacao* L. in Centro, Cunduacán and Comalcalco, locations in the state of Tabasco, Mexico. The molecular identity used was the region of the Internal Transcribed Spaces (ITS), ITS 1 and ITS 4.

Design/methodology/approach: The study identified 15 fungal strains, grouped into 13 different species, belonging to the *Ascomycota phylum*, distributed in three different classes: *Dothideomycetes*, *Eurotiomicetos* and *Sordariomycetes*. It is important to mention that it is the first record of *Endomelanconiopsis endophytica* and *freycinetiae* found in cacao in Tabasco. In addition, we also identified *Aspergillus foetidus*, *Fischeri*, *Delicatus arcoverdensis*; *Thielaviopsis ethacetica*, *Cophinforma atrovirens*, *Neurospora udagawae*, *Diaporthe miriciae*, *Nodulisporium indicum*, *Cophinforma atrovirens*; *Colletotrichum tainanense* y *hebeiense*.

Findings/conclusions: Many of these endophytic fungi produce secondary metabolites and antioxidants that can be used in the medical industry or for biological control of phytopathogenic diseases, such as *Moniliophthora roreri*.

Keywords: Cocoa, fungi, endophytes.

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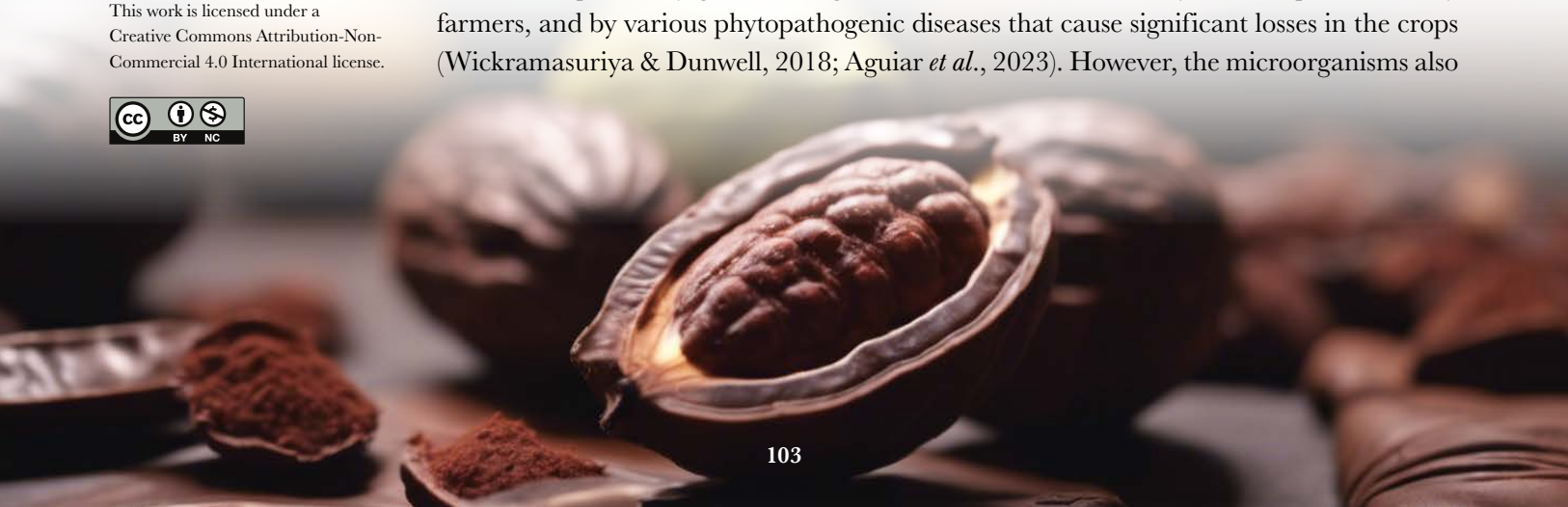
INTRODUCTION

The agrifood sector is one of the most important socioeconomic activities in the world because it provides a large diversity of food products to satisfy human needs. This sector has been impacted by global changes that influence the economy and the production by farmers, and by various phytopathogenic diseases that cause significant losses in the crops (Wickramasuriya & Dunwell, 2018; Aguiar *et al*., 2023). However, the microorganisms also



carry out an important role in the ecosystem; plants could not survive without mutualist microbes, since they improve the immune system, promote growth, and eliminate the diseases transmitted by the soil; the microbiome is considered as a gene reservoir. Cacao (*Theobroma cacao* L.) from the *Malvaceae* family, is one of the most important crops in the world and has faced various problems, primarily from phytopathogenic diseases (Aikpokpodion *et al.*, 2009). It is cultivated in more than 58 countries of Africa, America, Asia and Oceania. The International Cocoa Organization (ICCO) reported a global production of 4,923 thousand tons in 2021/2022 (MIDAGRI, 2022). In 2021, the Ministry of Agriculture and Rural Development (*Secretaría de Agricultura y Desarrollo Rural*, SEDER) reported that Mexico occupies the fourteenth producer at the global level with 28,106 tons of grain and 44,500 to 47,800 hectares of cacao; Tabasco, Chiapas and Guerrero are the main producing regions. Grain production has been affected primarily by phytopathogenic fungi with losses of 30 to 70% (Díaz *et al.*, 2020). Some important phytopathogens are: *Moniliophthora roreri*, *M. perniciosa*, (Bailey *et al.*, 2018); *Phytophthora palmivora*, *P. theobromicola*, and *Nodulosporium* sp., (Decloquement *et al.*, 2021; González *et al.*, 2019). Other fungi reported in the literature are endophytes which inhabit plants without causing apparent symptoms of a disease, in a balanced antagonistic relationship, in which nutrients and residence are provided for the fungus. In addition, the fungus favors the immune system of the host, produces secondary metabolites, and improves the resistance to pathogens (Tiwari & Bae, 2022). The following have been identified as endophytic fungi of plants: *Fusarium graminearum*, *F. equiseti*, *Lasiodiplodia jatrophicola* (Cruz *et al.*, 2022). In *T. cacao*, the following have been isolated: *C. gloeosporioides*, tropicale, theobromicola (Christian *et al.*, 2019); *L. theobromae*, *F. chlamydosporum*, *F. oxysporum*, *Verticillium luteo* (Rubini *et al.*, 2005), to mention a few. Because of this, and due to the great importance that fungi organisms have in plants, specifically in *T. cacao*, the objective of this study was focused in the isolation and the molecular identification of endophytic fungi of three cacao plantations in the state of Tabasco, with the aim of contributing knowledge about the fungal diversity of this important crop for Mexico and the state of Tabasco.

MATERIALS AND METHODS

Sampling sites

Three sites were selected for sampling in the state of Tabasco, Mexico (Figure 1): Centro (17° 58' 39.0" N; 93° 03' 45.0" W -Hacienda Buena Vista); Cunduacán (18° 06' 14.8" N; 93° 18' 26.3" W -Hacienda Río Seco); and Comalcalco (18° 15' 54.2" N; 93° 13' 39.9" W -Hacienda a Luz).

Collecting the plant material

The collection was done in March, 2019, with random sampling by selecting healthy and infected fruits and leaves, the latter with a slight infection; small dark spots with oily appearance or deformations; fruits that presented necrosis or white powder characteristic of a fungal disease were not selected (Aikpokpodion *et al.*, 2009). Five cacao plants per plantation were selected, and two fruits and two leaves were collected from each individual (a healthy one and an infected one), with a total of 60 samples. The leaves selected were

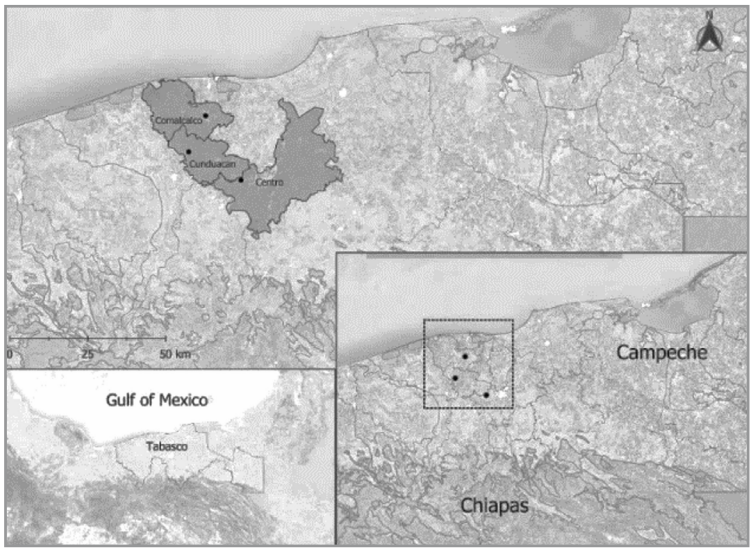


Figure 1. Location of the three collecting sites in the municipalities of Centro, Cunduacán and Comalcalco, in the state of Tabasco, Mexico.

cut with a pole previously disinfected with alcohol at 70%, while a pruning scissor was used for the fruits. The plant material was placed in Kraft paper bags, with the corresponding data, and conserved at a temperature of 4 °C. The isolation of fungi was carried out in the microbiology laboratory, while the molecular analyses were carried out in the genomic laboratory of Universidad Juárez Autónoma de Tabasco in the Academic Biological Sciences Division (*División Académica de Ciencias Biológicas*, UJAT-DACBiol).

Isolation of the fungal material

The processing of the samples was carried out under controlled sterile conditions in a laminar flow bell. The methodology for fungi isolation was the one proposed by Cañedo and Ames (2004), and Azuddin *et al.* (2021). Small fragments of 3 to 5 mm were cut from the borders with lesions and healthy tissue, using a sterile scalpel blade. Consecutively, the plant tissue was disinfected with hypochlorite at 1% and alcohol at 75%, each during one minute, and washed with sterile tri-distilled water (30 seconds). Four to five fragments were transferred to moisture chambers and some cuts were placed on a potato dextrose agar plate (PDA-Bioxon®). They were incubated at 27 °C for 3 to 5 days. The purification was done by transferring hyphae growth to PDA plates, to obtain new monosporic growth.

DNA extraction, PCR amplification, and DNA sequencing

The isolates obtained were transferred to 40 mL of potato dextrose broth (PDB) in Erlenmeyer flasks of 250 mL, incubating at room temperature for 3 to 7 days to obtain mycelium growth. The resulting mycelium was filtered with Miracloth paper (20-25 μm) washed twice in sterile tri-distilled water. Later, the mycelium is pulverized with liquid nitrogen (N_2) with the help of a porcelain pestle and mortar. The total genomic DNA was extracted from the mycelium of each of the individual isolates according to the protocol proposed by Stirling (2003). The quality of the DNA was analyzed with a spectrophotometer

at a wavelength of A260 nm and the purity based on the A260/280 rate. Electrophoresis in agarose gel at 1.5% dyed with ethidium bromide was used to verify the integrity of the DNA (0.5 μg/mL).

Amplification by Polymerase Chain Reaction (PCR)

The region of the Internal Transcribed Spaces (ITS), between ribosomal (rADN) 18S-5.8S and 5.8S-28S were amplified by PCR in each sample. The first were ITS1 (5´-TCCGTAGGTGAACCTGCGC-3´) and ITS4 (5´-TCCTCCGCTTATTGATATGC-3´), and the amplification protocol was the one proposed by White *et al.* (1990). The PCR amplification per sample consisted in: 15 μL of ultrapure water free of nucleases, 10 μL of 5X green: 1 μL of the following reagents: bovine serum albumin (BSA), 0.2mM dNTPs, MgCl at 1.5 mM, 10 μM of each starter, 1.25u of GoTaq® DNA polymerase and DNA at 100 ng. The amplifications obtained were verified by electrophoresis in Ultrapure™ Agarose 1000 at 2.5% w/v (1XTAE buffer), dyed with ethidium bromide (0.5 μg/mL), and visualized under UV light in a Bio-Print (Vilber®) transilluminator. The fragments were determined by comparison with a marker of 1-Kb (Invitrogen®). The sequencing was carried out with the Genetic 3500xl Analyzer (Applied Biosystems, Foster City, CA) at the Instituto Potosino de Investigación Científica y Tecnológica A.C. (IPICYT), in both directions ITS1 and ITS4. The ITS sequences were edited and assembled manually using the Bioedit 7.2.5 software (Hall, 1999). The sequences were aligned using the ClustalX 2.1 software (Thompson *et al.*, 1997), with the predetermined configuration. The set of sequences aligned built a phylogenetic tree with the sequences of endophytic fungi using the Molecular Evolutionary Genetics Analysis (MEGA) XI software (Tamura *et al.*, 2021). The ITS sequences were analyzed with searches in the Basic Local Alignment Search Tool (BLAST) system of the National Center for Biotechnology Information (NCBI, http://www.ncbi.nl-m.nih.gov).

RESULTS AND DISCUSSION

In total, 15 isolates from cacao (*T. cacao*) plantations were obtained, in the municipalities of Centro, Cunduacán and Comalcalco in Villahermosa, Tabasco, Mexico. Amplicons of 450 to 650 pb corresponding to the identification gene (ITS1 and ITS4) were obtained. The Blast analysis revealed that the fungi belong to the *Ascomycota phylum*, grouped into three classes: *Dothideomycetes*, *Eurotiomicetos* and *Sordariomicetos*. Table 1 shows the results obtained from the 15 fungi strains grouped into 13 different species; and the percentage of identity, total score, and number of access provided by the database from National Center for Biotechnology Information (NCBI) were also observed.

Figure 2 shows the phylogenetic analysis that was generated by the UPGMA method with a branch length of 3.3978, and the evolutionary distances were calculated using the method of Maximum Likelihood using 572 positions in the set of final data.

In the dendrogram developed from the sequences obtained, two evolutionary groups can be seen, the first of which is subdivided into two groups; the first group includes the families *Botryosphaeriaceae*, *Aspergillaceae*, *Glomerellaceae*, *Ceratocistidáceas*, *Hipoxiláceas* and *Sordariaceae*; the second group includes species from the *Aspergillaceae*, *Diaporthaceae* and

Table 1. Identification of isolate from the *Ascomycota phylum*, based on data obtained from the ITS rDNA sequences (https://www.ncbi.nlm.nih.gov/).

