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English version

Nutraceutical and nutritional characteristics of capulin segregants (*Prunus serotina*) fresh and processed

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Abstract

The capulin fruit (*Prunus serotina*; Family *Rosacea*) has been valued since pre-hispanic times for its medicinal properties, used in the treatment of some diseases. Although Mexico is part of the center of origin of the capulin, production and consumption of this fruit have decreased in recent years, becoming an underutilized fruit. There is little research on its nutritional and nutraceutical properties. The aim of this investigation was to evaluate the physicochemical properties, nutritional and nutraceuticals components of fresh and processed capulin fruits, from four segregants. Polar and equatorial diameter, peel color was determined by evaluating *L* (brightness), the angle of tone (*hue*) and color purity or *chromaticity* index (*chroma*), pH, and TSS; as well as the content of carbohydrates, ash, humidity, crude fiber, protein, and lipid content were quantified according to the AOAC guidelines. Mineral content was quantified by atomic emission spectrophotometry, phenolic compounds by the Folin-Ciocalteu method, anthocyanins by the pH differential method, and antioxidant activity by the ABTS method. The fruits showed high protein and fiber contents. Significant differences in nutraceutical content were found among the four types of segregants. The thermal process did not decrease the nutraceutical quality (except anthocyanins) of the four types of segregants, this only affected the nutritional attributes. Therefore, the segregants with the highest nutraceutical value were Puebla 5-28F and Puebla 5-3F, due to their high contents of phenolic compounds and anthocyanins. In conclusion, capulin fruits contain a wide variety of antioxidant and nutritional compounds, and their consumption could generate benefits for human health.

► **Keywords:** antioxidants, minerals, proximal, genetic segregation.

Introduction

Recently, in Mexico there has been a growing interest in the knowledge and management of underutilized fruit, also known as minor, secondary or alternative fruits, as it is the case of the capulin (*Prunus serotina*). Capulin belongs to the family *Rosaceae* and to the gender *Prunus*, where more than 200 species of commercially important species are found, such as cherry, peach, plum, among others, known as stone fruits (Potter, 2011). In 1951, McVaugh described five subspecies of *Prunus serotina*, the subspecies *capuli*, *serotina* and *virens*, coexist in several states of Mexico (Guzmán et al., 2020).

Prunus serotina is a deciduous tree native to America which grows in diverse regions in the wild or cultivated conditions, in cool semi-cold, humid and temperate climates. Its distribution includes southeastern Canada, northeastern United States, Ecuador, Colombia, Guatemala and the Sierra Madre Oriental, Occidental and the Neovolcanic axis of Mexico, (López-Hernández et al., 2024; Pathania et al., 2022). Currently, this species is naturalized in several countries around the world, including in various regions of Europe (Germany, Denmark, France, England, Lithuania, the Netherlands, Poland, Romania, Switzerland, among others) (Petitpierre et al., 2009). In the United States,

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capulin is known as wild or black cherry, in Europe as Mexican cherry (Petitpierre et al., 2009). In 2023, Mexico reported formal cultivation of capulin only in Veracruz, Mexico City, Puebla, Estado de Mexico and Jalisco, with a harvested area of 37 ha with a production of 114.28 t of this fruit (SIAP, 2024).

Capulin fruits are globose fleshy drupes, reddish to black in color depending on their state of ripeness, climacteric in nature and sweet and sour in taste; they also commonly contain cyanogenic glycosides (prunazine and amygdalin), excessive consumption, without thermal processing, can have adverse health effects (Telichowska et al., 2020). According to Swain et al. (1992), the presence of cyanogenic glycosides in some members of the genus *Prunus* is considered a defense mechanism of the plant against herbivores and pathogens through the release of hydrogen cyanide (HCN) and benzaldehyde. In the particular case of *P. serotina*, it is reported to accumulate high levels of cyanogenic glycosides in the ripe fruit; However, it lacks the enzymes amygdalin hydrolase (AH), prunasin hydrolase (PH) and mandelonitrile lyase (MDL) that by hydrolysis would release HCN, so these only contribute to the bitter taste that is compensated by the accumulation of sugars at maturity of consumption, in contrast, the seeds during the roasting process are destroyed by the temperature of the cyanogenic glycosides (Telichowska et al., 2020).

In general, the fruits of *P. serotina* are marketed fresh, dried, or in jams, liqueurs or syrups; the seeds are eaten roasted with salt as a snack (Ordaz-Galindo et al., 1999). Since pre-Hispanic times, its fruits have been traditionally used for the treatment of some diseases (respiratory, cardiac, stomach and hypertension) (García-Aguilar et al., 2015; Luna-Vázquez et al., 2013). In addition, the capulin fruit has attracted attention as a potential source of nutrients and antioxidants. Ordaz-Galindo et al. (1999) reported the presence of anthocyanins (cyanidin-3-glucoside and cyanidin-3-rutinoside) in the peel of *P. serotina* subsp. *capuli*. Moreover, Ibarra-Alvarado et al. (2009) point out the presence of antihypertensive compounds such as some phenolic ones (acid chlorogenic) in the fruit, metabolites that could justify its medicinal properties. Hernández Rodríguez et al. (2019) report that the content of flavonoids and phenolic compounds decreases in the last stages of fruit ripening, with a considerable increase of total anthocyanins of up to 1.4 mg cyanidina-3-glucósido · g⁻¹ dry weight. The high content of phenolic compounds (phenolic acids), flavonoids (anthocyanins, proanthocyanidins, catechins), essential oils and tannins (Jiménez et al.; 2011.; Luna-Vázquez et al., 2013) explain its use as a natural therapy for the treatment of neurodegenerative diseases, such as some types of cancer, immune system problems and cardiovascular diseases (Poti et al., 2019; Telichowska et al., 2020)). In addition, capulin seed is a significant source of minerals, unsaturated fatty acids (oleic, linoleic and α -eleostearic acid) and high-quality, highly bioavailable proteins (García-Aguilar et al., 2015).

On the other hand, genetic studies of *P. serotina* have reported the phenomenon of allopolyploidy (union of genomes from different species), so intraspecific hybrids of capulin in Mexico with characteristics of different subspecies are available (Fresnedo-Ramírez et al., 2011; Pairon & Jacquemart, 2005). In addition, the morphological variability of the capulin in central-western Mexico is a product of human selection, directed at anthropocentric characters of interest, so that fruits of several sizes, degree of sweetness, with epicarp from reddish to almost black colorations, are found in wild populations, in *in situ* managed and cultivated (Fresnedo-Ramírez et al., 2011; Guzmán et al., 2020).

In this context, the morphological variability of the capulin is useful for the genetic improvement of this species, aimed at obtaining selections with better fruit and seed quality, or nutritional and nutraceutical value. In this sense, the Colegio de Postgraduados has a capulin collection with several segregant lots derived from outstanding individuals from the state of Puebla, mainly for their physicochemical characteristics. The term segregant used in this research denominates to individuals that were born by growing seeds from the same tree (sexual reproduction). Therefore, the objective of this study was to evaluate the physicochemical characteristics, content of nutraceutical compounds, antioxidant capacity and nutritional composition in the fruits of four fresh and processed capulin segregants.

Materials and Methods

Plant Material

Fruits were collected at commercial maturity stage from four segregants (individuals born from different seeds of the same tree) of capulin (*Prunus serotina*) grown in the Fruit Orchard of the Colegio de Postgraduados, Campus Montecillos, municipality of Texcoco, Estado de México, México (19°27' N, 98°54' O, 2 245 msnm): Puebla 5-1 (P5-1F), Puebla 5-3 (P5-3F), Puebla 5-18 (P5-18F) y Puebla 5-28 (P5-28F). The four segregants were selected because they are offshoots of the same tree, under the same edaphoclimatic conditions.

Experimental Design

The physicochemical characterization of the fruits of four capulin segregants was carried out under a completely randomized experimental design with 25 replications, the experimental unit consisted of one fresh fruit with seed. The mineral content in the pulp of fresh fruits of four capulin segregants was carried out under a completely randomized design with three replications, considering 100 g of fruits with peel and without seeds of each fresh segregant as the experimental unit. The effect of thermal treatment on the proximal and nutraceutical characteristics of capulin fruits was evaluated using an asymmetric

factorial experimental design with completely randomized allocation for the study of the factors: capulin segregant (four segregants) and degree of processing (fresh and processed). A total of eight treatments were evaluated with three replications. The experimental unit was 100 g of capulin fruits with peel and seedless of each segregant, fresh or processed (Table 1).

Statistical analysis

The data were submitted to an analysis of variance (ANOVA) and Tukey's mean comparison test ($P < 0.05$), using the Statistical Analysis System program (SAS Institute Inc., 2002). The results of the evaluated variables were expressed as the mean standard deviation.

Sample preparation

For processing, the fruit in water was kept at 40 °C by 5 min in an electric grill (Corning, model PC-620D, USA). The processed and fresh fruits were frozen with liquid nitrogen and stored to -18 °C, until analyzed.

Physical-chemical characterization

Polar and equatorial diameters of 25 fresh fruits with peel and seed of each segregant were measured using an electronic caliper (Truper, model CALDI-6MP, Jilotepec, Mexico). Fruit weight was determined on an analytic balance (Adventurer Pro AV64C, Ohaus Corporation, New Jersey, USA). Similarly, these variables were measured in the pulp-free capulin seed.

Peel color of fruit from each segregant was determined by evaluating *L* (brightness), hue angle tone (*hue*) and color purity or chromaticity (*chroma*) with a digital colorimeter (Chroma Meter CR-400, model B8210363, Konica Minolta Sensing, Inc., Tokyo, Japan) as described by McGuire (1992).

Total Soluble Solids (TSS) were determined using a refractometer (Hand-Held Refractometer, N-1E, ATAGO,

Tokyo, Japan) and pH using a potentiometer (HI2211 pH/ORP Meter, Hanna Instruments, Woonsocket, RI, USA) as established by the AOAC (2005).

Quantification of minerals

Peeled and seedless capulin fruit from each segregant were dehydrated in a forced convection air oven (Binder®, model KB115 Tuttlingen, Germany) to 60 °C by 48 h. The dried and milled samples were subjected to di acid wet digestion diácida ($H_2SO_4:HClO_4$, 4:1 v/v and H_2O_2) in a Digestor™ (Tecator Kjeltéc FOSS, model DT 220, Hoega-naes, Sweden). The determination of B, Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn was performed according to the methodology described by Alcántar-González & Sandoval-Villa (1999) in an Induction Coupled Plasma – Atomic Emission Plasma Spectrophotometer (ICP-AES, Instrument Varian Liberty series II, Sydney, Australia).

Proximal analysis

Carbohydrate, ash, humidity, crude fiber, protein and lipid contents were determined according to AOAC (2005). The results were expressed as percentage of fresh weight.

Quantification of nutraceuticals

Preparation of methanolic extract. To 1g of fruit pulp with peel from each segregant (fresh and processed) was added 10 mL of aqueous MeOH to 80 % (v/v), the mixture was homogenized by shaking in a vortex (Barnstead International, model M16715, Iowa, USA). Subsequently, it was sonicated (Cole Parmer 8892, Illinois, USA) for 15 min at environment temperature and allowed to settle for 24 h. Finally, it was centrifuged (Cole-Parmer Instrument Company, model 8892, Vernon Hills, IL, USA) at 1 409 g (10 min) for nutraceutical quantification (Román-Cortés et al., 2018).