Isolation	GenBank number	Sample	GenBank ID	Size (pb)	Query Score (%)	Identity (%)	Place		
							Cunduacán	Centro	Comalcalco
H174	156272.1	Healthy leaf	CBS 120397 *Endomelanconiopsis endophytica*	526	100	99.81		X	
H171	158434.1	Healthy leaf	MFLUCC 17-0547 *Endomelanconiopsis freycinetiae*	479	98	98.76		X	
H177	156272.1	Healthy fruit	CBS 120397 *Endomelanconiopsis endophytica*	524	100	99.80		X	
H02	163668.1	Infected fruit	CBS 121.28 *Aspergillus foetidus*	365	88	98.46		X	
H53	137479.1	Infected fruit	NRRL 181 *Aspergillus fischeri*	441	72	77.61		X	
H100	155899.1	Infected fruit	IMI 50560 *Thielaviopsis ethacetica*	448	95	97.2	X		
H05	164291.1	Infected fruit	CBS 124934 *Cophinforma atrovirens*	430	98	99.53	X		
H148	103582.1	Infected fruit	CBS 309.91 *Neurospora udagawae*	358	93	95.85	X		
H130	147535.1	Healthy fruit	BRIP 54736 *Diaporthe miriciae*	437	97	97.18	X		
H76	160206.1	Infected fruit	CBS 101754 *Aspergillus delicatus*	262	85	94.25	X		
H60	166005.1	Healthy fruit	CBS 124.83 *Nodulisporium indicum*	461	99	95.81			X
H68	151816.1	Healthy leaf	JCM 19878 *Aspergillus arcoverdensis*	334	100	96			X
H301	164291.1	Healthy fruit	CBS 124934 *Cophinforma atrovirens*	337	78	93.54			X
H01	171185.1	Healthy fruit	CPC 30245 *Colletotrichum tainanense*	487	94	99.78			X
H120	160815.1	Healthy fruit	MFLUCC 13-0726 *Colletotrichum hebeiense*	480	91	98.86			X

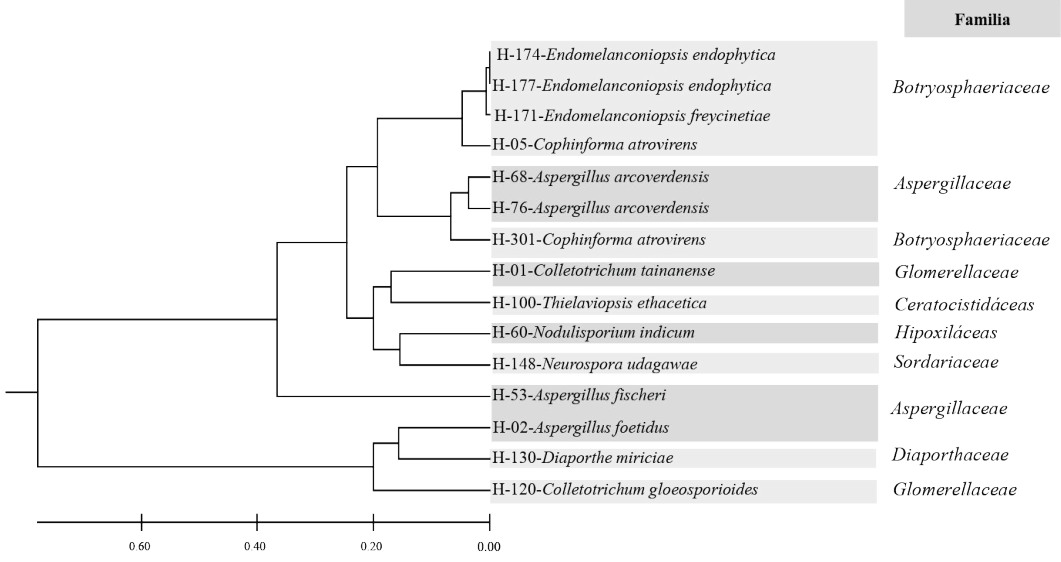


Figure 2. Representation of the phylogenetic proximity based on sequences of the ITS rDNA region of the species identified isolated in cacao trees of the state of Tabasco, using the UPGMA method; the evolutionary distances were calculated using the Maximum Likelihood method.

Glomerellaceae families, without geographic correlation. It is important to mention that no previous records of *Endomelanconiopsis endophytica*, *E. freycinetiae*, *Cophinforma atrovirens*, *Diaporthe miriciae*, *Neurospora udagawae* and *Colletotrichum tainanense* in Mexican cacao were found. These species have been isolated from tissues of plants from tropical and subtropical regions, similar to the climate in Tabasco (Macabeo *et al.*, 2020). The species *E. endophytica* was reported for the first time by Rojas *et al.* (2008), in the Republic of Panama, in healthy cacao leaves and in other plants of commercial interest such as *Ficus hirta* and tropical palm (Douanla & Scharnhorst, 2021; Sun *et al.*, 2016). *E. freycinetiae* has been identified in the *Pandanaceae* family, closely related with *E. endophytica* (Tibpromma *et al.*, 2018). They reside harmoniously in the plants and are considered antagonistic fungi, capable of inhibiting phytopathogens such as *Colletotrichum truncatum* and *F. oxysporum* (Azuddin *et al.*, 2021); in addition, they can segregate secondary metabolites with cytotoxic activity in cell lines and with antioxidant properties (Sun *et al.*, 2016). This study identified *N. indicum* from the Xylariales order (Bitzer *et al.*, 2008) in the database from the European Bioinformatics Institute (EMBL-EBI); until today it has been recorded in India and Vietnam. In Mexico, *Nodulosporium* sp. was isolated from infected cacao pods, recorded by González *et al.* (2019), causing symptoms of pod rotting and leaf dehydration. Another species isolated was *D. miriciae*, which has been described by Thompson *et al.* (2015) in plants of *Glycine max* and *Vigna radiata* in Australia. Regarding *N. udagawae* from the genus *Sordariaceae*, it is considered an endophytic fungus that colonizes soils, trees and dead shrubs (Fujimoto, 2018; Macabeo *et al.*, 2020). This study reports four species from the *Aspergillaceae* family; *Aspergillus foetidus*, *arcoverdensis*, *fischeri* and *delicatus*, from the *Aspergillus* genus, common in cacao plants and seeds (González *et al.*, 2019). On the other hand, *T. ethacetica* was identified, generalist pathogen with a wide variety of hosts, such as sugarcane, cacao and coconut. Its origin could probably be anthropogenic and it has been recorded in many countries in the five continents (Borges *et al.*, 2019; Mbenoun *et al.*, 2015). Two species from the *Colletotrichum* genus (*C. tainanense* and *hebeiense*) were isolated, frequently found in cacao, and this genus one of the most economically important (Landero *et al.*, 2017). Recent studies in Indonesia, Taiwan and India report *C. tainanense* as a pathogen in *Capsicum annuum* and *Punica granatum* L. (De Silva *et al.*, 2019; Manjunatha *et al.*, 2022), causing a disease called anthracnosis.

Finally, species such as *E. endophytica*, *A. arcoverdensis*, *D. miriciae* and *N. indicum* could be used for the biological control of plant diseases, primarily for cacao, since the literature expresses that they could present a series of chemical substances with antioxidant and anti-inflammatory activity, and of great interest in the pharmaceutical and cosmetic industry, and for biological control (Fujimoto, 2018; Reyes *et al.*, 2021). These results suggest that the agroforestry system sustains a large diversity of fungal species, and many of them could be used as biological control or for development in the pharmaceutical industry (Reyes *et al.*, 2021). It should be highlighted that it is necessary to perform more studies with the fungi identified, such as pathogenicity tests, metabolite detection, or to apply genomic methods based on molecular markers that allow identifying the possible allele variants associated with pathogenicity and aggressiveness of these species (Douanla-Meli & Scharnhorst, 2021).

CONCLUSIONS

This study reports 13 species of different endophytic fungi isolated from cacao trees in the state of Mexico. Non-pathogenic endophytic species are reported until now (*E. endophytica*, *E. freycinetiae*, *A. arcoverdensis*, *D. miriciae*), which with molecular and pathogenicity studies could be used as organisms for biological control of phytopathogens in trees of economic interest, among them cacao.

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Strategies to reduce the infestation of *Pseudohypocera kerteszi* (Diptera: Phoridae) in colonies of *Scaptotrigona mexicana* (Hymenoptera: Apidae)

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ABSTRACT

Objective: We intend to describe the strategies used for the control *Pseudohypocera kerteszi* in colonies of *Scaptotrigona mexicana* which help to reduce infestation during transfer, division, and harvest.

Design/methodology/approach: In this research, bibliographic sources of information on the control of *P. kerteszi* during the management of *S. mexicana* were used. The selected strategies were implemented by the authors of this manuscript, so they provided the experiences that were had in the control of *P. kerteszi* in the colonies of *S. mexicana*.

Results: We report six strategies applied to reduce the attack of *P. kerteszi*: 1) perform the transfer in a closed place, 2) minimize the damage to the offspring, 3) do not introduce food, 4) close the entrance to the nest for at least two days, 5) place vinegar traps, when necessary and in the initial stages of the infestation and 6) feed and clean bees the following days after the transfer or division.

Limitations on study/implications: Any limitation was involved in this study.

Findings/conclusions: The attack of *P. kerteszi* on *S. mexicana* colonies takes place during the transfer of nests, artificial division and the harvest of honey. Strategies to avoid infestation consist of using the appropriate box design, harvesting in an enclosed place, and avoiding breaking honey pots. During the critical stages of infestation, the revision must keep daily to clean the box, place vinegar traps, and make a manual control.

Keywords: Stingless bees, kleptoparasite, management, scuttle flies, meliponiculture.

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INTRODUCTION

Scaptotrigona mexicana (Guérin-Méneville, 1845) (Orden: Hymenoptera, Familia: Apidae) is an ecologically, economically and culturally important stingless bee (Quezada-Euán, May-Itzá & González-Acereto, 2001). In recent years, the destruction

of its habitats and attacks by natural predators have placed its populations at risk, thus the importance of their protection and conservation (Martínez-Fortún, Ruiz, Acosta & Vit, 2018). These bees naturally live in tree trunks, where natural enemies find it difficult to destroy their nest, yet may reduce its population. However, the commercial management of these bees in artificial housing (pots and poxes) present a high risk of their enemies completely destroying the colony if the necessary control measures are not taken.

One of the most relevant natural enemies of *Scaptotrigona mexicana* is the phorid *Pseudohypocera kerteszi* (Enderlein, 1912) (Order: Diptera, Family: Phoridae). An infestation of a bee colony by this pest is very difficult to control, since rescue interventions are arduous and not very efficient. In general terms, the elimination or burning of the colony is recommended to avoid the infestation of other colonies (Chaud-Netto, 1980; Moo-Valle, 2018).

P. kerteszi is also known as the phorid fly or "nenem" (in Mayan) (Pat, Anguebes, Hernández & Ramos, 2018). Its distribution in the Americas ranges from Mexico to Central America, and even South America (Robroek, De Jong, Arce & Sommeijer, 2003). In general, phorids are considered scavengers and saprophagous (Brown, 1992), making it a pest, particularly in the rainy season (Pat *et al.*, 2018).

The anatomy and life cycle of *P. kerteszi* is very characteristic. Its eggs are white and measure 1 mm. Larvae measure 1.6 mm in the first stage and 6 mm y the final stage and they are a dull white colour. Pupae measure 5 mm and they are light to dark maroon. Adults measure between 2.25 and 3 mm. Their development from eggs to adulthood takes, on average, between 12.8 and 16 days, whereas the egg stage lasts between 12.3 and 19.5 hours, the larval stage, between 6.9 and 7.2 days, and the pupa, 5.4 to 8.0 days. Adults present a prominent pronotum and a short abdomen that points downward, giving it a curve-shaped appearance. Females can lay anywhere from 31 to 102 eggs, with a viability of 72 to 82%, with a laying period of 35 to 45 days (Robroek, De Jong & Sommeijer, 2003; Wolff & Nava, 2007). The *P. kerteszi* female presents a very prolonged egg-laying apparatus, which lets it lay eggs in the cracks or fissures of boxes or certain structures of bees' nests, making it difficult for worker bees to remove the phorid's larvae from the colony.

There are three critical points in which *P. kerteszi* can infest the nest of a colony of *S. mexicana* under commercial management: 1) during the transfer of nests, 2) during the artificial division of the colony, and 3) during the harvest of the colony's products. The female phorids enter the colony through the entrance to the nest and past the guardian bees, since they are attracted by the acidic smell of the pollen, honey and larval food. Generally, on the female's first day inside the colony, it lays eggs, preferably in the pollen stored by the bees, in the involucre, in the cells of the honeycombs of the offspring destroyed by inadequate management, in broken pots with pollen and in garbage deposits (Moretto, 2000; Robinson, 1981; Robroek *et al.*, 2003; Tolsá & Ballesta, 2017; Wolff & Nava, 2007).

The aim of this work is to describe the strategies used to control *P. kerteszi* in *S. mexicana* colonies to reduce infestation and implement them in the transfer, division and harvest.

MATERIALS AND METHODS

The information on the strategies for the control of *P. kerteszi* was obtained by a bibliographical revision based on manuals, books and on the search of scientific articles in the referential databases Scopus, Web of Science Group, Google Scholar, Elsevier and Springer Link, using the following keywords: *P. kerteszi*, control, management, stingless bees, phorid and meliponiculture. On the other hand, the strategies chosen were implemented by the authors of the present manuscript, therefore contributing the experiences in the control of *P. kerteszi* in the colonies of *S. mexicana*, thus reducing the risk of infection by the phorid.

RESULTS AND DISCUSSION

Strategies to control *P. kerteszi* applied to transferred colonies. The transfer of the *S. mexicana* colony nests from clay pots to wooden boxes, complete with its food reserves, causes a high infestation of *P. kerteszi*, since this process destroys parts of the structure of the nest, which includes honey pots. This causes a disorganization of the members of the bee colonies, facilitating the access for *P. kerteszi* in the time taken to carry out the transfer.

The first strategy to avoid infestation by *P. kerteszi* is to carry out the transfer inside a small room made of mosquito nets, commonly called a "pavilion". A second strategy is to avoid introducing broken honey or pollen pots in the boxes in which the honeycombs are placed when performing the transfer (Moo-Valle, 2018). The third strategy consists in closing the entrance tube with wax for the first two days after the division, that is, blocking the entrance with the wax and resin that bees use to build their nests, since this time helps bees rebuild their nests and reorganize in their jobs inside the nest (Gennari, 2019). Generally, bees open the entrance themselves when the recovery of the nest is complete.

Strategies to control *P. kerteszi* during artificial division. It is recommended to carry out the division of the *S. mexicana* colonies when the colony no longer has enough space to develop. Once again, it is recommended to carry out the division inside a pavilion and avoid breaking the pots containing pollen or honey, since this may attract flies (Moo-Valle, 2018) (Figure 1).

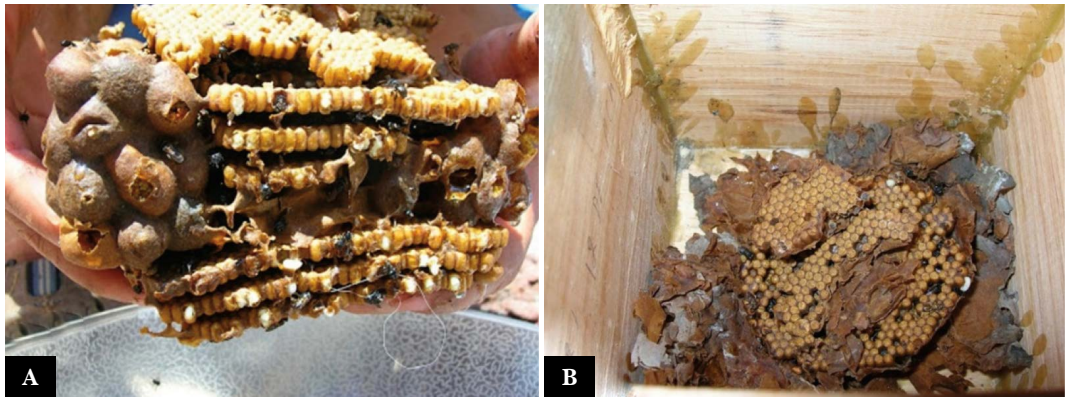

Figure 1. Artificial division of a nest of *S. mexicana*. A) Part of the offspring nest to be transferred to a wooden box, B) *S. mexicana* nest with involucre and without food pots. Photos: Juan Antonio Pérez Sato.

If a honeycomb with the offspring is damaged in any of the first stages, it is recommended against placing these inside the box, since the larval food may be released and this attracts the phorid (Shanahan & Guzmán, 2017). In addition, panels must be places with young offspring and in the pupal stage, in order to guarantee the emergence of new bees to strengthen the new colony in a short period (Moo-Valle, 2018). Likewise, worker bees, a queen bee and/or a queen cell must be placed for the colony to be quickly established and strengthened, and the bees must be fed 24 hours after the division is carried out. In addition, the box must be inspected every 3 days, and if a phorid is found, internal traps must be placed containing apple vinegar, and depending on their condition, clean the inside of the box, feed the bees and control the phorid by hand (Medina, Hart & Ratnieks, 2014).

Some important additional recommendations to avoid phorid infestations are: a) to provide fresh food with no fermented odours; b) feed with sugar syrup, which must be previously heated; c) do not provide excess food, to avoid fermentation and the attraction of phorids; d) provide wax from the same colony so it rebuilds its nest faster, particularly the tunnel that stretches between the entrance and the offspring chamber, so the guardian bees at the entrance have greater control over the phorids and e) keep the box sealed, avoiding cracks which *P. kerteszi* can use to enter it (Shanahan & Guzmán, 2017).