Determination of phenolic compounds. 0.5 mL of the methanolic extract was taken and 0.5 mL of Folin-Ciocalteu reagent (0.2N) and 4 mL of 0.7 M of Na_2CO_3 , were added,

Table 1. Physical attribute of fruits and seeds of four segregants of capulin (*Prunus serotina*).

Variable	Segregant			
	P5-1F	P5-3F	P5-18F	P5-28F
	<i>Fresh fruit with seed</i>			
Weight (g)	3.04 ± 0.53 b	3.39 ± 0.32 a	2.18 ± 0.28 d	2.73 ± 0.26 c
Equatorial diameter (mm)	17.58 ± 1.27 b	18.83 ± 0.78 a	16.51 ± 0.86 c	16.74 ± 0.57 c
Polar diameter (mm)	15.69 ± 0.78 b	16.29 ± 0.45 a	14.76 ± 0.60 c	15.07 ± 0.52 c
	<i>Seed</i>			
Weight (g)	0.38 ± 0.03 b	0.51 ± 0.06 a	0.32 ± 0.02 c	0.38 ± 0.02 b
Equatorial diameter (mm)	9.57 ± 0.26 b	10.72 ± 0.29 a	9.09 ± 0.28 c	9.48 ± 0.26 b
Polar diameter (mm)	10.84 ± 0.40 c	12.38 ± 0.45 a	10.08 ± 0.32 d	11.77 ± 0.38 b

Values represent the average of 25 replicates ± standard deviation. Means with the same letter, in the same row, are statistically equal (Tukey, 0.05).

the mixture was incubated at environment temperature in darkness by 2 h. Readings were taken on a UV/Vis spectrophotometer (Thermoscientific, Genesys 10s, Florida, USA) at 765 nm. Concentration was calculated from a standard curve ($y = 0.0068x - 0.0003$; $R^2 = 0.995$) based gallic acid (Waterman & Mole, 1994). Total phenolic content was expressed as mg gallic acid equivalents per 100 g of fresh weight (mg EAG · 100 g⁻¹ p.f.).

Quantification of flavonoids. It was carried out following the method reported by Chang et al. (2002). To 0.5 mL of methanolic extract was added 1.5 mL of methanol (95 %), 0.1 mL de AlCl₃ (10 % p/v), 0.1 mL 1 M de CH₃COOK y 2.8 mL of distilled water. The mixture was homogenized and incubated for 30 min to environment temperature in darkness. The absorbance was read at 415 nm in a UV/Vis spectrophotometer. The standard curve ($y = 0.007x - 0.0051$; $R^2 = 0.999$) was constructed based on quercetin. Results were expressed as mg quercetin equivalents in 100 g fresh weight (mg EQ · 100 g⁻¹ p.f.).

Quantification of anthocyanins. It was carried out by means of the differential pH method described by Giusti & Wrolstad (2001). Two 0.2 mL samples methanolic extract were taken; to the first one 1.8 mL of a buffer solution pH = 1.0 (KCl) was added to the second one a buffer solution pH = 4.5 (CH₃COOH/CH₃COONa · 3H₂O) was added. Both samples were measured for absorbance at 510 and 700 nm. The total absorbance (A_T) was calculated from the formula: $A_T = [(A_{510} - A_{700})_{pH=1.0}] - [(A_{510} - A_{700})_{pH=4.5}]$. The anthocyanin concentration was calculated by the equation: $\text{Anthocyanins}(\text{mg} \cdot \text{L}^{-1}) = (A_T \cdot \text{PM} \cdot \text{FD} \cdot 1000) / (\epsilon \cdot 1)$; where: A_T=total absorbance, P.M = molecular weight (449.2 g · mol⁻¹) of cyanidin-3-glucoside, FD = dilution factor (10), ϵ = molar absorptivity of the standard (26 900). Concentration was expressed as mg cyanidin-3-glucoside per 100 g fresh weight of capulin.

Quantification of vitamin C (ascorbic acid). It was determined in pulp with peel of fresh and processed fruits of the four segregants, following the methodology described by Dürüst et al. (1997). For the preparation of the extract, 1 g of plant material was placed in 10 mL of 0.4 % (p/v) oxalic acid. The mixture was sonicated for 15 min at environment temperature, then filtered. One mL of the extract was mixed with 1 mL of acetate buffer pH = 3 (3 g anhydrous sodium acetate in 7 mL water and 10 mL glacial acetic acid) and 8 mL dichloroindophenol (from a 12 mg · L⁻¹ aqueous solution), after 15 s, absorbance was measured at 520 nm in a spectrophotometer. Results were expressed as mg ascorbic acid per 100 g of fresh weight (mg EAA · 100⁻¹ p.f.), using an ascorbic acid standard curve ($y = 0.004x + 0.0011$; $R^2 = 0.997$).

Evaluation of antioxidant capacity. To 10 mL solution of the ABTS radical ^{•+}(acid 2,2'-azinobis (3-ethylbenzothiazolin)-6-sulfonic acid), 6.61 mg of K₂S₂O₄ was

added, the mixture was allowed to stand at environment temperature in darkness for 16 h (Re et al., 1999). 1 mL of the ABTS radical was taken and absolute ethanol was added until an absorbance of 0.7 ± 0.01 a was obtained at a wavelength of 734 nm. To 1 mL of the ABTS radical, 10 µL of the extract to be analyzed was added and the mixture was incubated at 30 °C in darkness for 7 min. Finally, absorbance reading at 734 nm was taken. A standard curve ($y = -0.2895x + 0.7583$; $R^2 = 0.9956$) was prepared based on trolox. The results were expressed in mg equivalents of trolox per 100 g of fresh weight (mg ET · 100 g⁻¹ p.f.). To calculate the percentage inhibition of the free radical ABTS ^{•+} the formula was used: % inhibition = $[(A_0 - A_F) / A_0] \cdot 100$, where: A₀ = initial absorbance of the free radical at 734 nm, A_F = final absorbance of the reaction with the sample.

Results and Discussion

Physical-chemical properties

Significant differences ($P \leq 0.05$) were found between the physicochemical characteristics of the fruits of the four segregants. The P5-3F segregant presented significantly higher fruit weight and size, the smallest and lightest fruits were found in the P5-18F segregant. The same trend was found for seed weight and size (Table 1). Information on the morphological characteristics of the Mexican capulin fruit is limited; however, Hernández Rodríguez et al. (2019) report lower values of weight, polar and equatorial diameters (< 2.8 g, 1.1 cm y 1.2 cm, respectively) of fruits at consumption maturity of *P. Serotina* collected in Zacatlán, Puebla, Mexico, the differences are probably due to their wild nature.

Fruits of segregant P5-1F obtained the highest significant values of total soluble solids (TSS °Brix) and pH, therefore, they were the fruits with the lowest sourness and probably the sweetest. Fruits of segregants P5-3F, P5-18F and P5-28F showed no significant differences in TSS content, while fruits of the segregant P5-3 obtained the lowest pH and TSS values (Table 2). According to Baxter et al. (2005), the increased sucrose discharged from the phloem is the main factor causing differences in soluble solids content (TSS) among fruits of several plants of the same species. This could explain the higher TSS content in the P5-1F segregant. On the other hand, the pH values obtained in this research were higher than those reported by Ordaz-Galindo et al. (1999) (pH 3.96) and Jiménez et al. (2011) (pH 4.20) in fresh pulp of the same species (*Prunus serotina* subsp *capuli*), this could be due to genetic variability (Ballistreri et al., 2013).

Significant differences ($P \leq 0.05$) were found in the color parameters (*hue* angle of tone, *chroma* and brightness) of the fruit peel (Table 2). Fruits of segregant P5-1F showed the significantly higher values of *chroma* and *hue* angle

of tone (17.16 and 28.35, respectively), which identified them as fruits with higher intensity of orange. Fruits of segregant P5-28F showed the statistically lowest values of *chroma* and *hue* (6.18 and 20.95, respectively), which identified them as redder and lower color intensity fruits. Brightness values allowed dividing the fruits of the four segregants into two groups, the significantly lighter fruits (P5-1F and P5-3F) and the darker fruits (P5-18F and P5-28F). Peel color is the most important attribute of quality and maturity in capulin fruits, associated with the presence of anthocyanins (Hernández Rodríguez et al., 2019; Jimenez et al., 2011).

Mineral content

Significant differences ($P \leq 0.05$) in mineral content were observed among the fresh fruits of the four segregants (Table 4). Segregant P5-1F presented the statistically higher concentration of P, K and Mg, in contrast, fruits of segregant P5-28F showed significantly higher Na, Ca, Fe and Cu contents; segregants P5-1F, P5-28F and P5-3F obtained the highest B contents (Table 3). Luna-Vázquez et al. (2013) reported higher values of K ($184.30 \pm 3.50 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ p.f.}$) and Na ($22.40 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ p.f.}$) in capulin fruits harvested in Huejotzingo, Puebla, Mexico; as well as, lower contents of P ($28.10 \pm 0.40 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ p.f.}$) and Ca ($12.90 \pm$

$1.90 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ p.f.}$); however, the Mg content ($21.20 \pm 0.40 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ p.f.}$) was similar to that obtained in the present research. The differences found with respect to the values reported in other investigations are mainly due to edaphoclimatic factors of the place of origin of the capulin harvest. Regarding the differences found in mineral concentrations among the four segregants, they could again be attributed to genetic differences as has been reported in other fruits and some vegetables (Reynoso-Camacho et al., 2006). There are no studies carried out on the variation of mineral content in capulin fruits in relationship to these factors, the present investigation is a contribution of mineral content in segregants. Therefore, the obtained results suggest that the consumption of capulin fruits could be an economical alternative for mineral intake in the population.

Proximal analysis

After fruit processing (40 °C for 5 min), ash content (minerals in the food) decreased only in three segregants P5-1P and P5-28P, as well as humidity content (Table 4); therefore, there was a loss of minerals (leachates) by transfer to the cooking water. According to Yagmur & Taskin (2011), most minerals in fruits and vegetables are water soluble, so it is common for these nutrients to pass from the tissue

Table 2. Total soluble solids content (°Brix), pH and color attributes in fresh fruit of four segregants of capulin (*Prunus serotina*).

Segregant	Total Soluble Solids (°Brix)	pH	Brightness (%)	Angle of tone Hue (°)	Chroma
P5-1F	15.88 ± 0.97 a	5.40 ± 0.18 a	23.34 ± 1.46 a	28.35 ± 3.82 a	17.16 ± 2.98 a
P5-3F	14.84 ± 1.43 b	4.21 ± 0.16 d	22.75 ± 1.69 a	19.38 ± 3.95 b	11.64 ± 3.82 b
P5-18F	14.92 ± 0.88 b	4.78 ± 0.16 b	21.22 ± 0.89 b	18.02 ± 2.93 b	9.44 ± 3.17 b
P5-28F	15.37 ± 0.88 ab	4.42 ± 0.20 c	20.95 ± 0.63 b	17.68 ± 2.68 b	6.18 ± 1.91 c

Values reported are the mean of 25 replicates ± standard deviation. Means with the same letter, in the same column are statistically equal (Tukey, 0.05).

Table 3. Mineral content (mg 100 g⁻¹ p.f.) in the fresh fruit pulp of four segregants of capulin (*Prunus serotina*).

Mineral	Segregant			
	P5-1F	P5-3F	P5-18F	P5-28F
P	40.28 ± 0.34 a	35.87 ± 0.24 c	35.85 ± 0.39 c	37.13 ± 0.41 b
K	106.72 ± 0.38 a	100.46 ± 0.46 c	104.99 ± 0.26 b	96.31 ± 0.17 d
Ca	22.63 ± 0.36 b	21.19 ± 0.25 c	16.43 ± 0.32 d	23.82 ± 0.43 a
Mg	24.57 ± 0.07 a	22.34 ± 0.21 b	16.97 ± 0.12 d	19.67 ± 0.42 c
Na	21.03 ± 0.39 ab	20.19 ± 0.42 b	17.45 ± 0.42 c	21.63 ± 0.05 a
Fe	0.67 ± 0.08 b	0.63 ± 0.00 b	0.53 ± 0.00 b	0.88 ± 0.04 a
Mn	0.16 ± 0.00 b	0.17 ± 0.00 b	0.20 ± 0.00 a	0.20 ± 0.01 a
Zn	0.28 ± 0.00 b	0.34 ± 0.00 a	0.30 ± 0.00 b	0.32 ± 0.00 a
Cu	0.02 ± 0.00 d	0.04 ± 0.00 b	0.03 ± 0.00 c	0.07 ± 0.00 a
B	0.99 ± 0.03 a	1.04 ± 0.00 a	0.90 ± 0.01 b	1.01 ± 0.02 a

Values represent the mean of 3 replicates; ± standard deviation. Means with the same letter, in the same row, are statistically equal (Tukey, 0.05).

Table 4. Proximal analysis (%) of the fresh fruits (F) and processed (P) fruits of four segregants of capulin (*Prunus serotina*).

Segregantng	Ashes	Humidity	Protein	Carbohydrates	Crude fiber
P5-1 F	0.771 ± 0.016 d	79.45 ± 0.26 b	1.79 ± 0.46 b	17.95 ± 0.25 ab	3.99 ± 0.06 ab
P 5-3 F	0.954 ± 0.004 a	81.21 ± 0.26 a	2.37 ± 0.01 a	15.52 ± 0.11 d	3.45 ± 0.06 d
P 5-18 F	0.911 ± 0.008 b	80.98 ± 0.66 a	1.82 ± 0.09 b	16.33 ± 0.76 cd	3.63 ± 0.17 cd
P5-28 F	0.912 ± 0.011 b	79.92 ± 0.02 b	1.83 ± 0.01 b	17.31 ± 0.10 bc	3.85 ± 0.01bc
P5-1 P	0.684 ± 0.008 e	79.26 ± 0.11 b	1.82 ± 0.01 b	18.21 ± 0.11 ab	4.04 ± 0.02 ab
P 5-3 P	0.856 ± 0.013 c	81.33 ± 0.09 a	1.17 ± 0.02 d	16.52 ± 0.09 cd	3.67 ± 0.02 cd
P5-18 P	0.841 ± 0.015 c	81.24 ± 0.16 a	1.40 ± 0.02 c	16.42 ± 0.13 cd	3.64 ± 0.03 cd
P5-28 P	0.821 ± 0.011c	78.95 ± 0.16 b	1.47 ± 0.02 c	18.74 ± 0.17 a	4.16 ± 0.04 a

Values represent the mean of 3 replicates; ± standard deviation. Means with the same letter, in the same column, are statistically equal (Tukey, 0.05).

to the process water; the external diffusion of minerals during cooking depends on the level of physical damage to the plant tissues and increases with thermal treatment in the cooking water; factors such as pH level, temperature, water-nutrient ratio, exposed surface area, among other factors affect mineral losses in the final product.

On the other hand, the fresh fruits of the four capulin segregants are an important source of carbohydrates, crude fiber, and protein (Table 4). No significant differences were found in fresh lipid content (F). Lipid content in all segregants was less than 0.03 % and therefore for l it was not reported in Table 5.

The P5-1F fruits had the highest carbohydrate and crude fiber concentrations among the studied segregants. Fruits of segregant P5-3F had the highest crude protein concentration. In this regard, Luna-Vázquez et al. (2013) reported lower carbohydrate values, similar crude fiber and higher protein values in capulin fruits (*P. serotina* subssp. *capuli*) (12.23, 3.58 and 2.10 %, respectively); but the fruit of the studied segregants presented higher values of carbohydrates and protein compared to those reported in cherry (*Prunus domestica*) (8.28 and 0.49 %, respectively) and grape (*Vitis vinifera*) (13.96 and 0.46 %, respectively).

Therefore, this fruit is a source of nutrients at low cost. It is important to highlight that the crude fiber value found in capulin of the studied segregants, between 18.36 to 19.41 % in dry weight, is higher than that reported by Blejan et al. (2023) in some by-products (dry mixture of peels, seeds and residual pulp after juice removal) of wild blueberries (*Vaccinium myrtillus* L.) and blackcurrants (*Ribes nigrum* L.) (11.84 and 15.50 % dry weight, respectively). Foods rich in fiber provide health benefits for the prevention and reduction of the risk of chronic diseases; the consumption of crude fiber has a laxative effect, that is why it is recommended by specialists to people suffering from constipation (Ioniță-Mîndrican et al., 2022). Regarding the effect of thermal treatment on carbohydrate and crude fiber contents, processed fruits (P) showed

higher concentrations compared to that of fresh fruit (F) (Table 4), due to the concentration of these nutrients as well as water loss from the fruit during thermal treatment. According to Ramalakshmi et al. (2021), nutrient loss during cooking depends on the temperature, length of treatment and the nutrient involved; carbohydrate loss during cooking is generally small and only after several minutes of cooking and at temperatures close to 100° C.

Finally, it is important to note that a reduction of up to 25 % in protein content was observed in processed fruits (P) with respect to fresh capulin (Table 4), with the exception of the fresh and processed segregant (P5-1F and P5-1P) whose content was statistically equal in both conditions. Considerable loss of soluble nutrients substances when dissolved or leached in the cooking water, such as proteins, water-soluble minerals and vitamins, is common (Deng et al., 2019).

Nutraceuticals content

Significant differences ($P \leq 0.05$) were found in the concentration of phenolic compounds, anthocyanins, flavonoids and vitamin C among the fresh fruits of the four segregants. Fresh fruit (F) of segregants P5-28F and P5-3F showed the highest concentrations of anthocyanins and flavonoid compounds. Regarding the concentrations of total flavonoids and vitamin C, the values were similar among all segregants, except for P5-1 F which presented the lowest concentrations of these metabolites (Table 5). Anttonen & Karjalainen, (2005) report that the content of phenolic components can vary significantly among cultivars of a species due to gene expression related to the biosynthesis of some metabolites in response to changes in the crop environment. Phenolic components in addition to their antioxidant capacity possess other mechanisms of action that explain their diverse beneficial effects on consumers (Potì et al., 2019).

Phytochemical studies of cultivars, varieties or segregants of a species allow planning breeding strategies, as

Chart 5. Content of nutraceutical compounds in fresh fruit (F) and processed (P) of four capulin segregants (*Prunus serotina*) by each 100 g of fresh weight.

Segregant	Phenolic Compounds (mg EAG)	Flavonoids (mg EQ)	Anthocyanins (mg ECyd-3-Gli)	Vitamin C (mg EAA)
P5-1 F	96.42 ± 3.09 e	28.39 ± 0.18 e	9.05 ± 0.20 e	33.87 ± 0.24 c
P5-3 F	331.57 ± 4.09 b	50.49 ± 0.83 a	19.69 ± 0.19 ^a	40.06 ± 0.55 a
P5-18 F	228.84 ± 5.95 d	48.68 ± 1.47 ab	16.46 ± 0.61 c	42.01 ± 0.93 a
P5-28 F	341.27 ± 3.09 b	50.25 ± 0.44 a	18.54 ± 0.32 ab	40.46 ± 0.77 a
P5-1 P	104.6 ± 2.14 e	31.30 ± 0.31d	8.44 ± 0.24 e	33.52 ± 0.34 c
P5-3 P	390.69 ± 3.24 a	49.58 ± 0.53 a	16.57 ± 0.34 c	40.06 ± 0.62 a
P5-18 P	305.27 ± 2.14 c	46.44 ± 0.53 b	10.77 ± 0.24 d	36.61 ± 0.40 b
P5-28 P	388.84 ± 0.86 a	42.01 ± 0.12 c	17.48 ± 0.29 bc	36.69 ± 0.54 b

Values represent the mean of 3 replicates; ± standard deviation. Means with the same letter, in the same column, are statistically equal (Tukey, 0.05). EAG: gallic acid equivalents, EQ: quercetin equivalents, ECyd-3-Gli: cyanidin-3-glucoside, EAA: ascorbic acid equivalents.

well as selecting individuals with high content of active ingredients or commercial interest as natural colorants, nutraceutical ingredients, and antioxidants for the food industry or to improve the content of healthy compounds in capulin fruits.

It is important to note that the values of phenolic compounds and flavonoids (4.69-17.64 mg EAG · g⁻¹ y 1.38-2.68 mg EQ · g⁻¹ p.s., respectively) found in the four studied segregants and transformed in the same concentration units were lower than those reported by Hernández Rodríguez et al. (2019) in wild capulin collected in Zacatlán, Puebla, Mexico (14.40-26.96 mg EAG · g⁻¹ p.s., 16.56-9.23 mg EQ · g⁻¹ p.s. and 0.04-0.66 mg cyanidin-3-glucoside (C-3-G) · g⁻¹ p.s.); in contrast, higher anthocyanin concentrations (0.44-1.05 mg C-3-G equivalents per g, dry weight) were found in all the studied segregants. Ordaz-Galindo et al. (1999) reported anthocyanin values in capulin fruits (*Prunus serotina*) (31.7 mg equivalents of cyanidin -3-glycoside · 100 g⁻¹ p.f.) similar to those reported in the present research.

On the other hand, the concentration of flavonoids found in all the segregants was lower than that of total phenolic components, possibly because some flavonoids could be found forming procyanidins (condensed tannins) as in other fruits (Cui et al., 2006).

The vitamin C content was statistically equal among the fresh fruits of segregants P5-3F, P5-18F and P5-28F, the lowest value of this metabolite was present in the fresh fruits of segregants P5-1F. There are no studies on the content of this vitamin in capulin.

On the other hand, thermal treatment had a significant effect on the content of nutraceutical compounds among the fruits of the four segregants. The content of phenolic components obtained a significant increase in the fruits of the four segregants post-thermal treatment (17 %), which

could be due to the concentration of these nutrients by the loss of water from the fruit during thermal treatment, as was observed in carbohydrates.

Segregants P5-3F and P5-18F showed no significant differences in flavonoid content after thermal treatment, while segregant P5-28P showed the greatest decrease in these metabolites. Flavonoids are also phenolic compounds with antioxidant potential found in vegetables and fruit; in the latest two decades epidemiological studies have shown a relationship between flavonoid consumption and the low incidence of degenerative diseases (Toh et al., 2013). The mechanisms of action of each group of phytochemicals in capulin are unknown, but the synergistic effect of these bioactives make it a food with notable functional properties, mainly the fruits of the segregant P5-3F and P5-28F.