Other general recommendations for the process of artificial division of a colony are the following: a) the two colonies obtained must each have an adequate population of bees so that strong colonies with worker bees of different ages are quickly formed, in order to have the ability to defend their colony. In case one of the colonies is found to be weak, it is to be moved to a place occupied by a strong one, and in this way, worker bees will enter that will strengthen the cleaning of the colony (González-Acereto, 2008); b) place honeycombs with mature offspring (pupae) about to emerge, from strong colonies, and c) carry out an early detection of *P. kerteszi*, thus the recommendation of carefully reviewing and observing the inside of the box, since phorids move quickly between reserves, honeycombs and the structure of the nest. In case adults are found inside the box, the different structures (offspring panels, reserves, etc.) that have been affected by the phorids must be removed immediately, and when the garbage containers of the infested honeycombs are cleaned, they must not be left near to the colonies, since this attracts more phorids (Figure 2), therefore they must be placed in a bag and discarded or buried in another, remote place.

Other actions recommended when infestation levels are low are a) to capture the adults, larvae and pupae of the phorids in the box manually; b) to blow between the structures in order to remove the adults and eliminate them; c) trap and eliminate the adults using a tulle fabric bag and d) place an adhesive glue in the entrance of the nest so the phorids are trapped when they try to enter (Guzmán, Balboa, Vandame, Albores & González-Acereto, 2011; Shanahan & Guzmán, 2017).

Recommendations when infestation levels are high are: a) to remove the colony from the meliponary so it does not contaminate the other colonies and b) clean the box and burn the infested honeycombs, since it will be difficult to eliminate the flies, which may wipe out the entire colony in a matter of a few days (Moo-Valle, 2018).

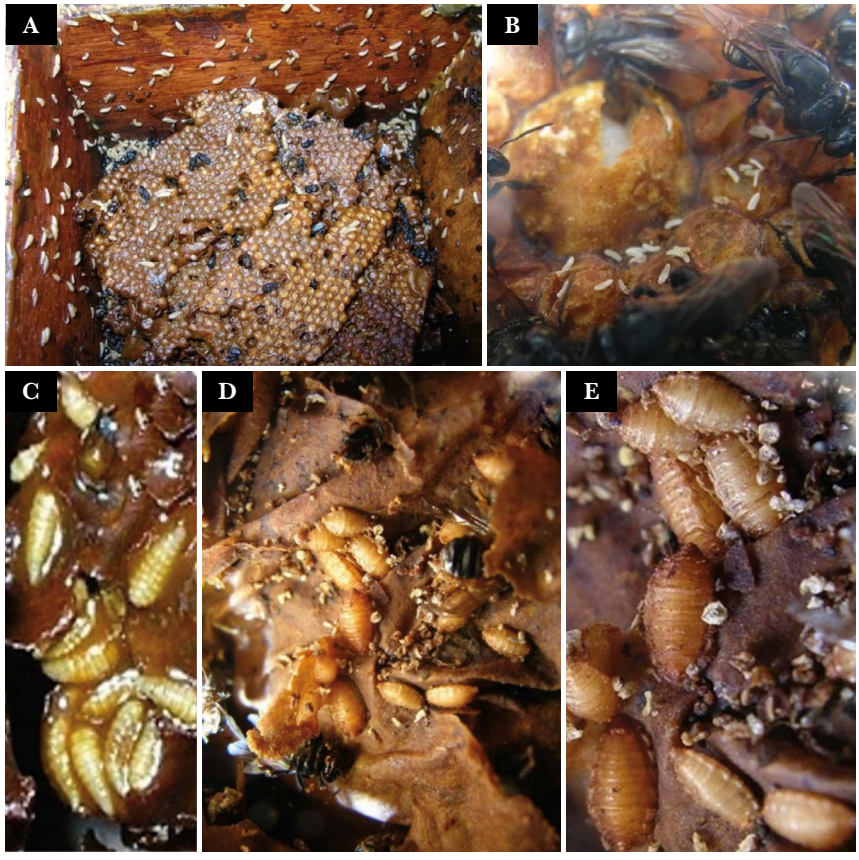

Figure 2. Infestation of *Pseudohypocera kerteszi*. A) *Scaptotrigona mexicana* nest with phorid larvae, B) *P. kerteszi* eggs on honey pots, C) Larvae of the phorid feeding off honey, D) Phorid prepupae, E) Phorid pupae. Photos: Juan Antonio Pérez Sato.

Strategies to control *P. kerteszi* applied during the harvest of honey. During the harvest of honey, the risk of infestation of the colonies by *P. kerteszi* has been observed to be low, since the honey-producing colonies have a high adult population. In addition, the design of the boxes in which the bee colonies are housed allows for a harvest of honey without affecting the structures that protect the offspring area, reducing infestation. However, in order to reduce the risk of infestation during the harvest, the following is recommended: a) to carry out this activity inside a pavilion, b) avoid breaking offspring honeycombs or honey and pollen pots during the revision and harvest of honey in the colony, c) performing the harvest as quickly as possible, trying to leave the colonies open the least time possible, mainly in the more humid seasons (the time of year in which the *S. mexicana* colonies are the most affected by *P. kerteszi* is in the months of June, July and August, which are part of the rainy season) and d) keeping the meliponary clean of organic matter, making sure to remove any pollen, honey or wax residues, since they may attract phorids (Medina *et al.*, 2014).

Other recommendations that can apply to any of the three critical stages by the infestation of flies in a colony are:

a) Use of natural repellents, using *Pluchea carolinensis* (Jacq.) D. Don, *Bursera simaruba* (L.) Sarg., *B. graveolens* (Kunth) Triana & Planch., *Croton humilis* L., *Psidium guajava* L., *Ruta chalepensis* L., *Euphorbia milii* Des Moul., *Ricinus communis* L., *Dieffenbachia picta* Schott y *Melia azedarach* L. plant leaf extracts. These are to be placed outside or on the inside corners of the box (González-Acereto, Quezada-Euán & Medina-Medina, 2006; González-Acereto & De Araujo, 2005).
b) Use of vinegar traps, since the *P. kerteszi* females are attracted to fermented pollen by its smell, and since one of the components of pollen is acetic acid, apple vinegar has been used as an attraction agent in traps placed inside the bee colony. Traps are made with small, plastic containers with airtight lids with holes punched in them, approximately 4 cm in height and 2 mm in diameter, in such a way that bees cannot fit through them; the container is filled with apple vinegar, to approximately two-thirds of its capacity (Guzmán *et al.*, 2011; Wolff & Nava, 2007) (Figure 3 A).

Other mixtures used for traps are: a) a mixture of white vinegar and water (30-50% vinegar) or apple vinegar (5% of acetic acid). These mixtures are placed in a small container without a lid, which is to be covered with a thin piece of fabric with small holes punched into it, and held onto the container with a rubber band. The flies will be attracted to the smell and will fall inside and die from drowning (De Oliveira, Venturieri & Contrera, 2013).

A third type of vinegar trap is made with a plastic funnel, which is placed upside down in the opening of a small jar containing vinegar and leaving a small orifice at the end of the funnel, making sure that bees cannot fit through it; the funnel is to be attached to the jar using scotch tape (Gennari, 2019) (Figure 3 B).

Figure 3. Traps with apple vinegar to control *P. kerteszi*, A) Trap with holes, B) Trap with a plastic funnel. Photos: Natalia Real Luna.

Depending on the degree of infestation, one or several containers may be placed inside the box, since the flies are attracted, they enter the trap and die from drowning. It is important to emphasize that the holes must allow flies to enter, but not bees. For a better control of the *P. kerteszi* flies, the vinegar traps must be replaced every 2 or 3 days and the adults that have previously entered the trap and died there must be removed. The holes in the lid must also be reopened, since bees can seal them with propolis or wax (De Oliveira *et al*., 2013; Ramos, Medina & May-Itzá, 2003). It is recommended to use the traps only when infestations are severe, and when flies are no longer found, the traps must be disposed of. If the number of adult flies is not reduced, the colony must be cleaned or transferred to another box. The vinegar trap method allows bees, without any adult flies, to efficiently clean the areas in which flies have laid eggs and where larvae are found. Vinegar reduces the adult populations, which favours bees, since they use large amounts of energy chasing adult flies.

CONCLUSIONS

The attack of *P. kerteszi* on *S. mexicana* colonies takes place during the transfer of nests, artificial division and the harvest of honey. The strategies to avoid infestation consist of: a) performing management procedures in a closed space, b) transferring the entire offspring area without damaging the structures that cover the offspring honeycombs, c) transferring mature and young offspring honeycombs without any damage, d) adding enough involucre to allow for the quick covering of the offspring area, e) closing the spaces of the box so as to leave it airtight, along with the entrance of the colony with wax for at least two days, d) placing vinegar traps inside the nest and e) avoid transferring in the rainy months, as well as to use the suitable box design. During the critical infestation stages, revision must be carried out on a daily basis in order to clean the box, place vinegar traps and perform a manual control.

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Physical and physiological indicators of the quality of soursop seeds (*Annona muricata* L.)

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ABSTRACT

Objective: The present study aimed to carry out the analysis of the physical and physiological quality of soursop seeds, since there is very little information on the subject.

Design/Methodology/Approach: The material was collected at physiological maturity. The seeds were extracted from fruits in commercial maturity. They were subjected to a physical and physiological quality analysis: physical purity, humidity content of the seed, weight of 1000 seeds, integrity test of the seed with the X-ray equipment, evaluation of germination and the evaluation of viability by the tetrazolium method. A completely randomized experimental design was used in all the physical quality variables and tetrazolium tests. Other hand, a completely randomized factorial design (3×7) was used in the germination evaluation.

Results: The viability results obtained by the tetrazolium method showed over 59% viable seeds, while in the germination test with the germinative pretreatments only 11.33% germination was obtained in the seeds from which the cover was removed.

Findings/ Conclusions: Therefore, it was concluded that the moment of obtaining the plant material is important for its germination.

Keywords: fruit trees, germination, viability, humidity content.

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INTRODUCTION

In Mexico, the soursop (*Annona muricata* L.), is considered the most important species of the Anonaceae family, due to its commercial value (Reyes *et al.*, 2018); since this is one of the fruits with the greatest commercial acceptance in the world due to its creamy pulp, sweetness and excellent flavor (Márquez *et al.*, 2013). These fruits are harvested at physiological maturity, but if they are harvested before this maturity, they do not ripen well and the pulp can acquire a bitter taste (Jiménez *et al.*, 2017).

Image by Marco Montoya at Pixabay

Moreover, the collection of good quality seed allows its use for its conservation (Elizalde *et al.*, 2017). Two types of tests can be found, those that measure the proportion of viable seeds (germination tests) and those that evaluate the state of viability, among them are the viability test by the tetrazolium method, which allows classifying the seeds as viable or not viable (Masetto *et al.*, 2007). This test is based on the activity of respiration dehydrogenases, which chemically reduce the colorless tetrazolium solution to formazan (Elizalde *et al.*, 2017), giving a red color to living tissue, pink tones to partially damaged tissue, and white to tissue not viable (ISTA, 2016).

The present work was proposed because there is little information related to this species. Therefore, the objective is to obtain information on the analysis of the physical and physiological quality of soursop seeds.

MATERIALS AND METHODS

This study was conducted at the National Genetic Resources Center, National Institute of Forestry, Agriculture and Livestock Research (CNRG-INIFAP) located in Tepatitlan de Morelos, Jalisco. Four samples were used, which were kept stored in a cool and dry place at room temperature.

Variables evaluated in the physical quality seeds analysis

Physical purity. The total sample was weighed and, later, the components of pure seed, inert material and other seeds were separated. Once each of the components were separated was weighed, expressing their value as a percentage (ISTA, 2016).

Weight of 1000 seeds. Eight replicates of 100 seeds were randomly taken; each was weighed on an analytical balance. Then the average of the eight replicates was obtained, and the variance, standard deviation, and coefficient of variation were also calculated with these values. If the coefficient of variation did not exceed 4% (for not chaffy seeds), the result was taken as valid. The result of the determination was obtained by multiplying by 10 the average weight obtained from the eight repetitions of 100 seeds (ISTA, 2016).

Humidity content. This was done using an electronic humidity meter (termobalance method). One gram of seed was weighed was crushed. Subsequently, the ground seed was placed in a thermobalance (AND-MS-70), three repetitions per sample were performed (Rao *et al.*, 2007).

X-rays. Four repetitions of 25 seeds were counted, which were used to be radiographed with X-ray equipment (Faxitron X-Ray MX-20®), with this test the physical integrity of the seed was evaluated, which the percentage of seeds full, empty, damaged by insects and mechanically damaged were evaluated.

Variables evaluated in the physiological quality seeds analysis

Viability. This analysis was carried out by the tetrazolium method (ISTA, 2016). The seeds were removed from the cover and soaked in distilled water for 24 h, after which a ventral longitudinal cut was made. For this, 20 seeds were used, with four repetitions. The seeds were incubated in a 1.0% tetrazolium solution for 24 h, at 30 °C, in the dark, and the staining obtained in the embryo was later evaluated.

The seed disinfection protocol consisted of scarifying the seeds, then they were left to soak for 24 h in distilled water, washed three times with soap (Axion Tricloro®) for ten minutes each. They were placed in a Captan® solution (3 $g \cdot L^{-1}$) for one hour and the excess was removed. Inside the laminar flow hood, they were subjected to a 70% ethanol solution for two minutes and later to a 1% commercial chlorine solution for ten minutes, the relevant rinses were performed to remove the excess. Subsequently, they were placed in a solution of citric and ascorbic acid at a concentration of 1 $mg \cdot L^{-1}$ each and were incubated for one hour. This was done in order to know if this protocol affected the viability of the seed.

Germination. Seeds were placed in transparent acrylic germinating boxes. A mixture of agrolite substrate and Canadian peat (peat most®) (2:1) was used. The seeds underwent different pre-germination treatments (Table 1).

15 seeds per box were placed and four repetitions per treatment were evaluated, this was done for sample 2, 3 and 4. The germination boxes were placed in a germination room at a temperature of 25±3 °C, with a photoperiod of 16 h light and 8 h dark. After sowing, they were irrigated with Captan® (3 $g \cdot L^{-1}$), to avoid the appearance of fungus. The germination percentage was evaluated in a period of 30 days.

A completely randomized experimental design was used for the physical quality variables and for the seed viability test; in the case of germination, a completely randomized factorial experimental design (3×7) was carried out, an analysis of variance was carried out and the comparisons of means (Tukey, $\alpha=0.05$) were carried out using the statistical software SAS version 9.3. The values expressed in percentage were transformed with the arcsine function $\left(\sqrt{X/100}\right)$.

RESULTS AND DISCUSSION

There are very few studies related to this subject, so the results were compared with other works that were carried out on similar seeds.

Variables evaluated in the physical quality seeds analysis

The characteristics of the seed samples showed a variability. Table 2 shows the analysis of the physical purity of the seeds, where an average initial weight of 612.685 g was obtained, with an average of 1,379 seeds and 95% pure seed.

Table 1. Pre-germination treatments applied to soursop seeds.

Number	Treatment
1	Complete seed
2	Complete seed with imbibition in water for 24 h
3	Seed without cover
4	Seed without cover with imbibition in water for 24 h
5	Seed with a small cut
6	Sulfuric acid application for five minutes
7	Application of gibberellins 100 ppm for 24 h

Table 2. Analysis of physical purity of soursop seeds.

Sample	Starting weight (g)	Inert matter weight (g)	Purity (%)	Number of total seeds
1	973.21	12.73	98.69	1,888
2	511.82	35.23	93.11	1,230
3	485.95	35.23	92.74	1,198
4	479.77	6.91	98.55	1,195
Average	612.685	22.53	95.77	1,379.25

Table 3 shows the weight of 1000 seeds, where sample 1 presented the highest weight with 541.42 g and samples 2 and 3 presented the lowest weight. Viveros *et al.*, (2015), in *Enterolobium cyclocarpum* (Parota) seeds found a weight of 1000 seeds of 836.4 g, a result higher than that obtained by Meza and Bautista (2007), in soursop seeds that was 336 g, data similar to that obtained in the present investigation in samples 2 and 3. Regarding the humidity content, sample 4 presented the highest value (36.46%) but samples 2 and 3 presented the lowest humidity content. Authors such as Viveros *et al.* (2015), in parota seeds found an average humidity content of 9.7% in whole seed and 5.3% in crushed seed, data below those obtained in the present work in soursop seeds, since in crushed seed it was obtained a humidity content greater than 27.8%, in a bibliographic review carried out by Magnitskiy and Plaza (2007), reported that the humidity content at the time of dissemination of recalcitrant seeds of tropical trees varies between 23% in *Pourouma cecropiifolia* Mart., 25% in *Bertholletia excelsa* Humb. Bonpl, 46-51% in *Euterpe espiritosantensis* Fernand palm, and 47-53% in *Eugenia dysenterica* D.C.

Regarding the X-ray analysis, samples 2 and 3 had 100% and 99% full seeds respectively, the damage caused by insects is minimal since the percentages obtained for this cause are less than 20% in samples 1 and 4. Viveros *et al.*, (2015), found in seeds of parota that 98% showed a developed embryo and 93% of the seeds with cotyledons in this condition.

Variables evaluated in the physiological quality seeds analysis

The results of the disinfection protocols did not show statistical differences. As observed in Figure 1, the seeds without disinfection protocol in sample 1 showed 86% viable seeds and in samples 2 and 3 they presented 61% viability. On the other hand, to the seeds that the disinfection protocol was applied to be introduced into *in vitro* culture, sample

Table 3. Comparison of Tukey means of the physical quality analyzes of four soursop seed samples.

Sample	Thousand seed weight (g)	Humidity content (%)	X-Rays analysis	
			Filled seeds (%)	Insect damaged seeds (%)
1	541.41 a	32.84 ab	81.00 b	19.00 a
2	386.30 c	28.34 b	100.00 a	0.00 b
3	383.74 c	27.82 b	99.00 a	1.00 b
4	410.72 b	36.46 a	82.00 b	18.00 a

Values with the same letter are statistically similar (Tukey, α=0.05).

1 exhibited 81% viable seeds and samples 2 and 3 presented 59 and 60% viable seeds, respectively. Lobo *et al.* (2007), found a percentage of viable soursop seeds of 69% and custard apple seeds of 79.5%. In the case of parota seeds, they found an average of 75.5% viability (Viveros *et al.*, 2015). In this investigation it was found that the viability percentage was higher than 58.75% and 61.2%, with disinfection protocol and without disinfection respectively, which means that they maintain a good percentage of viability, similar to that reported by Lobo *et al.* (2007).

Pre-germination treatments were carried out; however, germination did not increase.

As observed in Table 4, the percentages for all treatments were low. However, the cover removal treatment registered the highest percentage of germination with 11.33%, followed by the complete seed treatment with 7.50%. The seeds of tropical trees have inherent dormancy, which results in delayed and non-uniform germination (Joseph, 2014), such as soursop, which is why heterogeneous germination can be observed.

Joseph (2014) found that the earliest germination in soursop seeds soaked for 72 h in water was 22 days after sowing, with a percentage of 13%, and 25 days after sowing in seeds soaked in cold water for 96 h had 40% germination. Seeds were extracted from ripe fruits, which were pulped and washed, air-dried for 48 hours, and stored for 38 days. While Meza and Bautista (2004) found that soaking in water for 24 h and the control (without soaking)

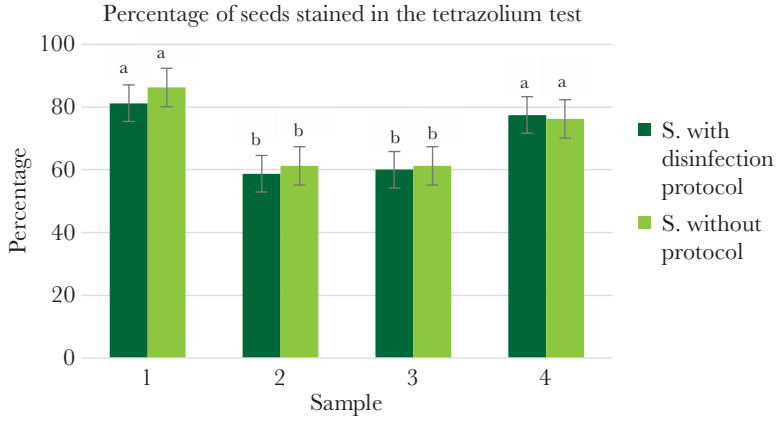

Figure 1. Seeds stained in the tetrazolium test. Values with the same letter are statistically similar (Tukey, α=0.05)

Table 4. Comparison of Tukey means of pre-germination treatments in soursop seeds.

	Treatment	Germination (%)
1	Complete seed	7.50 ab
2	Complete seed with imbibition in water for 24 h	5.53 ab
3	Seed without cover	11.33 a
4	Seed without with imbibition in water for 24 h	0.00 b
5	Seed with a small cut	2.50 b
6	Sulfuric acid application for five minutes	5.24 ab
7	Application of gibberellins 100 ppm for 24 h	3.03 b

Values with the same letter are statistically similar (Tukey, α=0.05).

simultaneously started germination at 17.66 days, but the treatment with scarification in sulfuric acid for 2 min was at 19.33 days.

Therefore, it is possible that, although a part of germination is determined by the conditions required for the embryo to emerge, it could be said that there is an interaction between the growth potential of the embryo and the restrictions imposed by the tissue that surrounds it (Lobo *et al.*, 2007). In the same way, it is important to mention that Meza and Bautista (2004) and Lobo *et al.* (2007), used seeds of fully ripe fruits and soft to the touch, for which they obtained a germination percentage higher than that was obtained in the present investigation since despite the fact that the fruits were physiologically mature, they did not feel soft.

CONCLUSIONS

The viability percentage of soursop seeds can be maintained above 60% in seeds stored at room temperature for three weeks after extraction. This viability percentage is not affected by the application of the seed disinfection protocol to be introduced to *in vitro* culture. And that the moment of harvesting the fruits for the extraction of the seed is vital for the seeds to germinate.

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Colegio de Postgraduados

Evaluation of microbiological safety in bioinputs produced in Mexico

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ABSTRACT

Objective: This work aimed to evaluate the microbiological safety of bioinputs produced in Mexico. The main reason for this evaluation is that bioinputs are products made from the region's manures, plant residues and raw materials. The transformation of these raw materials is carried out by microorganisms present. The process goes through three stages: initial, thermophilic and final. The thermophilic stage is critical because weeds and microorganisms with pathogenic potential disappear in processes under optimal conditions.

Methodology: 1345 bioinputs samples were received from different states of Mexico. The samples were evaluated for the presence of total and fecal coliforms and *Escherichia coli* under the provisions of the Official Mexican STANDARDS, NOM-210-SSA1-2014 and NOM-114-SSA1-1994.

Results: It was possible to identify 79% of the samples with Most Probable Number values <3 of total coliforms, fecal coliforms and *Escherichia coli*, the minimum permissible by the Official Mexican STANDARD NOM-210-SSA1-2014, also identified 99% of samples free of *Salmonella*.

Conclusions: The results obtained allow us to conclude that the bioinputs produced in Mexico are free of pathogens for humans, which can also be represented as innocuous bioinputs.

Keywords: Bioinputs, organic fertilizers, safety, fecal coliforms, *Salmonella*.

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INTRODUCTION

Sustainable agriculture has various strategies that propose reducing the use of agrochemicals (Carlise *et al.*, 2019), complementing their application with products of biological origin, such as bioinputs or organic fertilizers, whose production depends on renewable sources of raw materials present in the region of elaboration (Castro & Bertsch, 2009). Organic fertilizers and bioinputs are products made from cattle manure, stubble, plants, and microorganisms. We can find compost, bokashi, sulfocalcium broth, leachate, and others (Cabanillas *et al.*, 2017; Phooi *et al.*, 2022; Geisseler *et al.*, 2021). The main action of these bioinputs is to improve productivity, yield and health when applied to plants (Cabanillas *et al.*, 2017; Goulet *et al.*, 2021). Recycling organic waste products in agricultural soils is one of the most economical strategies and beneficial to the environment (Alvarenga *et al.*, 2017; Sayara *et al.*, 2020).

Bioinputs represent a beneficial natural fertilizer for agriculture. However, most are made from the fecal matter of farm animals (Yun *et al.*, 2007). Within the intestinal tract of

farm animals, many microorganisms with pathogenic potential are deposited in the fecal matter when discarded by the animals (Lefebvre *et al.*, 2006). The microbiological safety of bioinputs or organic fertilizers can be evaluated based on some microbiological indicators.

There are various reports in which *Salmonella* and *Escherichia coli* were identified from bioinputs (Goldstein *et al.*, 1988; Yun *et al.*, 2007). This indicates that bioinputs can potentially have pathogenic microorganisms, which can be transmitted to food.

For this reason, it is essential to detect potentially pathogenic microorganisms, such as total and fecal coliforms *Escherichia coli*, which are known to cause stomach infections, diarrhea, fever, abdominal cramps, and hemolytic uremic syndrome. In Mexico in 2017, there were more than eight thousand cases of bacterial food poisoning due to the consumption of raw vegetables and fecal coliforms (Martinez *et al.*, 2020). Due to this, this work aimed to evaluate the microbiological safety of bioinputs produced in Mexico.

MATERIALS AND METHODS

Collection and storage of samples

One thousand three hundred forty-five bio-input samples were collected in different states of the Mexican Republic; they were transported to the National Center for Genetic Resources of INIFAP (CNRG-INIFAP) and stored at 4 °C.

Preparation of serial dilutions

From the bioinputs, serial dilutions were made. The samples were homogenized; 10 g or 10 mL of each one was taken, depending on whether they were in a solid or liquid state, and they were diluted in 90 mL of sterile peptone water from here. Serial dilutions were made up to 10-7. This procedure was carried out by the Official Mexican STANDARD NOM-110-SSA1-1994.

Determination of total and fecal coliforms and *Escherichia coli*

The determination of total coliforms (OCT), fecal coliforms (OCF), as well as *E. coli* was carried out according to the Official Mexican Standard NOM-210-SSA1-2014, following the method approved for the estimation. Of the density of OCT, OCF and *E. coli* by the Most Probable Number technique.

Determination of *Salmonella* in bioinputs

The determination of *Salmonella* was carried out according to the Official Mexican Standard NOM-210-SSA1-2014, which consists of taking 25 g of the sample and transferring it to 225 mL of selective pre-enrichment medium (medium of selenite cystine), this was incubated for 24 h at 37 °C, and later it was streaked on Petri dishes with Brilliant Green Agar and XLD xylose lysine deoxycholate agar, they will be incubated at 37 °C and typical or atypical Salmonella colonies will be selected.

RESULTS AND DISCUSSION

One thousand three hundred forty-five bioinputs from different regions of the country were received (Figure 1). Region 4, made up of Chiapas, had the highest number of samples

for analysis, followed by Region 27, which belongs to San Luis Potosi, with 86 bioinputs, region 11 and region 23 with 80 bio inputs (Figure 1).

The bioinputs that were received were made up mainly of leachate (28%), Mountain Microorganisms (MM) (14%), bioles (10%), compost, humus, bocashi (8%), among others (Figure 2). The leachates were the bioinputs that were mainly produced and collected for the safety analysis, and this is due to their easy obtaining because they are the result of the application of water in the vermicomposting and compost piles; this allows to maintain the humidity of these organic fertilizers (Tejada-Gonzalez *et al.*, 2008), it is known that the application of leachates can be from a foliar application generating positive effects, such as the increase in chlorophyll, macro and micronutrients in tomato, rice and corn (Tejada and González 2004, 2006). The MM, for their part, are made up of colonies of fungi, bacteria and beneficial yeasts that are found in different ecosystems, such as forests, coffee plantations, and bamboo, among others (Suchini-Ramirez, 2012); these are extracted from the ecosystems and later reproduced in liquid cultures. It is known that applying these can increase the nutritional value and inhibit pathogens in plants of agricultural interest

Figure 1. Number of bioinputs received by region of Mexico.

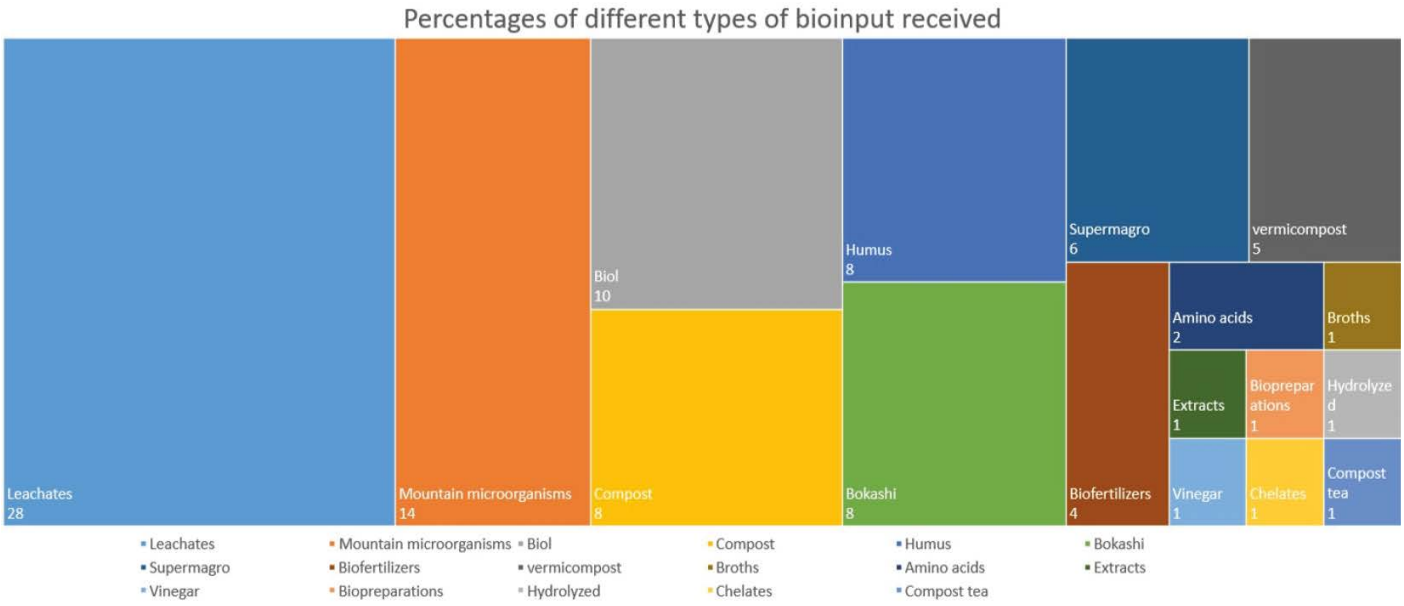

Figure 2. Percentages of bioinputs received from the Mexican Republic.

(Campo-Martínez *et al.*, 2014). One of the by-products of the anaerobic fermentation of manures is bioles (Cano-Hernández *et al.*, 2016), which are known to favor the growth of some crops such as spinach (*Spinacea oleracea*) (Siura *et al.*, 2009). These were the third bioinputs that were received in greater quantity.

Analysis of total coliforms, fecal and *E. coli*

One thousand three hundred forty-five samples were analyzed to identify the presence of total coliforms, considered bacterial indicators in samples that present fecal contamination. According to NOM-210-SSA1-2014, with the Most Probable Numbers (MPN) technique, MPN /g or mL <1100 means that the microorganisms present are outside the permissible limit and represent a human-use limitation. Of the 1345 samples, it was found that 78% and 79% of MPN/g or mL <3 of total coliforms and fecal coliforms, respectively. *E. coli*, for its part, is considered an indicator of fecal contamination, which can be found in animal waste and food waste (Lalander *et al.*, 2013; Mainoo *et al.*, 2009); in this study, we found 79% of bioinputs with <3 NMP/mL or g, 5% <23 NMP/mL or g, 4% <3.6 NMP/mL or g (Figure 3), which means a minimum load of pathogenic microorganisms, permissible by the standard (Figure 3). There are various reports on the high efficiency of removing coliforms present in bioinputs at the end of the process. For example, a bovine manure composting process was evaluated for two years, where the numbers of total coliforms and *E. coli* decreased as the process progressed; this was achieved because the thermophilic stage of the composting remained above 55 °C for 15 days (Larney *et al.*, 2003). On the other hand, fecal coliforms, *E. coli* and *Salmonella* were evaluated from composts obtained from residual water sludge, finding that temperatures of 57 and 61 °C, reached during composting, eliminated most of the pathogens present. At the beginning of the process (Banegas *et al.*, 2007).