The effect of the thermal treatment also resulted in a significant average decrease of 16 % of anthocyanin content in P5-3F and P5-18F segregants (Table 5). Anthocyanin levels may be affected by the temperature of process. Oliveira et al. (2010) observed a reduction in anthocyanin content in blueberries between 12 and 42 % during progressive heating from 12° to 99 °C for 60 min, the same phenomenon was found in the thermal treated capulin studied in the present research.

Vitamin C content (Table 6) showed significant differences only between fresh and processed fruits of segregants P5-18F and P5-28F, where a decrease in content was generated (12.8 and 9.3 %, respectively). The vitamin C content in fresh capulin fruits of the four analyzed segregants was higher than that reported by Garcia et al. (2006) in banana (8 – 16 mg · 100 g⁻¹) and green apple (3-30 mg · 100 g⁻¹). Vitamin C plays a very important role in human metabolism, it is essential for the development and function of the nervous system, it is part of the mechanisms of cicatrization, biosynthesis of collagen and different neurotransmitters (Kükürt & Gelen, 2024).

Table 6. Antioxidant capacity in fresh (F) and processed fruits (P) of four segregants of capulin (*Prunus serotina*).

Segregant	Antioxidant Capacity ($\mu\text{ mol} \cdot \text{ET}100 \text{ g}^{-1} \text{ pf}$)	Inhibition (%)
P5-1 F	1326.084 \pm 47.27 c	54.17 \pm 2.17 b
P5-3 F	1800.81 \pm 23.23 b	92.25 \pm 3.28 a
P5-18 F	1390.40 \pm 2.88 c	57.13 \pm 0.13 b
P5-28 F	2154.05 \pm 71.48 a	95.77 \pm 1.13 a
P5-1 P	1816.94 \pm 8.81 b	91.36 \pm 0.40 a
P5-3 P	2252.06 \pm 22.22 a	96.76 \pm 1.02 a
P5-18 P	2145.89 \pm 76.11 a	91.87 \pm 3.50 a
P5-28 P	2134.66 \pm 8.78 a	97.84 \pm 0.43 a

Values represent the mean of 3 replicates; \pm standard deviation. Means with the same letter, in the same column, are statistically equal (Tukey, 0.05). ET: trolox equivalents.

Finally, it is important to point that the fresh fruits of the P5-3F segregant were the ones in which nutraceutical compounds were less affected to the thermal treatment (except for the concentration of anthocyanins), which corresponded with higher antioxidant activity (Table 6), for this reason, it is recommended as a segregant with potential to be processed.

Antioxidant capacity

The antioxidant capacity of the fresh and processed fruits of the four segregants showed significant differences ($P \leq 0.05$) (Table 6). The highest antioxidant capacity values were observed in the fresh fruit of segregant P5-28F, followed by P5-3F. The fresh fruits of these segregants had the highest content of anthocyanidins, phenolic compounds, flavonoids and vitamin C. The processed fruits of the four segregants showed an increase of 28 % in the antioxidant capacity content; as well, in their average of free radical inhibitory capacity (34 %), which could be explained by the increase of phenolic compounds (17 %) as mentioned above in the present study. The antioxidant capacity obtained in fresh and processed fruits of the four capulin segregants ($1326.08 \pm 47.27 - 2252.06 \pm 22.22 \mu\text{mol ET} \cdot 100 \text{ g}^{-1} \text{ p.f.}$) was similar to the values reported by Luna-Vázquez et al. (2013) ($1455.2 \pm 92.5 - 2056.7 \pm 108.0 \mu\text{mol ET} \cdot 100 \text{ g}^{-1} \text{ p.f.}$) in fresh fruits of the same species. On the other hand, similar research carried by García-Mateos et al. (2013) on 20 different tejocote genotypes and Ballistreri et al. (2013) on 24 sweet cherries (*P. avium*), varieties showed that genetic factor could explain variations in the nutraceutical characteristics of capulin segregants.

Conclusions

The capulin fruit of the four studied segregants is a source of nutrients (protein, fiber, carbohydrates, P, K, Ca, Mg and Fe) and antioxidant compounds (phenolics, anthocyanins and vitamin C) at a low cost. The fruit of segregant P5-1F had the highest carbohydrate content, total soluble solids

and pH, important quality attributes for marketing a fruit; however, it had the lowest nutraceutical value in the fresh and processed condition. In contrast, the fruits of P5-3 segregant had the highest protein values and nutraceutical potential due to their high concentrations of phenolic compounds, flavonoids, anthocyanins, vitamin C and antioxidant activity in its fresh (P5-3F) and processed (P5-3P). Fresh fruits from segregants P5-3F and P5-28F were statistically superior in anthocyanin and flavonoid content, sharing this superiority in vitamin C content with fresh fruits from segregant P5-18F. The fresh fruits of the P5-28F showed the highest antioxidant capacity, associated with their high concentration of nutraceutical compounds. The factors thermal treatment (40 °C for 5 min) and capulin segregant had a joint significant effect on the nutritional and nutraceutical value of fresh and processed fruits. The fruits of P5-3P segregant tolerated the thermal treatment the most, so the fruits showed the least effects in the nutraceutical components and the highest value of antioxidant activity, for this reason it is recommended as a segregant with potential to be processed in order to generate added value.

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English version

Dendrochronology in Mexico over the last seventy years

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Abstract

The study of dendrochronology has intensified in recent years due to its close relationship with addressing environmental issues. Therefore, the objective of this study was to analyze the development of dendrochronological research in Mexico through a comprehensive literature review, which included search engines, websites, and publications from national and international journals. The collected information was organized into a database and classified into nine categories to analyze study frequencies, species, and locations. A total of 229 documents published between 1944 and 2021 were identified, with a significant increase during the period from 2009 to 2021, when dendroclimatology emerged as the most studied subdiscipline. In total, 429 different chronologies were documented, primarily distributed in the Sierra Madre Occidental. The best-represented reconstructed period spanned from 1801 to 2019. The most frequently studied species were *Pseudotsuga menziesii* and *Pinus hartwegii*. The most common application of this science has been precipitation reconstruction. Due to its extensive biodiversity, Mexico has significant potential for dendrochronological research. However, it is essential to expand both the physical and intellectual boundaries of this field and broaden its spatial scope within the country.

► **Keywords:** tree rings, chronologies, dendroclimatology, dendroecology, climate reconstruction.

Introduction

The development of forest ecosystems is primarily determined by environmental factors. When these factors align with optimal conditions, tree growth improves; conversely, under adverse conditions, growth tends to be limited (Villanueva-Díaz et al., 2011). The radial growth of woody forest species, particularly conifers, leads to the formation of growth rings. These anatomical structures inherently record temporal variability and influence of factors such as climate, wildfires, pests, diseases, geomorphological processes,

competition, air pollution, and human management, among others (Franco-Ramos & Vázquez-Selem, 2017). Among these factors, climate has the greatest influence on natural forests (Rojas-García et al., 2020).

Dendrochronology is a science that examines temporal variability of physical, structural, and compositional changes in tree growth rings and their relationship with environmental conditions of the sites where they develop. This discipline makes it possible to reconstruct and study a part of the environmental history underlying the development of the

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trees, as it allows for the analysis of various climatic variables and ecological processes spanning from hundreds to thousands of years (Douglas, 1941).

Dating growth rings of species sensitive to specific environmental conditions has become a trusted tool for the historical analysis of these conditions, as it offers a wider temporal perspective than instrumental records (Villanueva-Díaz et al., 2002). Depending on the approach and application of dendrochronological series, this science is categorized into various fields, including dendroclimatology, dendroecology, dendropyrology, dendrochemistry, dendrogeomorphology, dendrohydrology, dendrovolcanology, and dendroarchaeology (Amoroso & Suarez, 2015).

The study of dendrochronology in Mexico began in the 1940s with the work of Schulman (1944). In the early 1960s, new chronologies were established during the expedition to Casas Grandes in Chihuahua, where a notable 500-year floating chronology was developed (Bannister & Scott, 1964). In the 1970s, the “Mexican Tree Ring Project” led to the development of several chronologies (Therrell, 2003), although their application has remained limited (Villanueva-Díaz et al., 2011). During this time, Naylor (1971) conducted a preliminary study in Oaxaca, but was unable to generate successful chronological advances.

At the beginning of the 21st century, the global interest in understanding the historical variability of climate and other environmental factors influencing forest dynamics (such as wildfires and atmospheric circulation phenomena) spurred the study of dendrochronology in Mexico. This was achieved through technical and financial contributions of international organizations (Villanueva-Díaz et al., 2002). In the last two decades, the use of multiple forest species with dendrochronological potential across various approaches has demonstrated Mexico’s viability for the development of this science (Reyes-Basilio et al., 2020). These advancements have positioned the country as one of the leading nations in dendrochronological research in Latin America (Fo et al., 2009).

In this context, the objective of this article was to analyze the spatial-temporal evolution of dendrochronology studies in Mexico through a comprehensive literature review and the analysis of relevant scientific products. The goal was to generate information that provides a comprehensive understanding about the application of this science in the country, to diagnose research needs and opportunities, and to provide solid bases for decision-making for the development of dendrochronology.

Materials and methods

For this study, scientific publications (research articles) as well as gray literature (books and chapters, conference proceedings, technical brochures, and preprints) from

1944 to 2021 were considered. The latter, although less formal, represent relevant contributions from the scientific community that, directly and indirectly, are related to the progress of dendrochronology in Mexico (Montes de Oca-Montano, 2018). Information was searched and obtained through specialized search engines such as Science Direct, Scopus, Wiley Online Library, and Springer; additionally, websites like Google Scholar, Redalyc, SciELO, and PubMed were consulted. Special attention was paid to the contents of the main national forestry journals: *Revista Mexicana de Ciencias Forestales*, *Madera y Bosques*, *Revista Chapingo Serie Ciencias Forestales y del Ambiente*, *Agrociencia*, and *Investigaciones Geográficas*, as well as high-impact international journals: *Dendrochronologia*, *Tree-Ring Research*, *Trees y Forest*.

For the search, the following keywords were used in both Spanish and English: “dendrochronology”, “growth rings”, “Mexico”, “dendroclimatology”, “dendroecology”, “radial growth”, “chronologies”, “climatic reconstruction”, “tree rings”, “fire scars” y “paleoclimatology”, as well as their combinations using the logical operator AND. As inclusion criteria, all publications referring to dendrochronology in Mexico were considered.

The information collected was organized into a database in Microsoft Excel®, using macros to semi-automate the process. The information fields corresponded to two criteria: documents and content. The first criterion included a unique identifier composed of consecutive numbers, title, year of publication, journal, authors and institution of origin. For the content of the documents, the following was considered: study site (entity, latitude, longitude and altitude), species studied, type of vegetation, variable measured in the rings (ring width, earlywood, latewood, chemical composition, fire scars or geomorphic processes, among others), chronology range and period, reconstructed variable, and range and period of reconstruction.

Subsequently, the information was classified into nine categories based on the analysis of growth ring chronologies: dendroclimatology, dendroecology, dendropyrology, dendrochemistry, dendrogeomorphology, dendrohydrology, dendrovolcanology, dendroarchaeology, and others (which included systematic reviews, meta-analyses, sites suitable for dendrochronological studies, and reconstruction of agro-food variables). Each category was analyzed based on frequencies, species, and locations studied.

Results and discussion

A total of 229 documents related to dendrochronology in Mexico were identified. Of these, 90 % (206) were scientific articles, while books or book chapters accounted for 6.6 % (15), proceedings from scientific dissemination events made up 3 % (seven), and preprints represented 0.4 % (one).

The scientific articles were published in a wide range of indexed journals. A total of 83 different journals were identified, mainly foreign (74.4 %). This reflects the quality and scientific rigor of research in this field in Mexico, because these articles met the requirements for publishing their results in leading journals. More than half (53.66 %) of the published articles appeared in foreign journals, primarily from the United States (Figure 1). The national journals with the highest number of publications were *Revista Mexicana de Ciencias Forestales, Madera y Bosques*, and *Revista Chapingo Serie Ciencias Forestales y del Ambiente*.

Between 1944 and 2021, scientific production on dendrochronology in Mexico concentrated in 33 years. The average production during this period was 2.66 articles per year (205 articles in total). Although the first studies were conducted in the 1940s (Schulman, 1944), the initial uncertainty about Mexico's potential for the development of this discipline led to intermittent progress and the generation of a limited number of chronologies throughout the 20th century (Bannister & Scott, 1964; Villanueva-Díaz et al., 2000; Naylor, 1971). In contrast, the 21st century saw a significant shift in the research trend of this science.

Three periods of scientific production in dendrochronology in Mexico were identified (Figure 2). The first period (from 1944 to 1998) showed limited development and low productivity in dendrochronological research in the country. During these 54 years, only 12 scientific products

were recorded (5 % of the total). According to Villanueva-Díaz et al. (2000), just over 40 chronologies were generated during this period.

In the second period (from 1999 to 2008), a substantial improvement was observed, with 17 % (39) of the 229 scientific products found published. Villanueva-Díaz et al. (2009) classify this period as one in which the knowledge of the application of dendrochronology in Mexico was strengthened. This resurgence was driven by four factors: 1) growing scientific interest in climate elements and their temporal variation, atmospheric circulation phenomena (Villanueva-Díaz et al., 2002), temporal availability of water resources (Villanueva-Díaz et al., 2007), and influence of other environmental factors on natural resources; 2) the establishment of a dendrochronology laboratory in Mexico; 3) national and international funding for research projects (Villanueva-Díaz et al., 2008); and 4) collaboration and technical support of international organizations (Villanueva-Díaz et al., 2011).

In the third period (from 2009 to 2021), the rise of dendrochronology in Mexico was consolidated, with 77.7 % (178) of the scientific products considered in this study being published.

The temporal analysis identified nine categories or research approaches, with the results emphasizing the predominance of studies focused on the relationship

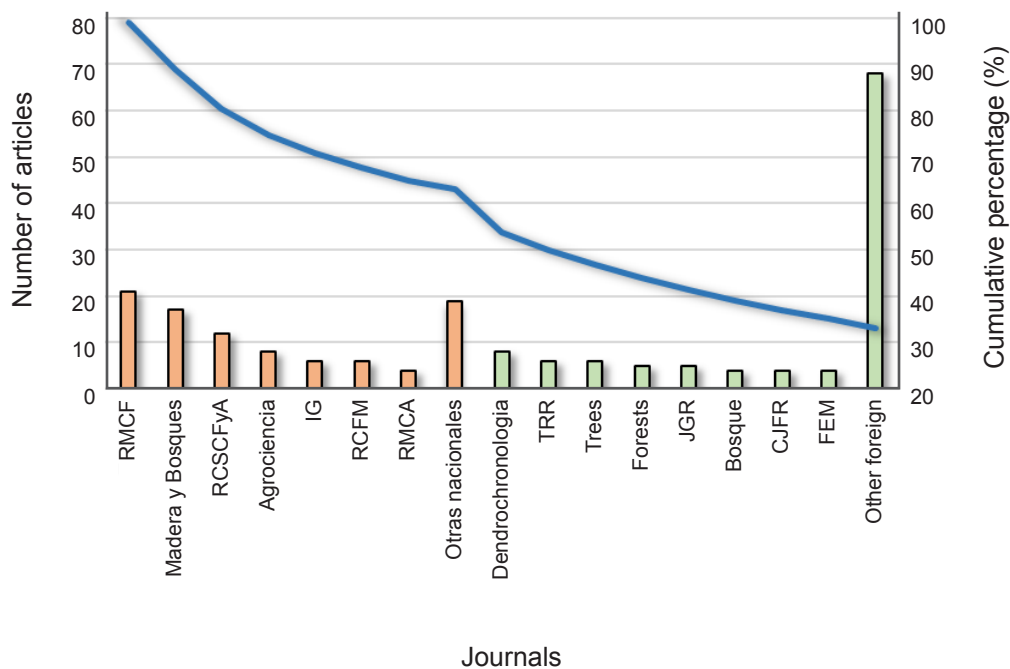


Figure 1. Distribution of the number of articles and national (orange) and foreign (green) journals that have published research on dendrochronology in Mexico. The blue line reflects the cumulative percentage. RMCF: *Revista Mexicana de Ciencias Forestales*; RCSCFyA: *Revista Chapingo Serie Ciencias Forestales y del Ambiente*; IG: *Investigaciones Geográficas*; RCFM: *Revista de Ciencias Forestales en México*; RMCA: *Revista Mexicana de Ciencias Agrícolas*; TRR: *Tree-Ring Research*; JGR: *Journal of Geophysical Research*; CJFR: *Canadian Journal of Forest Research*; FEM: *Forest Ecology and Management*.

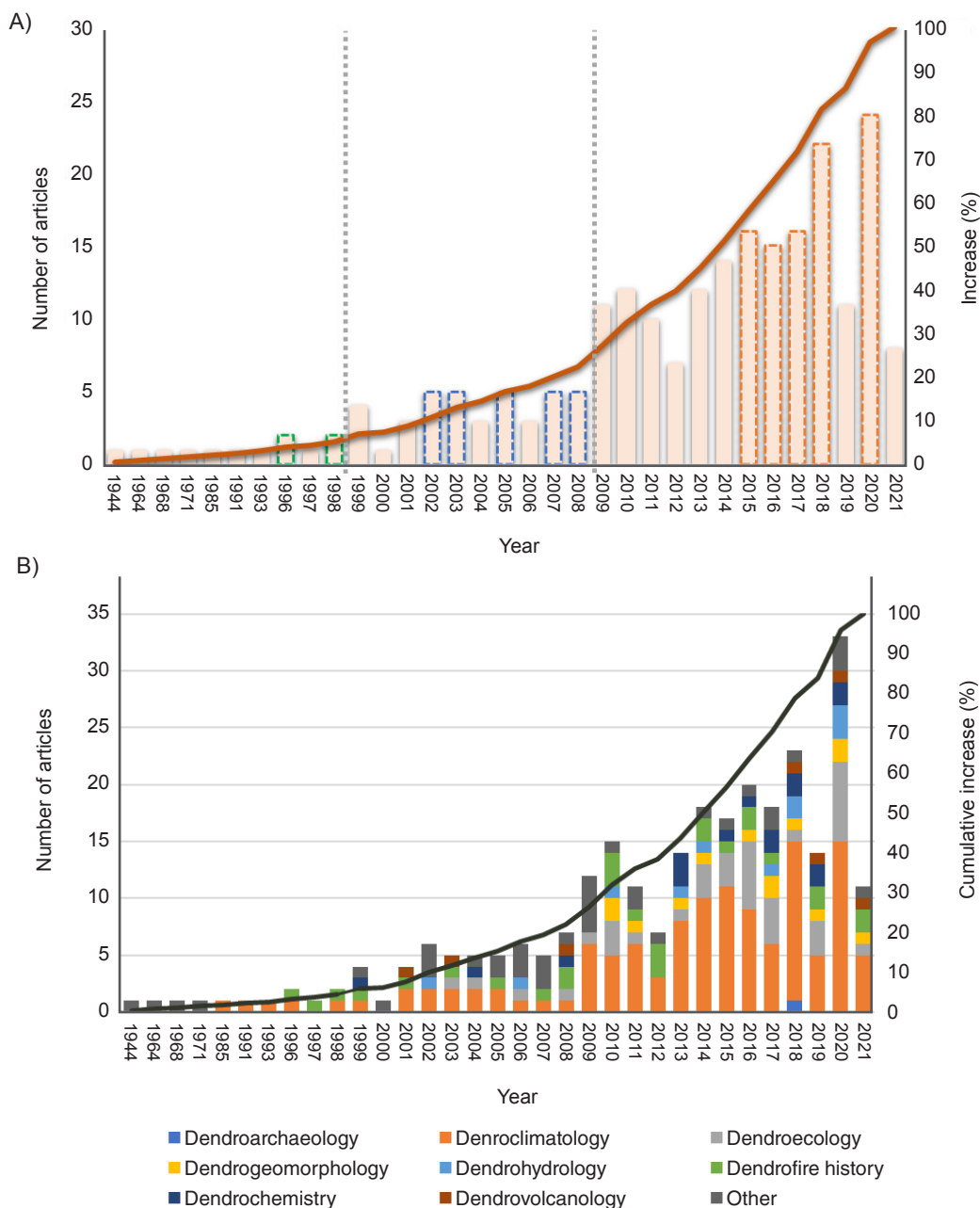


Figure 2. Scientific production related to dendrochronology in Mexico: A) three periods of scientific production (highlighted bars indicate outstanding production) and B) production according to the dendrochronology category.

between ring width and climate variables. Other approaches include research on the influence of environmental variables other than climate (Grissino-Mayer et al., 2005), dendrochronological potential, dissemination of methodologies, and innovation studies (Figure 2B).

Dendroclimatology

Dendroclimatology is based on the physiological ability of certain tree species to record climatic variability in their growth rings, making them genuine meteorological bio-stations (Cerano-Paredes et al., 2016, 2021). This physiological ability allows them to record dominant climate

conditions, as well as interannual variations at a specific spatial scale and with seasonal resolution (Manzanilla-Quñones et al., 2020). Dendrochronological methods help detect the relationship between ring width and climate variables, leading to the emergence of dendroclimatology. This subdiscipline is essential for analyzing global climate variability, the influence of atmospheric circulation phenomena, and their effects on natural resources (Cerano-Paredes et al., 2011; Gómez-Guerrero et al., 2013).

In Mexico, dendroclimatology represents the most developed approach within this science, with 53 % (122) of the scientific products analyzed. Research is mainly

concentrated in the north and center of the country and has made it possible to evaluate how climatic variables influence forest development in these regions. In addition, they have made possible the reconstruction of climatic variables, such as seasonal precipitation and seasonal rainfall. (Cardoza-Martínez et al., 2018; Cerano-Paredes et al., 2011, 2021; Chávez-Gándara et al., 2017; Díaz-Ramírez et al., 2016; Irby et al., 2013; Manzanilla-Quiñones et al., 2018; Villanueva-Díaz et al., 2007, 2008, 2009).

Other climatic variables, such as temperature (Villanueva-Díaz et al., 2020) and evaporation (Pompa-García et al., 2013), have also shown a relationship with the development of growth rings. This connection has facilitated the identification of extreme events, such as drought occurrences (Martínez-Sifuentes et al., 2019).

The influence of atmospheric circulation patterns on the climatic variability of Mexico has generated significant interest (Pompa-García et al., 2015). These dendroclimatic studies have enabled the analysis of phenomena such as El Niño-Southern Oscillation (ENSO) (Cerano-Paredes et al., 2011; Pompa-García et al., 2013, 2015). Additionally, the relationship between adverse events and climate variability has been explored (Burns et al., 2014). Overall, the application of dendroclimatology has triggered the creation of an extensive network of chronologies and has allowed the evaluation of dendrochronological potential of a wide range of species (Acosta-Hernández et al., 2020).