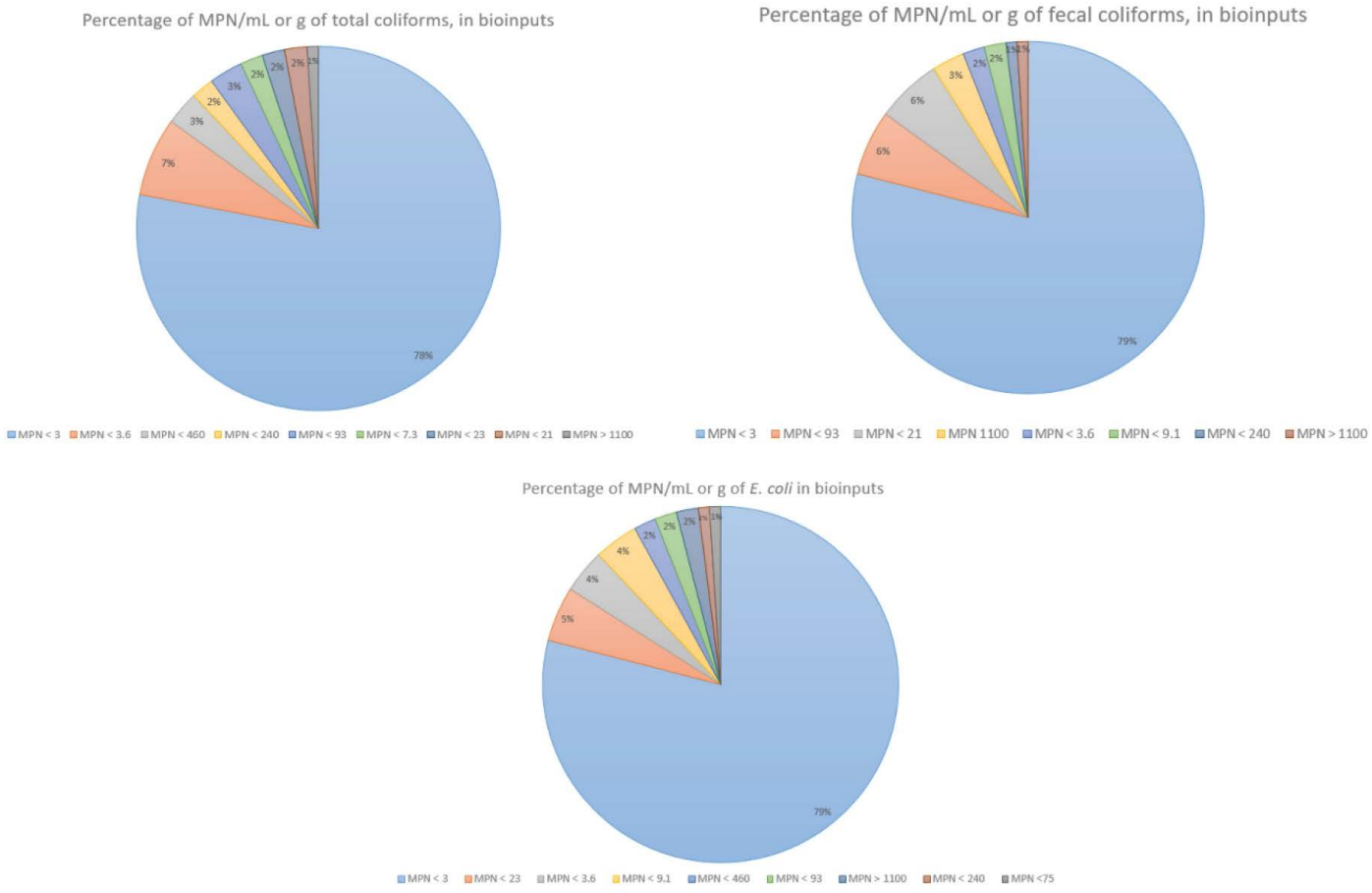

Figure 3. Percentages of MPN/mL or g of total, fecal coliforms, and *Escherichia coli*, present in the bioinputs.

Regarding the maximum allowable by the standard, 1% NMP/g or mL >1100 of total fecal coliforms and 2% NMP/g or mL >1100 of fecal coliforms and *E. coli* were identified in all the bio inputs analyzed (Figure 3), which indicates that this could have a potential risk to human health. As in this report, NMP sludge composting of >1100 g^{-1} has been identified in other works; these results are explained by some factors that contributed to the high levels of pathogens, such as the large size of facilities and waste piles. Composting and immaturity of the compost (Brinton *et al.*, 2000) probably also intervened in the 1% of the bio inputs we found with values >1100 NMP/mL or g.

Salmonella is considered a severe problem of the hygienic quality of compost and bioinputs; in this work, it was found that 99% of the samples were free of it. Domínguez and Edwards 2004 indicated that temperatures above 30 °C prevent the presence of *Salmonella* in composting processes; it has also been reported that the use of worms such as *Eisenia fetida* in vermicomposting processes decreases the presence of *Salmonella* by up to 99% (Brown & Mitchell, 1981), this is probably due to the antimicrobial response of gram-negative bacteria from the gizzard to the intestinal tract of earthworms (Soobhany *et al.*, 2018).

CONCLUSIONS

This research's analysis indicates that the fermentation process in the different processes of leachate, biol, compost, and vermicompost was efficient, reaching temperatures above 55 °C in the thermophilic stage, which is related to the decrease in total coliforms. Fecal coliforms, *E. coli* and *Salmonella* obtained values less than 1100 NMP/mL or g, which is allowed by NOM-210-SSA1-2014.

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Perception of and adaptability to the effects of climate change in a rural community of the State of Mexico

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ABSTRACT

Objective: To analyze the population's perception of recent changes in climate patterns and the actions they have taken to adapt to these changes in the El Saltillo community, Municipality of Jilotepec.

Design/methodology/approach: A survey was carried out between September and October 2022, answered online. Social networking and WhatsApp groups were used to disseminate the invitation to participate in the survey; 96 responses were received, of which 30 were answered on site together with the respondent. Aspects such as socioeconomic data, conceptualization and perception of climate change and adaptability actions were measured.

Results: All the survey respondents mentioned that they perceive strong changes in droughts and frosts in the last 10-15 years. Of them, 96% stated that they had heard the term climate change in different media; however, it is not a concept that is used to explain the changes that take place in the community. As a result of these changes, the population has chosen to build or enlarge rainwater container mounds for agricultural and livestock use, improve the physical condition of the stables, and begin to use precocious corn seed or more adapted varieties of improved corn.

Limitations on study/implications: The application of surveys online is a feasible and economical option that implies the need to implement data validation, control and verification mechanisms, as well as sampling of the results.

Findings/conclusions: Adaptation strategies to climate change were identified, showing that they are not spontaneous actions and that they have emerged empirically through daily contact with the phenomenon.

Keywords: Food security, agricultural vulnerability, online surveys.

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INTRODUCTION

Climate change is one of the most important themes both in the political agenda and in public opinion. The negative effects at the global level have become topics of analysis in global conferences to the degree that various groups of international experts have been established to understand the phenomenon, but mostly to attempt to give solutions to the quandary. In the United Nations (UN) Conference of the Parties on climate change (COP27,

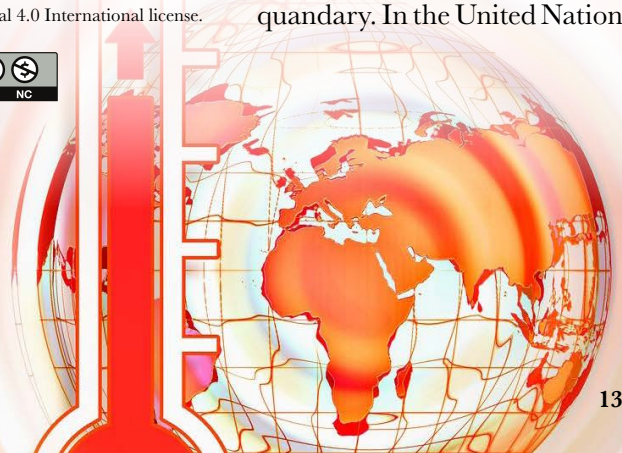

Image by Gerd Altmann at Pixabay

Egypt, 2022), the Secretary-General of the organization pointed out that: "People and the planet are trapped by climate change, half of the global population lives in places that are very vulnerable to climate change, extreme climate events have surpassed the level of tolerance of animals and plants, the result has been an increase of insecurity in the access to foods and water, particularly in the most vulnerable regions like Africa, Asia, South America and Central America" (UN, 2022). Various studies such as those by Hallegatte *et al.* (2016) and the IPCC (2014) point out that by 2030, Climate Change (CC) will lead more than 100 million people in the world to poverty, because the increase in temperatures and in the availability of water increase the intensity and the frequency of natural disasters, and food crises increase the risk of diseases transmitted by water, among many other impacts. Hoffmann (2020) mentions that "CC and natural disasters exacerbate inequality in the population because, among other things, the poor (countries and communities) are often more exposed to CC, suffer greater losses in proportion with wealth, and are less resilient and more susceptible to increase their poverty".

Studies such as those by Seaman *et al.* (2014) and Guajardo-Panes *et al.* (2018) describe that the effects of CC will be especially severe in rural communities of the least developed countries whose incomes depend on agriculture and become a risk in food security. In this sense, the IPCC (2014) points out that "agriculture is one of the activities that will be most affected by CC, because of the impacts that high temperatures, droughts and storms are expected to have on plant and animal production". In studies such as those by Ortega-Gómez *et al.* (2019) and Ortiz-Paniagua *et al.* (2018) the authors assume that, in face of the evident decrease in agricultural yields and increase in presence of pests and diseases in the crops as a result of the increase in water stress, agricultural producers will face the challenge of remaining competitive in the commercial and productive scope, at the same time that threats increase, such as the unknown behavior of hydro-meteorological factors and the uncertainty in the market's performance. In Pinilla-Herrera *et al.* (2012), the authors mention that "since the second half of the 20th century there have been reports at the global, regional and local level about the space-time alteration of the patterns of behavior of meteorological phenomena (storms, hailstorms and frosts, among others) and of climatological variables (temperature, humidity and precipitation). Around this, the scientific knowledge has specified and demonstrated that its main causes are the occurrence and intensity of phenomena of climatic variability by the ENOS (El Niño–Southern Oscillation) cycle and the widely spread climate change".

Mexico's geographic situation makes the country a highly vulnerable zone as a result of phenomena originated by CC. Zamora-Martínez (2015) mentions that "In Mexico the systematization of the information and the analysis of data referring to CC indicate, among other things, that meteorological drought will increase in some regions, forest ecosystems will present changes in 50% of the surface, rainfed agriculture will be severely reduced, and in the population, it will be evident in the quality of health". According to the National Climate Change Strategy (*Estrategia Nacional de Cambio Climático*, ENCC, 2013), the government of Mexico fixed the adaptation to its effects as one of the pillars of the federal strategy against CC, and it includes reducing the vulnerability of the social sector as an action line. In this sense, understanding and defining the vulnerability, in addition to

having the ability to measure it, becomes an essential aspect to address the consequences through risk management.

In the State of Mexico, studies like those by Pérez *et al.* (2007) have documented that the thermal amplitude (difference between daily minimum and maximum temperature) has increased in region of Toluca in more than 30.0 °C due to the effect of urban growth and the change in land use, while inside the city of Toluca there have been "heat islands" which refer to the temperature within the city being warmer than in the periphery by approximately 2 °C at the same hour. Monterroso-Rivas *et al.* (2011), through the use of various climate change scenarios, evaluate the vulnerability of the agricultural sector at the municipal level in Mexico; the region of Jilotepec and its surroundings appear as with High vulnerability for corn farming, and these results agree with the publication by Espinosa-Rodríguez *et al.* (2020).

Climate change also has strong repercussions in the livelihood, traditions, culture and ways of thinking of the population, particularly in rural, poor and marginalized zones, due to migration towards urban centers of the country in search for better living conditions, primarily of the young generations. As mentioned by Landa *et al.* (2008), "Climate variability is not the main cause of the socio-environmental quandary that is experienced in several regions of the country, although it is a factor that favors the appearance of conflicts in the population. There are other factors such as the negative effects of farming policies, unemployment, deficiency in health services, social conflicts and poverty, which increase the vulnerability of the population in the presence of changing conditions in the availability of water and in climate".

According to Retamal *et al.* (2011), "the framework of scientific approaches to climate change develops in three different research lines: (a) Physical sciences of climate change; (2) Impacts, adaptation and vulnerability; and (3) Mitigation. The last two approaches are the ways of responding to the potential impacts of CC and require behavioral changes from citizens and cultural changes from society. Therefore, behind these changes there should be a positive perception of the risk introduced by climate change, a level of information that backs this valuation and a degree of awareness to design and implement strategies for mitigation and adaptation, as well as to maintain them for some time". Therefore, the study of climate change requires a line of study that approaches the perception of citizens, since the successful application of any strategy, both mitigation and adaptation, demands understanding the level of sensitivity, information and comprehension about climate change by those who will adopt such strategies, those who will evaluate their performance, and those who will benefit from their application.

When analyzing the perception of the population of the dangers from climate change, two aspects are considered: adaptation measures and mitigation measures. Adaptation, according to the definition by IPCC, is "the adjustment of natural and human systems in response to climate change to moderate its negative effects and exploit its benefits" (Pascual-Bellido, 2017). On the other hand, Libert-Amico *et al.* (2018) mention that "adaptation is equivalent to developing the abilities in different social sectors to adjust the variability to climatic extremes and to climate change, with the aim of taking advantage of positive effects and moderating potential damage". The perception of changes in the climate of a

region, as well as the adaptation of daily activities that tend to reduce the negative effects and take advantage of the positive, are linked to the traditions of each society, and each strategy is a conscious and rational act by those who implement it, although influenced by the social context as a local strategy for subsistence. In Ávila-Flores *et al.* (2015) the authors argue that it is essential, in studies directed at understanding how the adult population accepts its responsibility in face of threats, to know whether they really perceive them as the institutions responsible for risk management expect them to; that is, to understand if society considers itself to be vulnerable, to what and to which extent, as well as knowing what is needed to reassess their modes of prevention and recovery.

MATERIALS AND METHODS

Study area

The research study was carried out in the locality of El Saltillo, belonging to the ejido Aldama, municipality of Jilotepec, State of Mexico. This is a rural community located 90 km to the NE of Mexico City (Figure 1). Agriculture produces mainly native corn with a rainfed regime. According to the General Population and Housing Census 2020 (INEGI), the population is approximately 870 inhabitants distributed in 220 households, and the territorial extension is 1,384 Ha. The type of climate of the community of El Saltillo is temperate sub-humid, C(w2)(w)b(i)g, sub-humid with long summer, winter rainfall under 5%, and the highest temperature manifests before the summer solstice (Casa, 1997).

Data compilation and survey

The survey titled "Perception and adaptability to Climate Change in the community of El Saltillo" was applied, which was answered online and with the aim of measuring

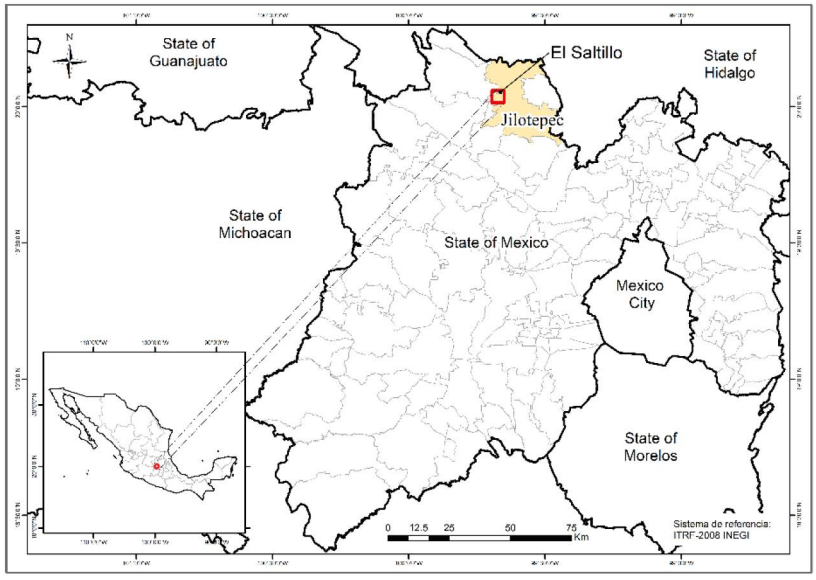

Figure 1. Location of the study zone.
Source of data: Prepared by the authors with cartographic data from INEGI.

the perception of change in climatic conditions in the zone, as well as understanding adaptation practices in the farming sector, in addition to the household or family itself. The method of dissemination and invitation to participate in the survey was through the ejido authorities who, when informed about the aim of the study, communicated the invitation to participate in the survey through the community's different WhatsApp groups. The control mechanisms of the survey were to request one person per family to respond, as well as the head of the household to ensure that, in addition to the confidentiality of their data, this would correspond only to the survey control and not to the body of the survey. Mandatory and control survey questions were asked. The period of application was from September 15 to October 30, 2022, and 96 surveys were answered in total, of which 30 were answered on site and directly with the survey respondent as a way of validation and sampling. The 96 surveys represent 43.6% of the households of the locality of study.

The survey was structured in six sections, the first and only as control mechanism, called A) general data (name and address of the survey respondent). The next sections were: B) socioeconomic data (to understand the characteristics of the survey respondents), C) conceptualization of climate change (to define what is understood as CC and how widespread the phenomenon is), D) perception of climate change (to know if such changes have been noticed in the community and how intense), and E) adaptability to climate change and vulnerability (to analyze the actions they have taken to face such changes).

RESULTS AND DISCUSSION

Of the survey respondents, 96% manifested having heard the term Climate Change (CC) mentioned in media like television, radio, internet, etc. However, CC is not a concept that is used to explain the changes observed in the precipitation and temperature patterns of the locality. All the people surveyed manifested they have perceived changes in the last 10-15 years, particularly in phenomena such as droughts (more severe and prolonged), delay and decrease in rainfall ("the rains come later and later and we do not get downpours as before"), as well as frosts (more severe and earlier than before), although only 5% of the survey respondents mentioned that it is a local phenomenon that only affects the community or the State of Mexico and this corresponds to people with scarce or no education (Table 1). When it comes to the origin of such changes in rainfall and precipitation patterns, 93% of the answers mentioned that they are caused by the activity of men or industrial activities and the other 7% said they ignore the cause or attribute it to divine matters or God.

Table 1 presents the main social characteristics of those who responded the survey, grouped by age range. The capacity of the population to act in the presence of emergencies is related to aspects such as level of preparation and age range, where older people and with lower academic preparation will understand the phenomenon to a lesser degree, they will be more vulnerable to the negative effects and will not be able to implement actions for adaptability; similarly with the young generations, particularly with university studies, who prefer to devote themselves to other activities and to abandon the farmland.

Table 2 shows how the inhabitants of the community conceive the changes in patterns of rainfall and temperature; the concept of rainfall is the one that all the survey respondents mention that has basically decreased since at least 10 years ago, similar to drought which

Table 1. Main characteristics of the population that answered the survey.

Question	Concept	Ages (years)					Total cases
		Less than 30	30 to 39	40 to 49	50 to 64	More than 65	
Educational level	Uneducated	0	0	0	0	6	6
	Primary level	0	0	3	17	4	24
	Secundary level	0	2	19	8	0	29
	Baccalaurate level	1	6	9	13	0	29
	University level	3	0	2	3	0	8
Have you heard about CC?	No	0	0	0	0	4	4
	Yes	4	8	33	41	6	92
Do you think CC occurs only in?	El Saltillo	0	0	0	0	1	1
	State of Mexico	0	0	3	1	0	4
	All Mexico	0	0	0	1	5	6
	The whole world	4	8	30	39	4	85
What do you think causes CC?	I do not know	0	0	0	1	4	5
	I have not noticed any changes	0	0	0	0	1	1
	All the changes are natural	0	0	0	4	1	5
	Because of divine reasons (God)	0	0	0	0	2	2
	Because of industrial activity	0	1	6	7	0	14
	Because of man's activity	4	7	27	29	2	69

Source of data: Prepared by the authors with field research work.

has practically increased in the same period. In these two concepts there was no response that they remain the same as always.

As Table 2 shows, the meteorological frosts have an increasing trend and the hailstorms seemingly do not have greater changes since there is no clear differentiation when it comes

Table 2. Perception of climate change.

Phenomenon / Numbers of cases		Change periods (years)				
		Since 5	Since 10	Since 15	For more than 15	It's always changed
Rainfall	Increase	0	0	0	0	0
	Decrease	21	39	21	9	6
Drought	Increase	21	36	21	8	6
	Decrease	0	3	0	1	0
Frost	Increase	13	20	12	6	3
	Decrease	4	3	4	0	0
	As always	4	16	5	3	3
Hailstorm	Increase	7	13	5	4	0
	Decrease	9	13	13	4	4
	As always	5	13	3	1	2

Source of data: Prepared by the authors with field research work.

to the change rate or period of influence. To measure which phenomena affect certain aspects of life in the community to a greater extent, they were asked which phenomena affect more the community, the crop, the livestock, and the family (Figure 2).

Figure 2 shows the level of affectation of some phenomena in percentage. For example, the decrease in rainfall (decrease in the amount of rain per event, or "there are no more downpours as before", common comment among inhabitants of the community) affects the entire community equally; however, prolonged droughts seem to affect more the crops and the livestock. On the other hand, the increase in temperature has more effects on the family or on the people.

When it comes to adaptation to the negative effects of the changes in temperature and precipitation patterns, 82% answered yes to the question of: "As a result of the changes in climatic conditions that you mentioned, have you modified your daily activities in the field?" For the specific question about what actions they have implemented in the plot (it could be more than one action), 63.7% mentioned building or enlarging rain water container mounds; 28.3% said using more precocious corn seeds and better adapted to drought; 5.3% rationing water for irrigation, and here it is important to mention that a group of producers implemented drip irrigation systems with rain water; 2.6% manifested sowing a second agricultural product (such as oats or barley) in case that "the seasonal rainfall is not enough for corn to grow". Regarding the actions to protect the livestock from

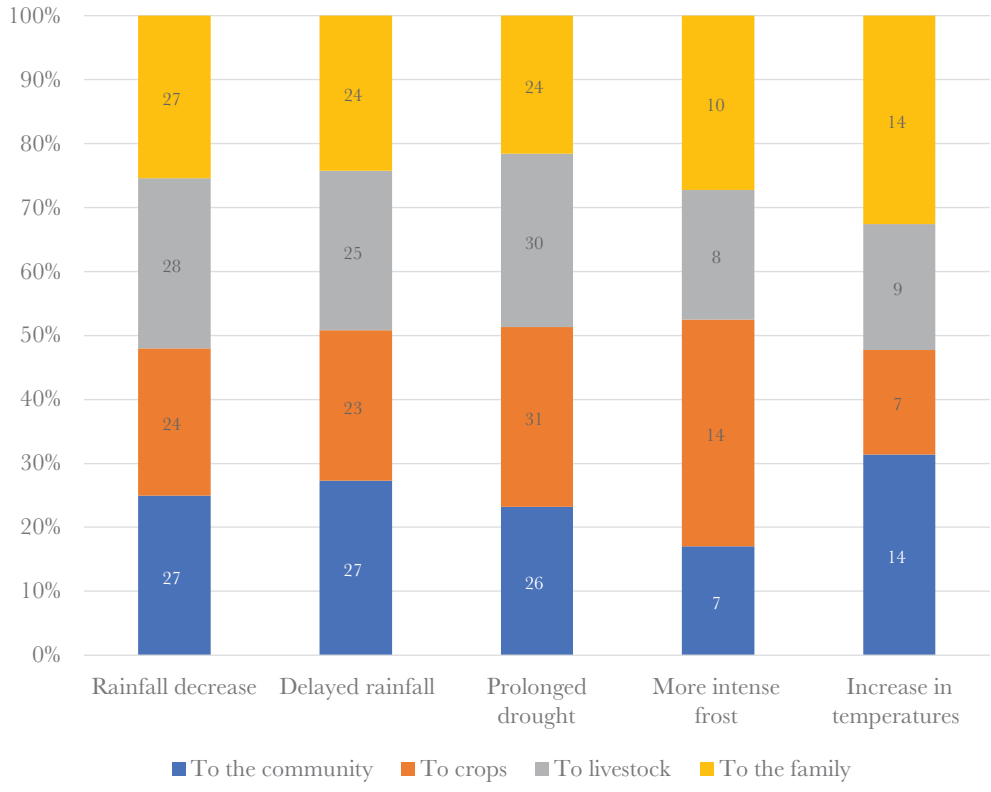

Figure 2. Level of affectation (%) of natural phenomena on some aspects of life of the inhabitants of the El Saltillo community.
Source of data: Prepared by the authors with research work.

drought, 49.5% of the people said they have built or enlarged the rain water container mounds both for irrigation and for the livestock, and 48.6% mentioned that they reduced the number of livestock, to feed fewer animals.

To protect the livestock from frosts, 75.4% mentioned that they have improved the stable with actions like placing a roof and/or a type of floor that allows keeping the livestock dry and warm; 15.6% said that during winter time, they add vitamins to the livestock feed to prevent illness. When it comes to actions to face the drought in the home or household, 74.4% mentioned that they have been forced to build cisterns or purchase water tanks to store water for exclusive use in the household; 15.3% have made home improvements in aspects like introducing piped water, placing a tile roof instead of metal sheets with the aim of keeping the house fresh. In this regard, 10.2% of the survey respondents answered that they have not taken any actions.

In the topic referring to the vulnerability from climate change in the agricultural sector, all the farmers mentioned that corn is their main crop. Figure 3 shows that 64.6% of the producers sow only corn (monocrop) and only 35.4% sow corn with another fodder product. This value could be less representative because in the year when the survey was applied, 2022, the drought forced several producers to sow oats, barley or another fodder instead of corn, with the aim of producing feed for the livestock.

Figure 3 shows that the number of producers who sow native corn varieties is 88.5%, which makes these varieties the main agricultural product of the region. Of the total surface sown, 95.7% is destined to producing corn. The average agricultural yields are 2.5 Ton/ha for native corn varieties and 6.6 Ton/ha for improved varieties.

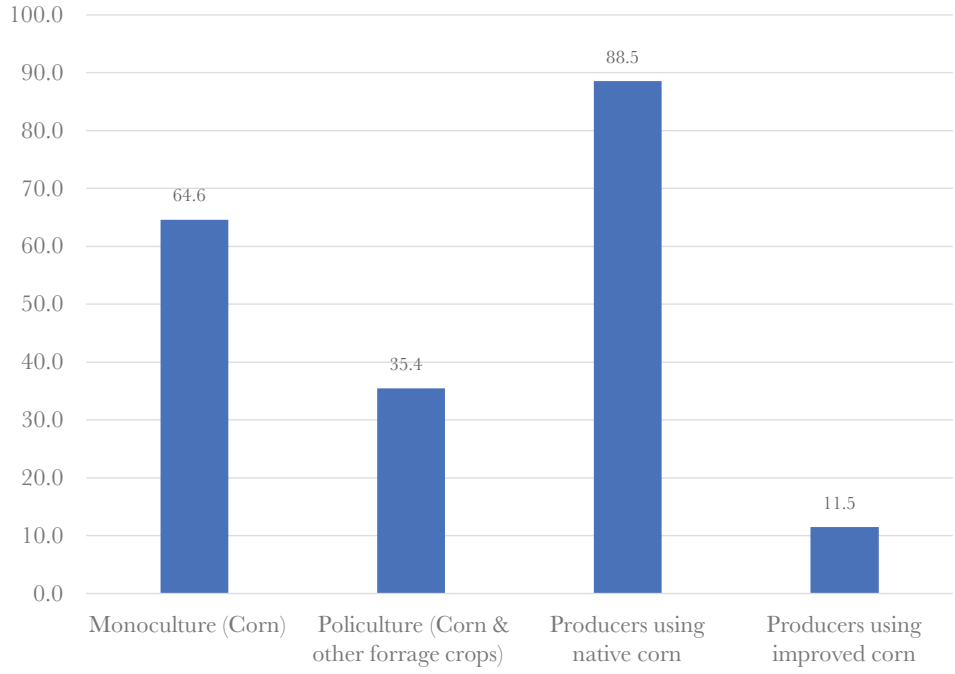

Figure 3. Comparison between producers who sow native and improved varieties of corn.
Source of data: Prepared by the authors with field work.

Various studies such as those by Noriega-Navarrete *et al.* (2021), Monterroso-Rivas *et al.* (2011) and IPCC (2014) consider rainfed agriculture, including corn growing, as a highly vulnerable sector to the negative effects derived from CC. Despite the importance of the corn crop in the study zone, the negative effects of CC place the agricultural sector in a vulnerable situation, 98% of the survey respondents mentioned that the inhabitants are not organized to face the changes in climate conditions, it is only a topic that comes out in talks between neighbors or family members and that no government institution, research center or university, has approached them to inform them of the issue or to guide them in how to adapt better to these changes.

CONCLUSIONS

This study evidenced aspects such as how the population of the community has become aware and has empirical knowledge of changes in the natural patterns of temperature and precipitation, although without calling it Climate Change. These changes affect them in economic and social aspects. There is a generalized lack of knowledge about the causes for changes in climate and, therefore, it is an issue that does not go beyond the news, and which is not clearly relevant in the local reality. It is also true that producers have seen agricultural yields decrease, particularly in the native corn varieties, which has caused the adoption of improved varieties particularly among people whose main destination of production is to feed livestock. Unfortunately, it is a fact that this will worsen in the medium and long term according to scenarios of CC that point to an increase of temperature and decrease in rainfall in the region.

The study also allowed identifying strategies for adaptation to the negative effects of CC, evidencing that they are not spontaneous actions and that they have emerged empirically through daily contact with the phenomenon. As mentioned by Pinilla-Herrera *et al.* (2012), "In face of the technical documentation of the perceptions and the adaptability on alterations of climatic variables, it is important to conclude that from a scientific viewpoint, consistencies will continue to be found between the objective and the subjective, since the local knowledge —not measurable— establishes another form of knowledge that is justified in the experience, and therefore, they are valid, verifiable and credible understandings". Therefore, the application of measures of prevention, mitigation or adaptation to CC is a long and varied process that depends not only on the ability to comprehend but also on the resources available, priorities of those involved, and society's organization, in addition of course to the support from governments and research institutions.

The application of the online survey is an option in studies where data about one or many phenomena that are happening in a community need to be obtained, such as the perception and the adaptability of the population in face of changes in climatic patterns or Climate Change; therefore, the application of online surveys is a feasible option that is inexpensive, easy to obtain, easy to quantify, graph, interpret and analyze, although mechanisms for validation and sampling must be implemented.

Climate Change is one of the greatest challenges that humanity faces today since it affects each and all of society's activities, although food security is perhaps one of the more critical issues and which forces authorities, politicians, scientists, decision makers and

the population at large to implement urgent measures to revert the problem. As a result of this, there is a need to elaborate detailed and local studies on the present and future affectations from climate change but also on how to involve society in general to generate action strategies.

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Is there a "Made in Mexico" model for innovation transfer or diffusion among farmers?

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ABSTRACT

Objective: To determine the existence or absence of a model or several models for the transfer of innovations that have been developed and evaluated in Mexico.

Design/methodology/approach: Using the SCOPUS® metadatabase, a search was conducted with the words innovation AND farmer AND Mexico. It resulted in 70 articles, of which only 35 met the selection criteria.

Results: The articles used concepts, frames of reference, and, to a lesser degree, theories to support their research. The highest number of published cases dealt with the MasAgro technological hub model in maize and the GGAVATT model for group-oriented work in livestock.

Limitations on study/implications: Using a metadata base that is not open access limits the results, since technical reports, books, and other documents that might otherwise enrich the discussion are left aside.

Findings/conclusions: There is still much to be theorized in order to create new models adapted to other product systems that could promote technology transfer from the institutions of the sector and researchers to farmers.

Keywords: Agriculture Innovation Systems, extensionist, technological model.

INTRODUCTION

One of the greatest challenges that researchers face when developing new technologies, varieties, products, or services for the agri-food sector is achieving the adoption of the technology by users. Studies on the adoption of agricultural technology have their beginnings in the United States during the seventies. The researchers who made the most significant contributions were sociologists interested in distinguishing the characteristics of possible adopters of technology, opinion leaders, as well as their perspectives, adoption rates, and the communication channels they used (Marra *et al*., 2003).