Dendroecology

Trees can record certain ecological processes, and dendroecology, as a specialized branch of dendrochronology, focuses on their study (Rojas-García et al., 2020). This discipline has been used to identify the temporal occurrence of natural processes that influence the development of growth rings, making it possible to determine the significance of these processes for the development of forest ecosystems. In Mexico, dendroecology has had several applications. By analyzing stand structures and applying dendrochronology, researchers have estimated forest ages (Villanueva-Díaz et al., 2010), biomass quantities (Correa-Díaz et al., 2019, 2020; Martínez-Sifuentes et al., 2019), forest productivity (Arreola-Ortiz & Nívar-Cháidez, 2010; Castruita-Esparza et al., 2016; Gómez-Guerrero et al., 2015; Reyes-Cortés et al., 2020) and the capacity of forests to capture carbon (García-Bedolla et al., 2015; Reyes-Basilio et al., 2020).

Other applications include evaluating the effects of extreme climatic events on the development of natural resources (Acosta-Hernández et al., 2020; Pacheco et al., 2020; Pompa-García et al., 2017; Rodríguez-Ramírez et al., 2018), assessing the impact of pests on tree radial growth (López-Sánchez et al., 2017), and analyzing wood anatomy and density (Morgado-González et al., 2019; Rodríguez-Ramírez et al., 2020).

Dendropyrochronology

Fire plays a crucial ecological role in certain forest ecosystems, and its benefits have been recognized by the scientific community. Dendropyrochronology provides tools for dating wildfires by analyzing the scars left by fire in tree growth rings. This allows for the estimation of parameters related to historical fire regimes (Cerano-Paredes et al., 2019) and provides a solid foundation for detecting anthropogenic disturbances, as well as for developing fire management strategies in forests (Sáenz-Ceja & Pérez-Salicrup, 2019).

In Mexico, this subdiscipline is becoming increasingly common, although studies remain scarce compared to the country's vast forested area (Cerano-Paredes et al., 2021; Sáenz-Ceja & Pérez-Salicrup, 2019). Most studies have identified changes in fire regimes, highlighting periods of fire exclusion, particularly in recent decades (Cerano-Paredes et al., 2021; Fulé & Covington, 1999; Sáenz-Ceja & Pérez-Salicrup, 2019; Yocom et al., 2014). These changes, mainly attributed to human activity, have raised concerns within the scientific community, as prolonged fire suppression can increase the risk of more severe future events due to the accumulation of combustible materials. Moreover, fire is an integral part of the ecology of many forests (Cerano-Paredes et al., 2021). Several studies have recommended incorporating fire management practices, such as prescribed burns, to reduce fuel loads and mitigate the risk of severe wildfires (Cerano-Paredes et al., 2021; Ponce-Calderón et al., 2021).

Some studies, such as that of Skinner et al. (2008) have explored the potential correlation between climate and fire occurrence. However, Fulé and Covington (1999) argue that this relationship is weak. These differences may be attributed to the specific conditions of each study site, including geographic location, topography, and vegetation type. Additionally, Stephens et al. (2010) analyzed the formation of fire scars and found that only a small proportion of trees exhibited scarring.

Dendrochemistry

Trees can absorb chemical components present in water, soil, and air. Variations in pollution levels or in the chemical composition of any of these media can be reflected in the chemical content of growth rings (Reyes-Camarillo et al., 2020). Through dendrochronological analysis, it is possible to determine the temporal variability of the chemical content in tree rings. Dendrochemistry is the subdiscipline that, with the support of chemical methods, allows for the evaluation of trees' physiological responses to variations in chemical elements (Correa-Díaz et al., 2020; Gómez-Guerrero et al., 2013).

In Mexico, dendrochemistry has received little attention in recent years. Studies have focused on three main areas: 1)

analysis of stable isotopes and tree physiology (Beramendi-Orosco et al., 2018; Correa-Díaz et al., 2020; Gómez-Guerrero et al., 2013; Pacheco et al., 2020), 2) the impact of environmental pollution on chemical concentration in tree rings (Flores et al., 2017; Morton-Bermea et al., 2016), and 3) the determination of chemical element composition (Sheppard et al., 2008).

Dendrogeomorphology

In mountainous areas, characterized by steep slopes, massive movements of rocky and edaphic material are common, favored by gravity and geological, climatic and anthropic factors. These processes can cause damage and the removal of forest masses. Rock falls, landslides and debris flows can cause alterations in trees, which can be expressed as scarring, abrupt growth reduction, eccentric growth and traumatic resin ducts. Each alteration can be dated through the analysis of growth rings, which facilitates the reconstruction of past geomorphological processes (Stoffel et al., 2011).

In the mountains of Mexico, especially in the Sierra Transversal, debris flows, torrential floods (Franco-Ramos et al., 2019; Martínez-Sifuentes et al., 2019) and lahars (Bollschweiler et al., 2010; Franco-Ramos et al., 2016) have been reconstructed. Additionally, determining the age of trees inhabiting newly formed geomorphological surfaces has allowed estimating the minimum age of these structures (Franco-Ramos & Vázquez-Selem, 2017).

Dendrohydrology

Dendrohydrology is used as a reliable tool for reconstructing river flows, groundwater levels, lake level changes, and floods. By analyzing growth rings, it is possible to generate dendrohydrological reconstructions that provide insights into water resource availability (Villanueva-Díaz et al., 2018). In Mexico, this subdiscipline has been applied to reconstruct streamflow volumes, water body gauge levels, and runoff patterns (Therrell et al., 2006; Villanueva-Díaz et al., 2020).

Dendrovolcanology

The emission of ash and gases from volcanic eruptions can cause alterations in regional environmental conditions. When trees are partially or completely covered by volcanic ash, they may either perish or record abrupt changes in their development, such as the suppression of ring growth (Biondi et al., 2003).

Dendrochronological analysis allows for the reconstruction of eruptive events and the assessment of their effects on forest ecosystems (Torbensohn, 2015) by linking volcanic eruptions with tree rings. However, for dendrovolcanolo-

gical studies, it is crucial to distinguish between climate-induced stress signals and those caused by volcanic activity (Biondi et al., 2003).

In Mexico, dendrovolcanology has gained importance through the analysis of the effects of the volcanoes along the Trans-Mexican Volcanic Belt on trees in the region. These studies have enabled the reconstruction of eruptive events (Alcalá-Reygosa et al., 2018; Alfaro-Sánchez et al., 2020; Sheppard et al., 2008) and the tracing of pyroclastic flows (Franco-Ramos et al., 2019). Furthermore, it has been shown that volcanic eruptions lead to changes in the chemical composition of growth rings, which is why these studies are often complemented with dendrochemical analyses (Alfaro-Sánchez et al., 2020; Carlón-Allende et al., 2015).

Dendroarchaeology

In the past, wood played a crucial role in construction, especially from species that form growth rings. This characteristic has allowed dendrochronology to be used at archaeological sites with ancient wood, for dating historical buildings and other archaeological applications (Bernabei & Macchioni, 2012). While this tool is useful for extending chronologies and inferring historical events, the application of dendroarchaeology in Mexico has seen limited success. Villanueva-Díaz et al. (2011) note that until the early 2010s, there was only one successful example of this subdiscipline applied to prehistoric ruins in Casas Grandes, Chihuahua. Additionally, with the help of the “Wiggle Matching” radiocarbon, it was possible to date ancient wood obtained from two sites in the Malpaso Valley, Zacatecas (Turkon et al., 2018).

Other categories

The studies include those focused on systematic reviews and meta-analyses specific to Mexico (Acosta-Hernández et al., 2017; Villanueva-Díaz et al., 2000), as well as reviews on the development of dendrochronology in a broader region (Bannister & Scott, 1964; Giraldo-Jiménez, 2011; Rojas-García et al., 2020; Schulman, 1944). Additionally, suitable sites for dendrochronological studies (Carlón-Allende et al., 2015) and for the reconstruction of agro-food variables (Therrell et al., 2006) have been identified.

Figure 3 shows spatial distribution and volume of studies related to different categories of dendrochronology. Most of the research has been conducted in the central and northern parts of the country, with a higher concentration in the latter due to the predominance of dendroclimatic studies, both in terms of volume and spatial applicability.

A total of 429 chronologies were identified, primarily distributed across three physiographic provinces: the Sierra

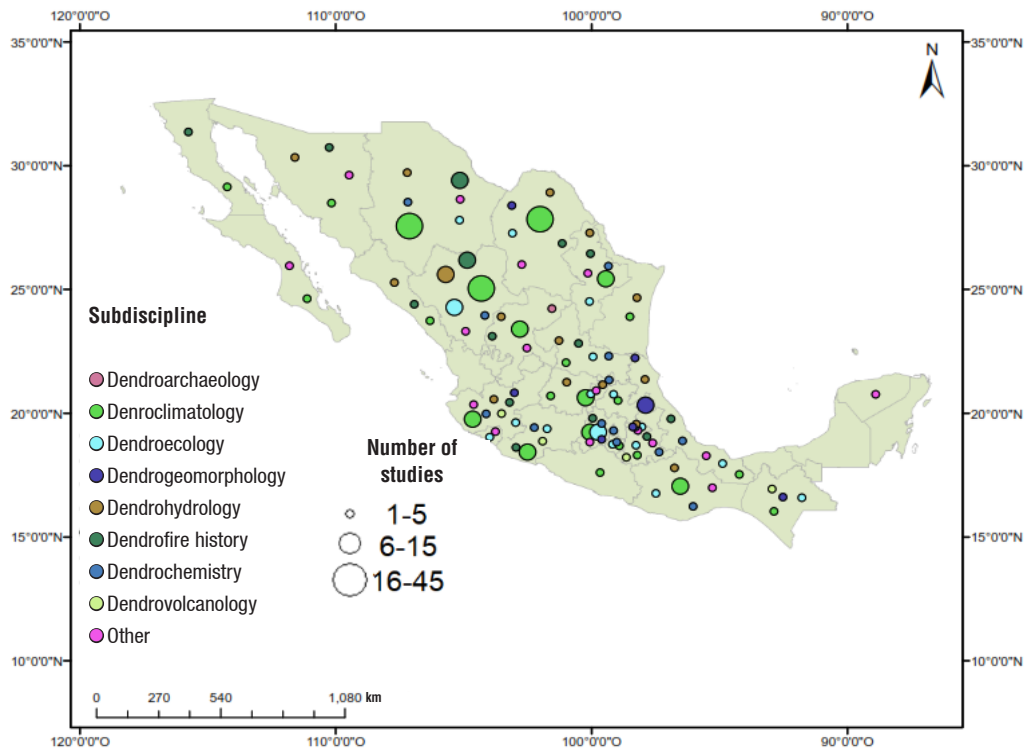


Figure 3. Spatial distribution of dendrochronology subdisciplines in Mexico.

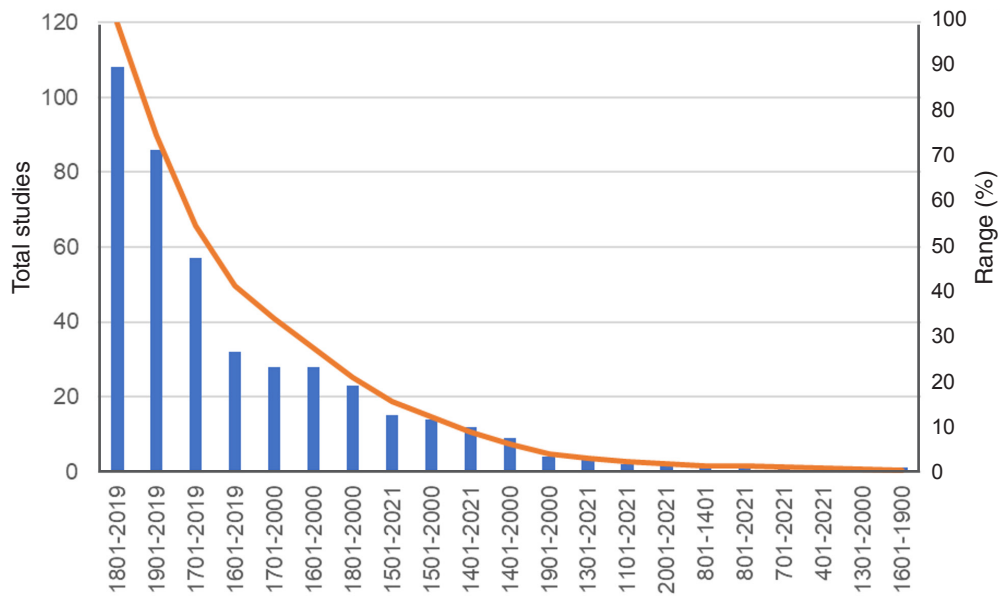


Figure 4. Range (period of years) of tree ring chronologies in Mexico

Madre Occidental (44.76 %), the Trans-Mexican Volcanic Belt (22.38 %), and the Sierra Madre Oriental (19.58 %). The remaining 13.28% is distributed across other provinces in the country. Chronologies have been developed in 28 states of Mexico, with Durango, Chihuahua, and Coahuila accounting for just over half (51 %). Regarding the species used, 53 different species have been reported, with the majority belonging to the conifer group (84.9 %), specifically the genus *Pinus* (60.38 %). Only eight broadleaf species have been used to create chronologies in Mexico, and three species dominate in terms of use: *Pseudotsuga menziesii* (23.78 %), *Pinus hartwegii* (10.26 %) and *Taxodium mucronatum* (8.86 %).

The chronologies span from the year 467 to 2019, covering a period of 1537 years. The longest chronology was created from the growth rings of *Taxodium mucronatum* in Los Peroles, San Luis Potosí (Villanueva-Díaz et al., 2007). However, since most of the chronologies are concentrated between 1801 and 2019, this period is the best represented (Figure 4). In Mexico, precipitation is the variable most closely related to the variability of tree rings, making it the most reconstructed variable.

Conclusions

Dendrochronological studies in Mexico have experienced notable growth since 1999, successfully exploring various approaches. However, there remains a dominance of climate variables as the primary factors influencing the variability in tree ring widths. Other research approaches in dendrochronology present opportunities for new studies in other regions of the country, particularly in the south.

Broadleaf species with dendrochronological potential need to be studied more in-depth, especially those found in tropical environments, where climatic seasonality is less pronounced. The vast biodiversity of Mexico's forest ecosystems offers an opportunity to expand knowledge in this area.

Dendrochronology in Mexico has significant potential for development. Future studies could focus on comparing the number of studies and species analyzed at an international level. It is essential to broaden both the physical and intellectual boundaries, as well as expand the spatial scope of dendrochronological research in the country.

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English version

Glyphosate in the media: the battle for public information

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Abstract

Glyphosate (GLF), one of the most widely used herbicides worldwide, has been the subject of controversy in Mexico due to its potential impact on human health, the environment and agricultural production. The objective of this study was to describe and analyze the information published in the Federal Public Administration (APF) press releases and in the main digital newspapers in Mexico regarding GLF, with the purpose of identifying predominant positions, their evolution, and competing interests. A content analysis assisted by artificial intelligence (AI) and web taxonomy tools was conducted on more than 150 information sources published between February 2023 and April 2025. The information was systematized into six categories: political-ideological, legal, commercial, academic, environmental, and public health. The results showed that the government's position went from a decrease in imports to a ban, and then to the repeal of the decrees due to the lack of viable substitutes, which generated disputes within the framework of the Free Trade Agreement between Mexico, United States and Canada Free (T-MEC). While academia proposes alternatives, the agrochemical industry warns of negative impacts on production and prices. Public positions reflect contradictions both between and within sectors. The debate over GLF in Mexico is marked by political, scientific and economic tensions, which hinders informed and consensual decision-making.

► **Keywords:** decree, digital newspapers, herbicides, journalism, agroecological transition.

Introduction

In Mexico, the use of pesticides and fertilizers is considered essential to maintain agricultural productivity and to ensure food supplies for both the domestic and export markets. However, there are no accurate official statistics on their use, which represents a public problem (Silveira-Gramont et al., 2018). The available information is based on sales estimates from companies, which report an annual volume of close to 31 thousand tons (Arellano-Aguilar & Rendón-von Osten, 2016), used in approximately 72 % of the cultivated area in the country (around 32 million hectares) (Hernández, 2024a; Instituto Nacional de Estadística y Geografía [INEGI], 2023).

Annual sales of these inputs are estimated at 18 billion pesos (mmdp), distributed equally between agrochemicals and pesticides. Among the most widely used are insecticides (4 billion pesos), herbicides (3 billion pesos) and fungicides (2 billion pesos), as well as inorganic fertilizers, which are key to increasing agricultural yields (INEGI, 2019).

Globally, the most widely used herbicide is glyphosate (GLF). Its global use exceeds 747 million tons (mt) annually, and it is the basis for approximately 750 commercial formulations (González-Díaz, 2025). The application of this herbicide is used primarily on genetically modified (GM) crops such as corn, cotton and soybean, as well as citrus fruits, grasslands,

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sorghum and sugarcane (Mordor Intelligence, 2024). In Mexico, GLF is estimated to be used in more than 31,000 farms, with an approximate annual consumption of 2 million liters. The states that use it most frequently are Veracruz, Chiapas, Campeche, Guerrero and Tabasco, mainly for corn cultivation (CONACYT, 2019; Encino, 2024; Agrarian Prosecutor's Office, 2021, 2024). Its popularity has grown due to its efficiency in pre and post emergence applications, both for land preparation and for accelerating crop drying prior to harvest (González-Ortega & Fuentes-Ponce, 2022). This growth has been driven by the introduction of GM crops and the reduction of their price (Bonny, 2016; General Coordination of Pollution and Environmental Health [CGCSA], 2018).

For decades, GLF was considered safe for human health and ecosystems (Torres-Cruz, 2024), even less toxic (at normal doses) than alcohol or certain greenhouse gases (Joaquín, 2024). However, the International Agency for Research on Cancer (IARC) warned of its possible link to carcinogenic diseases in humans and in laboratory animals, which was confirmed by the World Health Organization (WHO). The European Food Safety Authority later argued that the research conducted lacked scientific rigor (National Geographic, 2017).

At the institutional level, the Intersecretarial Commission on Biosafety of Genetically Modified Organisms (Comisión Intersecretarial de Bioseguridad de los Organismos Genéticamente Modificados CIBIOGEM, by its Spanish abbreviation) of the National Council for the Humanities, Science and Technology (Consejo Nacional de Humanidades, Ciencia y Tecnología CONAHCYT, now the Secretariat of Science, Humanities, Technology, and Innovation [Secretaría de Ciencia, Humanidades, Tecnología e Innovación SECIHTI, by its Spanish abbreviation]) has documented more than 600 research papers on the effects of GLF on health, the environment and food (CIBIOGEM, 2024). In this context, GLF has become a topic of political, academic, commercial, environmental and public health discussion. On one hand, the productive-commercial benefits of its use are mentioned; on the other, the possible negative implications on ecosystems, food and public health are questioned. Therefore, the objective of this work was to analyze the public information disseminated about

GLF in Mexico between February 2023 and April 2025, with the aim of describing the main positions generated by the presidential decree proposing its restriction and subsequent repeal.

Methodology

The review was based on the methodologies proposed by Campoverde et al. (2022) and Pérez-Vázquez et al. (2024) and the web taxonomy technique. The research focused on the analysis of official and media information related to GLF in Mexico. The main sources considered were the Official Journal of the Federation (Diario Oficial de la Federación [DOF, by its Spanish abbreviation]) and press releases issued by different federal government agencies, including: the Ministry of Environment and Natural Resources (Secretaría de Medio Ambiente y Recursos Naturales SEMARNAT by its Spanish abbreviation), the Ministry of Health (Secretaría de Salud SS by its Spanish abbreviation), the Ministry of Agriculture and Rural Development (Secretaría de Agricultura y Desarrollo Rural SADER by its Spanish abbreviation), the Federal Commission for the Protection of Sanitary Risks (Comisión Federal para la Protección de Riesgos Sanitarios COFEPRIS by its Spanish abbreviation) and CONAHCYT (now SECIHTI). Likewise, journalistic information from the main national newspapers was included: *El Financiero*, *El Universal*, *La Jornada*, *Milenio*, *El Economista*, *El Herald de México*, *El Sol de México*, *Reforma*, *Excelsior* and *Forbes México* (SCImago Media Rankings, 2024). Relevant complementary sources were also consulted.

Data collection was based on Google search alerts, with keywords related to GLF in the political, legal, commercial, academic, environmental and public health fields. The information search was conducted from February 2023 to April 2025, considering the enactment of the current decree on glyphosate restriction (February 2023) until the announcement of the new decree banning certain herbicides in 2025. In a first analysis, 193 sources of information were obtained; subsequently, the most relevant information was then filtered for the presentation of the results. The data were analyzed with the support of artificial intelligence (AI) to identify trend analysis, sentiment, impact, source comparison and media coverage (Table 1).

Table 1. Types of analysis and characteristics of information.

Type of analysis	Description
Trend	Most cited and mentioned topics
Sentiment	General tone of the news (positive, negative or neutral)
Impact	Consequence or effect of the news found
Comparison of sources	How different media outlets have covered the issue
Media coverage	Frequency of appearance in different media

Source: Authors' self-made with artificial intelligence support.

The information was also grouped into six categories: political-ideological, legal, commercial, academic, environmental and public health.

Results and discussion

The trend analysis indicated that public information about GLF in Mexico is mainly related to political-ideological, legal and public health aspects. This suggests that the government has set the agenda, followed by the academic sector. The sentiment analysis showed an average polarity of -0.10 ; that is, most public information addresses the negative effects of the herbicide. However, conflicting views were identified within the same sector. Regarding the impact analysis, the environment and health issues presented the highest degree of resonance in the sources consulted, demonstrating social concern. Meanwhile, the political and legal categories reflect the existence of conflict surrounding government decisions.

The comparison of information sources and the analysis of coverage revealed that three media outlets concentrated the highest proportion of publications on GLF: *La Jornada* (26 %), *El Universal* (23 %), and *Milenio* (21 %) (Table 2).

Table 3 shows the categories in which the different positions on GLF in Mexico were classified. These categories made it possible to identify the tensions between different agents, whose positions oscillate between the defense of the use of the herbicide for productive and economic reasons, and its rejection due to environmental and health risks.