The concept of innovation has been widely used in science, as governments, companies, or organizations that finance science seek to ensure that the technologies they support have an innovative component. In the agricultural sector, innovation has also played an important role among researchers. In relation to innovations and their transfer, the focus has gone from studying innovation *per se* (technology-oriented approach), to understanding its users, taking into account the combination of technological and non-technological aspects (systemic, holistic, user-based approaches)

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(Schut *et al.*, 2014). The aim of the present study was to understand, in the case of Mexico, how research related to innovations that have focused on farmers has been addressed, whether the focus has been on innovation, or on more complex approaches, and whether these have been proposals developed in Mexico or models imported from abroad.

Theoretical and conceptual framework

Farmer-level reasons for the adoption of innovations

After the initial studies, there is a wide range of research that has established that the adoption of innovations is affected by various factors. For example, in a literature review on the factors that affect the adoption of innovations in small farmers in Africa (Fadeyi *et al.*, 2022), the authors found 29 factors that could be classified into five groups: farmer characteristics, production unit characteristics, technology characteristics, as well as institutional and financial factors. Of these, the most commonly mentioned factors were finance, gender, age, education, size of the production unit, and access to extension. One case is the adoption of agricultural innovations in Ethiopia, where farmers with the following characteristics were more likely to adopt innovations: higher level of education, larger families, more participation in activities outside their production unit, more livestock, access to extension services, advisory services, credit, optimal roads, production units close to their homes, and less income from remittances (Zegeye *et al.*, 2022). In another study carried out in China, the authors found an increase in the adoption rate of innovations for precision fertilization when extension services based on information and communication technologies were used (Li *et al.*, 2022); in a study on adoption of irrigation systems in Lebanon, the authors found that an increase in risk perception reduced the adoption of innovations (Sabbagh and Gutierrez, 2022). Another factor is the level of knowledge that the farmer has about a given innovation, which has been positively correlated with the adoption of that innovation (Khan *et al.*, 2022); as well as the farmer's ability to process information, which is correlated to his or her age (Wu *et al.*, 2022), and whether or not they belong to a cooperative (Adebayo *et al.*, 2022).

Systemic-level reasons for the adoption of innovations

Other studies have stressed the role of the State, as well as of the institutions. For example, they become critical for facilitating the collaboration and participation of diverse actors in the value chains when digital innovations are adopted. The State, the institutions, as well as the context surrounding the users, have contributed to a situation in which the process of diffusion and adoption of innovations not only focuses on the user, nor on the innovation to be transferred, but rather it has been reported that the adoption of innovations must be addressed under a systematic approach (Schut, Rodenburg *et al.*, 2014).

Agricultural innovation systems, which analyze technological, economic, and institutional changes in agriculture, represent one of the existing approaches from a systematic perspective (Klerkx *et al.*, 2010).

This approach has been and continues to be used to analyze agricultural systems around the world (Klerkx *et al.*, 2023). In one study, the authors found that each country

has agricultural innovation systems that are unique and that suffer from problems which, although they might seem common, are not actually common for everyone (Hermans *et al.*, 2015).

Other approaches are: 1) farming systems (FS), which emerged in the eighties and nineties and focuses on the production unit. FSs require experts and technologies that are specific to a specific context; and 2) agricultural knowledge and information systems (AKISs), which emerged in the nineties and seek to empower producers with a value chain approach, joint production, and joint learning. It employs a participatory approach but does not consider the power relations between the actors or their inequalities (Schut, Rodenburg *et al.*, 2014).

As mentioned above, the transfer of innovations and their adoption by users has been analyzed at various levels: the innovation by itself, the individual, and at a systemic level that can even reach the national scale. In the case of Mexico, there are several studies that address technology innovation processes; however, the level of their analysis has not been explored to date, nor whether they have a systemic vision or whether they have proposed models to understand and promote innovation transfer processes in the sector. The objective of this systematic literature review was to determine the existence or absence of a model or several models for the transfer of innovations that have been developed in Mexico.

MATERIALS AND METHODS

Search

The articles that were included in the study were obtained through a search performed in the SCOPUS database on February 17, 2023, using innovation AND farmer AND Mexico as search words, resulting in 70 articles. The search was limited to these terms because we wanted to investigate experiences with technology transfer models in farmers and not specific experiences of "diffusion" or "extensionism" since these concepts could have limited the search or might have already been part of a technology transfer model.

Data analysis

The research question became the framework under which the literature was analyzed, categorized, and coded to highlight the way in which the innovation was approached and whether it had been transferred under a model. And if it had, establish the characteristics of the model. Articles were coded and organized by topic using an inductive approach.

Scope

To be included in the study, the articles had to show evidence of processes of innovation transfer to farmers. An important restriction was that the studies should not have an anthropological/historical focus that could refer to ancient Mexico. The articles could be published in English or Spanish, and could address agricultural, livestock, or fishing components. Based on the above criteria, only 35 of the 70 articles were retained for analysis.

RESULTS AND DISCUSSION

The oldest publications that address the topic of innovations, farmers, and Mexico begin in 2006 with a growing trend until 2022, 71.4% of the publications being concentrated in the last six years. They focused on several agri-food products (Figure 1), but mainly on two product systems: maize (seed, cultivation, or combinations with other crops); and livestock (in dual-purpose systems, for meat or milk).

Geographically, the studies were located at various levels, from those that made comparisons at the binational level (Mexico-Peru or Thailand-Mexico), at the Latin American level, at the National level, or those that covered various locations in several states (Guanajuato and Michoacán; 10 states of Mexico), regions (the Mexican tropics, the Central Valleys of Oaxaca, or the Purépecha region). In total, experiences were documented in 16 states of the republic (Figure 2).

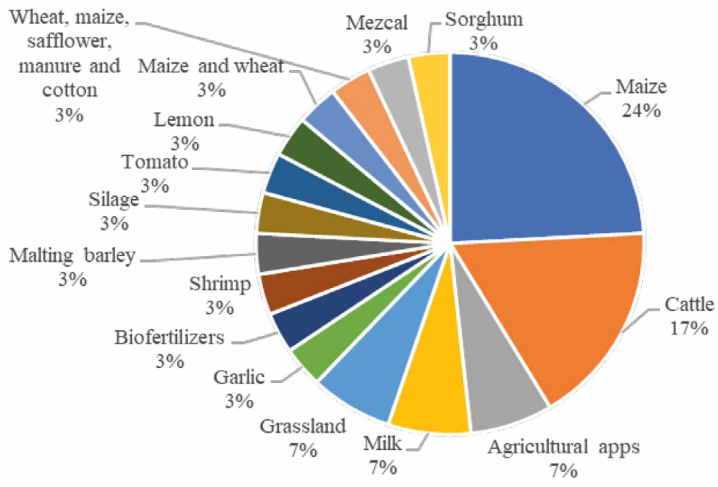

Figure 1. Studied products in publications referring to innovation, farmers, and Mexico.

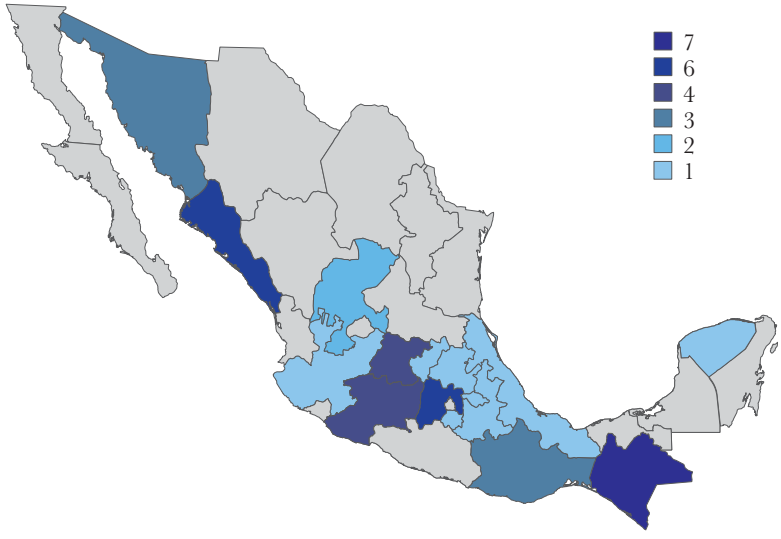

Figure 2. States where publications referring to innovation, producers, and Mexico were located.

Of the total of articles, 62.8% of the studies focused on: farmers (28.5%), ranchers (25.7%), heads of household (2.9%), mezcalilleras —the woman who produces and likes mezcal— (2.9%), and stakeholders (2.9%). The rest of the studies focused on institutions such as foundations or innovation centers. Most of the articles based their research on concepts (51.4%), followed by frameworks (37.1%) and theories (11.5%); the most frequent concept was that of technology adoption, while the most common framework was social network analysis. The theories used were the Theory of Reasoned Action (TRA), the Theory of Goal Orientation, the Theory of Planned Behavior (TPB), the Unified Theory of Acceptance and Use of Technology (UTAUT), and the Unified Theory of Acceptance and Use of Technology Modified (UTAUT2).

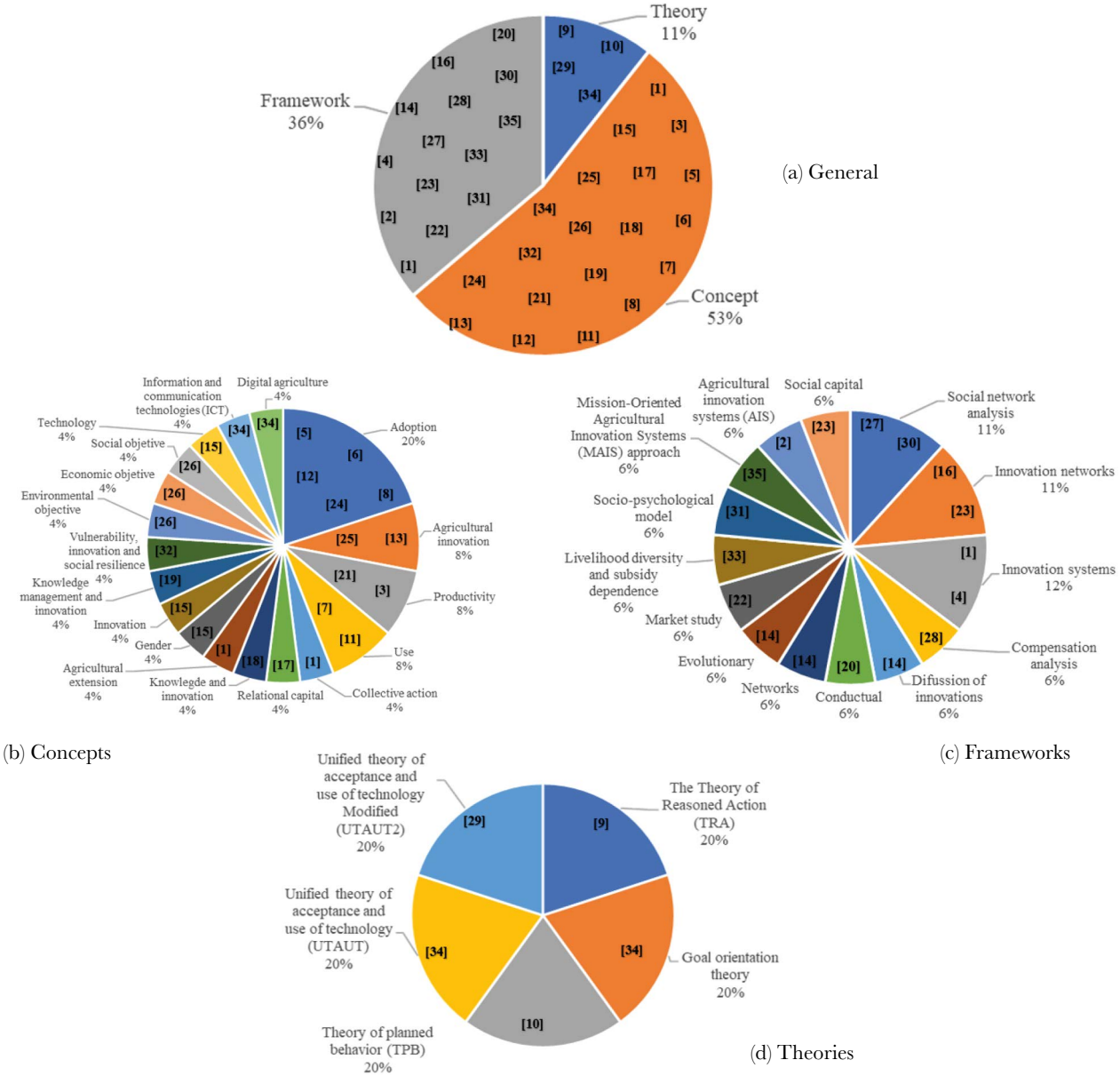

Figure 3. Distribution of articles according to the basis of their research (theories, concepts, or frameworks).

Technologies, systems, and technology transfer systems were documented. The technologies were biofertilizers, silage, improved maize, new varieties, improved grass, crop residues, improved seeds, and technological applications. The systems were agroforestry, conservation agriculture, technological kits, aquaculture parks, sowing in double row and piled furrows (SSDHP, Spanish initials), and technology for milk production; the technology transfer systems analyzed were MasAgro, GGAVATTs, and MAIS.

With regard to the developers of the technology, systems, and/or technology transfer systems, CIMMYT (International Maize and Wheat Improvement Center, Spanish initials) developed MasAgro and is responsible for 8 of the 35 articles. The second most important is INIFAP with 7 of the 35 and one co-authored between CIMMYT and INIFAP. The following institutions had one development: CONANP (National Commission of Protected Natural Areas, Spanish initials), FIRA, CINVESTAV Mérida in collaboration with the Kellogg Foundation, and a private company, the rest made no indication in this regard, so they were attributed to the authors but not to an institution.

Finally, in the global analysis of the articles, the approach followed by the authors was determined in terms of whether they were descriptive, theoretical, explanatory, or predictive. Most of the articles were descriptive and only one was theoretical. In the greatest number of cases, the authors' aim was for their contributions to be explanatory, and to a lesser extent predictive. Descriptive articles focused on descriptions of the technologies, the adoption made by users, and the results found in the field; that is, they concentrated on the technology, while explanatory articles tended to establish the characteristics of the farmers that cause a technology to be adopted or not. Finally, predictive articles were those that make it possible to establish how the adoption of the technology or the technology might behave in other cases (Table 1).

The adoption of technology is relevant to the achievement of changes in rural areas. This systematic literature review seeks to present elements to discuss the existence of a "made in Mexico" model. The results show three models in particular: MasAgro, MAIS, and GGAVATT. We shall begin with the first: MasAgro is a sustainable modernization of a traditional agriculture program developed jointly between CIMMYT and the Mexican government that began in 2010 and aims to contribute to the country's food self-sufficiency (Camacho-Villa, Almekinders *et al.*, 2016).

In particular, it focuses on traditional maize farmers who can transition to a more commercial and profitable production through the use of more modern practices such as improved varieties, comprehensive soil fertilization, improved tillage methods, and their integration into more profitable markets (Donnet, Becerril *et al.*, 2017). In order to attain this, they establish "hubs" or innovation platforms, under the principles of agricultural innovation systems (AISs, which were developed by researchers from The Netherlands). This program initially focused on technology, and later transitioned to a more system-oriented approach (Camacho-Villa, Almekinders *et al.*, 2016).

MasAgro, although it has been replicated in various parts of the country, has not produced the expected impact. Furthermore, the program was not developed in Mexico, but rather contains elements of other CIMMYT experiences in various places around the world. The international focus of the program, which can be considered its strength, shows

Table 1. Focus of the articles on innovation, farmer, and Mexico.

No.	Author(s) (year)	Descriptive	Theoretical	Explanatory	Predictive
[1]	Hellin (2012)	✓			
[2]	Hellin and Camacho (2017)		✓		
[3]	Cuanalo de la Cerda and Siniarska (2006)	✓			✓
[4]	Speratti *et al.* (2015)	✓		✓	
[5]	Cuevas-Reyes (2019)	✓		✓	✓
[6]	Sánchez-Toledano *et al.* (2018)	✓		✓	✓
[7]	Cuevas-Reyes *et al.* (2021)	✓		✓	✓
[8]	Martínez-Garcia *et al.* (2020)	✓		✓	✓
[9]	Martínez-García *et al.* (2013)	✓		✓	✓
[10]	Juárez-Morales *et al.* (2017)	✓		✓	✓
[11]	Reyes Cuevas *et al.* (2013)	✓		✓	✓
[12]	García *et al.* (2012)	✓		✓	✓
[13]	Dutrénit *et al.* (2012)	✓		✓	
[14]	Díaz-José *et al.* (2016)	✓		✓	
[15]	Contreraś medina *et al.* (2021)	✓		✓	
[16]	Monsalvo-Velázquez *et al.* (2014)	✓		✓	
[17]	Roldán-Suárez *et al.* (2018)	✓		✓	
[18]	Lebel *et al.* (2016)	✓			
]19]	Lopez *et al.* (2020)	✓		✓	
[20]	Sánchez-Toledano *et al.* (2021)	✓		✓	✓
[21]	Donnet *et al.* (2017)			✓	
[22]	Barragán-Ocaña and del-Valle-Rivera (2016)				
[23]	Zarazúa *et al.* (2012)	✓		✓	
[24]	Sánchez-Toledano *et al.* (2017b)			✓	✓
[25]	Camacho-Villa *et al.* (2016)	✓			
[26]	Sánchez-Toledano *et al.* (2017a)			✓	✓
[27]	Oriana *et al.* (2021)			✓	✓
[28]	Speelman *et al.* (2006)	✓			
[29]	Molina-Maturano *et al.* (2021)			✓	✓
[30]	Villarroel-Molina *et al.* (2021)			✓	✓
[31]	Martínez-García *et al.* (2018)			✓	
[32]	Díaz-José *et al.* (2018)	✓			
[33]	Zabala *et al.* (2022)			✓	✓
[34]	Molina-Maturano *et al.* (2022)			✓	
[35]	Castillo-Martínez *et al.* (2022)	✓		✓	

that it is not a purely "made in Mexico" product. The transition from programs focused on technology to those with a holistic vision in the program is evident, yet the primary orientation of the program continues to be toward technology (improved varieties of maize and conservation tillage).

The second case is MAIS, a model that focuses on the evaluation of different aspects from a holistic perspective in order to establish an agenda for research and innovation, as well as for the production system. This approach was developed as an extension of the principles of agricultural innovation systems (AISs) by the same group of researchers in the Netherlands. It remains as a methodological guide to understand a reality in order to propose public policies (Castillo-Martínez, Díaz-José *et al.*, 2022), but it does not constitute a tested or pilot model.

Finally, the GGAVATTs are Livestock Farmers' Groups for the Validation and Transfer of Technologies, which conform to a model developed several decades ago by the INIFAP (National Institute for Forestry, Agriculture, and Livestock Research, Spanish acronym) in which livestock farmers were organized into groups with a common interest, that of adopting technology (reproduction, feeding, management, health, quality, management, and use of agricultural lands). The studies show that the GGAVATTs have been constituted as homogeneous groups with well-defined structures and with higher levels of technology adoption than livestock farmers that did not belong to them (Villarroel-Molina, De-Pablos-Heredero *et al.*, 2021). The greatest limitations of this model are that it has only been used in livestock farming and that a central axis is to group farmers. This becomes complex for other cases in which organization among farmers has been a historical problem.

The GGAVATT model has components that are like those of MasAgro, which is to have a clear innovation, a technological package to transfer and disseminate, as well as technical support (technicians and researchers) to monitor the processes. This is similar to the innovation ecosystem (Fursov and Linton, 2022). Although the two differ in their objectives, GGAVATT seeks to improve the standard of living of the livestock farmers and MasAgro originally sought to increase competitiveness; then they focused on food sovereignty and poverty reduction, adapting the justification of the impact of their models to the prevailing government discourse. Half of the rest of the research focuses on the factors that determine the adoption of innovations by farmers. These can be divided into those that focus on innovation and the rest on testing various concepts mentioned in the results section. This makes it possible to demonstrate that the focus of research has been on technology as well as on technological and non-technological aspects (Schut, Rodenburg *et al.*, 2014), with few models that have been evaluated or adopted by companies, institutions, or NGOs outside the institutions that created them.

CONCLUSIONS

This systematic literature review aimed to determine whether there is a "made in Mexico" technology transfer model. The answer to that question could be said to be yes, if we consider the GGAVATTs as a model to validate and transfer innovations as well as to organize livestock farmers. However, it remains to be determined whether this model is reproducible for other non-livestock farmers and in contexts where farmer organization

is a complex issue. In conclusion, there remains much to be theorized in order to create new models adapted to other product systems that could promote technology transfer from institutions, companies, and NGOs to farmers.

It should also be noted that, except for one publication, the rest are not theoretical in nature; that is, the level of discussion does not reach the point of rejecting or improving existing models such as the Agricultural Innovation System (AIS) or social networks. Or perhaps proposing a tropicalized version of them, in order to use them as research frameworks to characterize or validate what was implemented in the field, especially with regard to the publications associated with MasAgro.

One of the limitations of this review is that it was restricted by the keywords used during the search, hindering the ability to include other publications that might have shed light on other models for the transfer and diffusion dissemination of innovations to farmers. Likewise, the use of a metadata base that was not open access also limited the results, since technical reports, books, and other documents that might have enriched the discussion were left out of the analysis.

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Colegio de Postgraduados

Heliconia SCARLET: a mexican variety for cut flower and gardening

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ABSTRACT

Objective: To carry out the morphological characterization of *Heliconia uxpanapensis* × *Heliconia latispatha* var. Scarlet for varietal registration purposes.

Design, Methodology and Approximation: By means of rhizomes division of 71-1 plant from a plot of *H. uxpanapensis* × *H. latispatha* segregant plants (F2), twelve plants (tillers) were generated, planted, and cultivated in open field under 30% of natural shade. Morphological characterization was methodic conducted using the Technical Guide descriptors for Heliconias designed by the National Seed Inspection and Certification Service (SNICS, 2023).

Results: Morphological characters of the clones from the 71-1 segregant plant, tested with the SNICS Technical Guide descriptors, were constant so that a differentiation could be make between the Scarlet and Karely, a reference variety found in the Guide. Scarlet variety's primary distinctive characteristics are the red color, high brilliance, and the revolute-involute margins of their bracts. Their inflorescence morphological characteristic suggest they can be cultivated for cut flower and gardening.

Study limitations and implications: In order for Scarlet variety express, their characteristics of intense color and bracts brightness, they have to be cultivated under 30% of shade.

Findings and conclusions: Based on the Technical Guide descriptors for heliconias varietal description (SNICS, 2023), the Scarlet variety differentiates from the Karely (reference variety) as it presents a stability-distinction-homogeneity. For this reason, the varietal registration seems appropriate.

Keywords: bract brightness, cut flower, *Heliconia* Karely.

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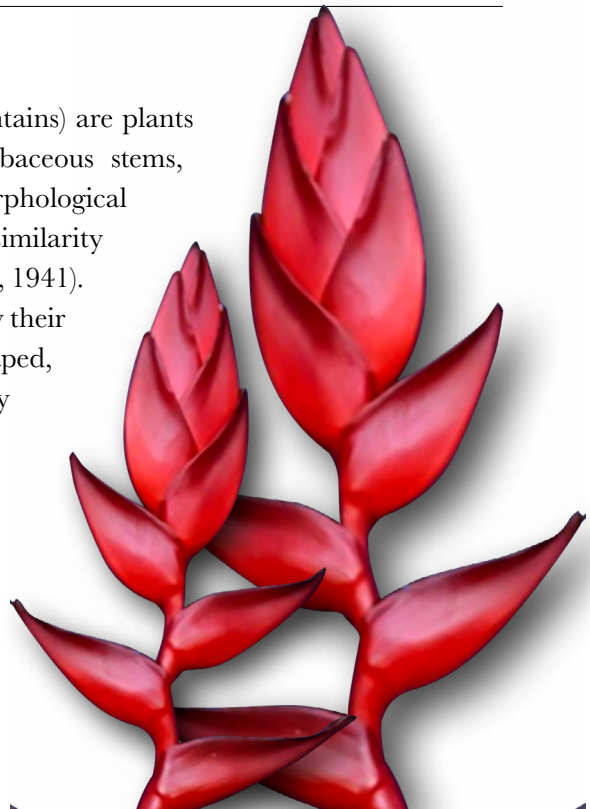
INTRODUCTION

Species from the *Heliconia* genus (little plantains) are plants that characterize themselves for having herbaceous stems, broad and large leaves, and high degree of morphological similarity to plantains (Musaseae family); such similarity was taxonomically backed up until 1941 (Nakai, 1941). Species from the heliconias, be differentiated by their erect or pending inflorescences consisting of shaped, textured, tinged, and colored bracts; their rarity and beauty let them to be used in gardening, but their post-harvest durability makes them ideal for cut flower.



Around 220 species of heliconias which integrate the genus (Berry and Kress, 1991; Kress *et al.*, 1999) grow up naturally in the rainforests and subtropical forests of

the American continent between the Tropic of Cancer and Tropic of Capricorn parallels, geographically expanded from northern Argentina to northeast Mexico (Anderson, 1989). The greatest diversity is found in the territories of Colombia, Ecuador, and Venezuela; although it is worth mentioning that Colombia concentrates nearly 100 species (Kress *et al.*, 1999). These countries, apart from Costa Rica, have positioned themselves as the suppliers for most varieties existing in today's market and consolidated as worldwide producers and suppliers of tropical flowers (Diaz *et al.*, 2002).

Within the Mexican territory, there is a transition happening from tropical to cold climate that makes it possible to find at least twelve species of heliconias in the wild, along with their corresponding intraspecific diversity. Climate conditions, in addition to deep and fertile soils, favors the cultivation of commercial varieties, especially in Tabasco (Saldaña & Hernández, 2004), Chiapas and Veracruz. As previously cited, the varieties currently grown are coming from Central and South American countries.

Throughout INIFAP, Mexico generated its first heliconia variety called Karely in 2022, a variety registered in the National Catalogue for Plant Varieties.

To obtain the Breeder's Certificate for new heliconias varieties, a stability-distinction-homogeneity (SDH) test must be conducted using the SNICS Technical Guide, which works under the authority of the Department of Agriculture in Mexico. Therefore, the objective of the current work was to carry out a morphological characterization of the *Heliconia uxpanapensis* × *Heliconia latispatha* var. Scarlet for varietal registration purposes.

MATERIALS AND METHODS

From a segregant population (F2) of *Heliconia uxpanapensis* × *Heliconia latispatha* (cited by Ortiz-Curiel *et al.*, 2022), the 71-1 plant was selected due to its inflorescence deep red, brightness of its bracts and stem height; by means of rhizome division, such plant was asexually multiplied from which 12 plants were put directly into soil under 30% of shade. The site showed the following features: sandy franc soil (79.9% sand) where climate conditions are humid tropic with an average annual rainfall of 4,443 m and average temperatures of 26.6 °C, reaching up to 35.5 °C from February to April.

Originally, the morphological characterization was methodic conducted using the descriptors suggested by Avendaño-Arrazate *et al.* (2017). Subsequently, such characterization for SDH purposes was carried out based on the Technical Guide descriptors for Heliconias (2023) created by the SNICS.

RESULTS AND DISCUSION

Heliconia Var. Scarlet Varietal Description

Heliconia var. Scarlet, besides their deep red color and brightness of their bracts, have a tall-posture plant like. The red color growing in the midway, which included the apex in each bract, was slightly intensified —this did not imply the presence of a second color— the revolute margin orientation in this section associated to high brightness, generated a difference in the reflection of light; consequently, visual contrasts were intensified. These characteristics make *Heliconia* Scarlet different from their biological parents and from *Heliconia* Var. Karely, a reference variety (Figure 1).

Figure 1. *Heliconia* Var. Scarlet and *Heliconia* Var. Karely. A) Inflorescence in a plot of Var. Scarlet, B) Inflorescence zooming, C) Involute-revolute margin orientation of the bracts, D) Color in flower, and E) *Heliconia* var. Karely (reference variety).

At maturity, *Heliconia* var. Scarlet presented up to 16 bracts in their inflorescence phyllotaxy distichous, which a rachis at zigzagging medium level. The plants' impressiveness, the contrast between the green in leaves and the deep red in the inflorescence, make them ideal for gardening. Generally, the first bract had a highly extension as the flag leaf; however, it did not present any foliar blades at the terminal end of this structure, as it happens with *Heliconia latispatha* varieties. The *Heliconia* var. Scarlet preserves similar trademarks to its female parent: *Heliconia uxpanapensis*.

Commercially speaking, their beauty and red color make the Scarlet variety suitable for cut flower. For instance, red heliconias are highly marketed preferable (Linares *et al.*, 2017; Diaz *et al.*, 2002). The length of Scarlet variety' stems, which exceed the 100 cm, are appropriate for the mainstream market since hotels, restaurants and hall's owners request tall flowering heliconias to have their ample spaces decorated (Baltazar-Bernal *et al.*, 2011).

The inflorescence formed by bracts in distichous position favors their packing and lessening any friction or bruising damages, a relevant feature for commercial varieties such as *Heliconia wagneriana*, *H. bihai*, *H. stricta*, *H. ortotricha*, *H. caribaea* and Karely (Ortiz-Curiel *et al.*, 2022).

For a suitable expression of color and characteristics inherent to Scarlet variety, they must be cultivated using an agroforestry system where shade conditions should be about 30% as Grajales-Solís and Montejo-Rodríguez (2008) have suggested not only for

Table 1. Morphological characterization of the *Heliconia uxpanapensis* Gutiérrez × *H. latispatha* var. Scarlet using the Technical Guide for Heliconias Varietal Description (SNICS, 2023).

Characteristics	Var. Scarlet	Note
Plant: degree of tillering	Medium	5
Plant: growth habit	Erect	1
Plant: height (144 cm)	Medium	5
Pseudostem: thickness (4.8 cm)	Medium	5
Pseudostem: antocianina pigmentation	Present	9
Pseudostem: intensity of antocianina pigmentation	Strong	4
Leaf: length (151.9 cm)	Long	7
Leaf: width (42.85 cm)	Broad	7
Leaf: length/width relation (3.5)	High	7
Petiole: length (136.9 cm)	Long	7
Rolled leaf: antocianina pigmentation in the underside margin	Present	9
Rolled leaf: intensity of antocianina pigmentation in the underside margin	Weak	3
Inflorescence: flag leaf	Present	9
Flag leaf: blade	Absent	1
Inflorescence: rotation	Absent	1
Inflorescencce: degree of zigzagging in main axis	Medium	5
Inflorescence: number of bracts (14)	Plenty	7
Inflorescence: width (23.4 cm)	Medium	5
Inflorescence: bracts separation (3.3 cm)	Short	3
Bract: margin orientation	Medium	5
Bract: height (3.8 cm)	Medium	5
Bract: length (14.27 cm)	Medium	5
Bract: width (2.9 cm)	Medium	5
Bract: brilliance	Strong	7
Bract: number of colors	One	1
Bract: primary color	Red	3
Flower: length (joined tepals) (5.6 cm)	Medium	5
Flower: num. of colors in free tepal	One	1
Flower: free tepal primary color	Yellow	3
Flower: curvature of long axis in free tepal	Medium	5
Flower: curvature level in nectar storage	Weak	3
Flower: num. of color in joined tepals	One	1
Ovary: color	Yellow	2

diverse heliconia species and varieties but also for other tropical flowers growing within these agroforestry systems. Rhizomes and propagation material are kept at the INIFAP Rosario Izapa Experimental station. In line with the assessment results, we began the registration process of the Heliconia Scarlet in the National Catalogue for Domestic Varieties and the Breeder Certification before the National Service for Seed Inspection and Certification in Mexico.

CONCLUSIONS

SNICS morphological descriptors confirm there are two distinguishing morphological characterization for this variety: their deep red color and brilliance. Apart from meeting the SDH test, these two traits make *Heliconia* Scarlet ideal for cut flowers and gardening. With its register, the *Heliconia* var. Scarlet will be the second generated variety of heliconias in Mexico.

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