Political-ideological category

In 2020, the Mexican government issued a first presidential decree for the gradual substitution of GLF use, as well as to regulate its acquisition, distribution, and importation. This decree also prohibited the use of GM corn and

entrusted the interministerial coordination (SEMARNAT, SS, SADER, CONAHCYT, and COFEPRIS) to create the legal mechanisms for its prohibition in 2023 (DOF, 2020). In February 2023, a second decree was published, repealing the previous one and establishing new strategies for the use of GLF and GM maize. The new instrument detailed measures to restrict the distribution and importation of the herbicide, and set March 31, 2024 as the transition deadline (DOF, 2023).

Under this strategy framework, two types of GLF were differentiated: technical and formulated. The first is the concentrated active ingredient, while the second is the mixture of technical GLF plus adjuvants (such as diluents, surfactants and solvents), where 1 L of formulation contains 360 g of the concentrated ingredient (CONACYT, 2021). According to official records, in 2021 the import of 16,500 t of formulated and 1,200 t of technical was authorized; in 2022, these figures were reduced to 8,263.09 and 628.62 t, respectively (DOF, 2024); while in 2023 only 4,131 t of formulated and 314 t of technical were imported (COFEPRIS, 2023; CONAHCYT, 2024a). This is with the aim of a gradual reduction until its prohibition in 2024 (COFEPRIS, 2023; SEMARNAT, 2020b).

Given the lack of viable alternatives, the government postponed the herbicide ban from March 2024 (Secretaría de Economía [SE], 2024). This decision was supported by the National Agricultural Council (Consejo Nacional Agropecuario CNA, by its Spanish abbreviation), which represents the main agricultural companies in the country, considering that any substitute for GLF must be widely distributed, competitively priced and proven efficiency (Hernández, 2024b). For its part, the Mexican Union of Agrochemical Manufacturers and Formulators (Unión Mexicana de Fabricantes y Formuladores de Agroquímicos UMFFAAC, by its Spanish abbreviation) stated that the use of agrochemicals is necessary to maintain and increase production volumes, as they help to avoid losses of up to

Table 2. Position of each media outlet on glyphosate in Mexico (February 2023 to April 2025).

Source	General position	Polarity of sentiment
<i>La Jornada</i>	Critical	-0.20
<i>El Universal</i>	Critical	-0.10
<i>Milenio</i>	Neutral	0.00
<i>El Financiero</i>	Pro-economic	0.10
<i>El Economista</i>	Pro-economic	0.15
<i>Forbes México</i>	Neutral	0.00
<i>Excelsior</i>	Neutral	0.05
<i>Reforma</i>	Pro-industry	0.20
<i>El Heraldo de México</i>	Pro-industry	0.20
<i>EL Sol de México</i>	Critical	-0.10

Source: Authors' self-made with artificial intelligence support.

Table 3. Classification of positions in public information on glyphosate (GLF) in Mexico (February 2023 to April 2025).

Categories	Main positions
Political-ideological	<ul style="list-style-type: none"> • The government, through decrees, went from reducing, prohibiting, postponing, and even repealing the use of GLF. • It is considered that GLF regulation is based on a political-ideological conception, rather than on scientific, technical or productive criteria. • It is expected that the Constitution will be amended to regulate the use of GLF, and avoid further litigation and trade disputes under the framework of the T-MEC.
Legal	<ul style="list-style-type: none"> • From 2016 to 2023 there are lawsuits and legal disputes in national and international courts. • Mexico lost a T-MEC litigation on the restriction of GLF and GM corn. • Monsanto faced and dismissed lawsuits against the Mexican government regarding GLF.
Commercial	<ul style="list-style-type: none"> • The industry maintains that there are no viable alternatives that match GLF in terms of effectiveness, availability and price. • Globally, the glyphosate market is worth at over \$9 billion annually, and \$3 billion pesos in Mexico. • Eliminating GLF without a real alternative will negatively impact food production and prices.
Academic	<ul style="list-style-type: none"> • It presents opposing positions for and against the herbicide. • The scientific veracity is argued, but the safety for the environment, soil, food and human health is not proven. • Bio-herbicides have been developed (Contronat: argues 90 % effectiveness) and alternatives for an agroecological transition to reduce and replaces glyphosate.
Environmental	<ul style="list-style-type: none"> • There is concern about the negative impact of GLF on biodiversity, soil, water, and food. • Reducing and banning herbicides is necessary to protect ecosystems and native species. • The effects on the environment would have future economic implications for various agricultural subsectors; however, the economic aspect takes precedence over environmental considerations.
Public health	<ul style="list-style-type: none"> • International organizations have linked GLF to cancer, as well as kidney, reproductive, liver and brain diseases. • Prolonged exposure, as well as improper dosage, application and clothing can cause the presence of GLF in the human body. • Presence of GLF in food, water bodies and people has been documented, but it is argued that there is no medical or scientific evidence.

Source: Authors' self-made with artificial intelligence support.

40 % due to drought, 28 % due to pests, 27 % due to diseases and 42 % due to weeds (Hernández, 2024c). Other positions consider that the decrees were driven more by a political ideological issue than by the preservation or protection of health and the environment (Mota, 2024).

Legal category

Internationally, Bayer’s acquisition of Monsanto generated approximately 100,000 lawsuits, with a total amount estimated at \$10 billion dollars, for damages allegedly caused by the use of GLF (Nava, 2024; Reuters, 2024). Although Bayer agreed to cover these amounts, it clarified that this did not imply an admission of guilt, but rather a strategy to close the litigation (Barragán, 2024). The company argues that there is no scientific evidence to support harm to health or the environment (Petovel, 2024; Salazar-López & Aldana-Madrid, 2011).

In Mexico, the restrictive GLF import policy reached the Supreme Court of Justice of the Nation (Suprema Corte de Justicia de la Nación SCJN, by its Spanish abbreviation) (Aguirre, 2024). After the judicial process, Monsanto stopped legal action against the 2020 decree, which represented a triumph for the federal government (Agencia Reforma, 2024a; CONAHCYT, 2024b).

resented a triumph for the federal government (Agencia Reforma, 2024a; CONAHCYT, 2024b).

In November 2023, the governments of Mexico and the United States initiated a trade dispute within the framework of the T-MEC, related to import restrictions on GM maize and the use of GLF (Amador, 2023; Ordaz, 2024). In late 2024, it was announced that Mexico had lost the legal dispute, which was considered by various sectors as a triumph for commercial agricultural biotechnology (Matamoros, 2024). The main argument of the ruling was that Mexico did not present sufficient scientific evidence on the negative effects of the herbicide on human health and the environment (Balderas, 2024; Morales, 2024). On the other hand, CONAHCYT argued that there is scientific evidence and that the US government did not prove the safety of the herbicide (Poy, 2024; Wise, 2024).

After the ruling, Mexico had until February 2025 to challenge it (Balderas, 2024; Cosío, 2024), as the government considered that public health and Indigenous rights were not taken into account (Ribeiro, 2024a). In this sense, the defense would raise the issue from a scientific and cultural perspective (Aguirre, 2024; Junta de Coordinación Política

[JUCOPO], 2024), with the objective of strengthening national agricultural and food sovereignty (Monreal, 2024). If Mexico loses this lawsuit, the dispute that both countries have been maintaining since 2020 would come to an end (Radio Fórmula, 2024). It should be noted that in México the planting of GM maize is prohibited, but not its importation, which means that the decree of February 2023 lacks support (Villalobos, 2025).

Commercial category

GLF is a water-soluble organic acid derived from the phosphonomethyl derivative of the amino acid glycine, whose active ingredient is N-(phosphonomethyl) glycine (Davoren & Schiestl, 2018). It was developed by Monsanto company in 1974 under the brand name Roundup, which held the patent until 2000 (Nava, 2024). Currently, the main companies dominating the market are Bayer CropScience AG, BASF Corporation, Syngenta AG, Adama Agriculture Solutions Ltd., and UPL Limited. In 2023, the value of the global GLF market was estimated at 8 500 million dollars, and by 2024 it increased to 9 000 million dollars, reaffirming itself as a stable market due to the lack of viable alternatives for both GM and conventional crops (Mordor Intelligence, 2024). However, it faces challenges such as oversupply, regulatory pressure in several countries and falling prices (Katsaris, 2024).

According to the Action Network on Pesticides and Alternatives in Mexico (RAPAM by its Spanish abbreviation), more than 9,500 types of pesticides are used (Animal Político, 2024), and 85 % of the market is concentrated in Bayer and Corteva (merger of DuPont/Pioneer and Dow) (Mordor Intelligence, 2023; Ribeiro, 2024a). There are around 36 commercial brands of GLF in the country and more than 400 products that use it as a base ingredient, including Faena, Cacique 480, Nobel 62 %, Lafam, and Eurosato. The available presentations are: soluble, soluble granules, soluble powder, concentrated solution, and aqueous solution (Arellano-Aguilar & Montero-Montoya, 2017). Considering the above, it is thought that the GLF remains in the market due to economic pressure through the generation of doubts about the veracity of scientific studies (Pomar, 2024).

Academic category

Internationally, there are three bioherbicides approved by the European Union (EU) and the United States: Beloukha, Burn Out Formula 2 and Weed Zap (Procuraduría Agraria, 2024). In Mexico, at the end of the 2019-2024 six-year term, CONAHCYT, together with Promotora Técnica Industrial, SA de CV, announced the product “Contronat” as the first Mexican bioherbicide registered with COFEPRIS. In addition, the development of other formulations with 90 % effectiveness were reported to be in some phase of

registration: Herbi-O, Kill Herbs, Herbitech, Zekatryn and Sec Natural, developed by Agroplanet, Agribest, Biotech, Megainsumos and Seragram, respectively (CONAHCYT, 2024c; Villaseñor, 2024).

In view of these advances, the Chamber of Deputies requested that COFEPRIS expedite the corresponding procedures (Martínez, 2024). Likewise, state congresses were urged to inform producers about herbicides in the process of being banned (AC Noticias, 2024). However, the SE, SEMARNAT, SADER, and COFEPRIS acknowledged that no substitute with equivalent characteristics to GLF has been found (Barragán, 2024; Ribeiro, 2024b).

In addition to the development of bioherbicides, agroecological strategies for weed management have been promoted, including cultural and mechanical practices (Ramírez, 2024; SADER, 2024), extension work through field schools (Domínguez, 2024), and the use of geographic information systems and drones (AVC Noticias, 2024; Domínguez, 2024). Meanwhile, education and research institutions have developed projects to identify active ingredients that would allow the formulation of bioherbicides (Salinas, 2024) and to generate lines of research aimed at GLF free agriculture (Digital Roadmap, 2024).

On the other hand, there are positions that question the viability and effectiveness of these alternatives, and highlight the possibility of generating adverse economic impacts (Yuste, 2024). The UMFFAAC argues that the GLF ban would not improve the health of the population, but would increase the price of grains due to the drop in production (Hernández, 2024b). Moreover, the Protection of Crops, Science and Technology (PROCCYT, by its Spanish abbreviation) assures that GLF has been used for more than 40 years without adverse effects on health or the environment (Miranda, 2024), while the National Union of Agricultural Workers (UNTA, by its Spanish abbreviation) approved the measures taken by the federal government (Soriano, 2025b).

Environmental category

In 2020, the fifth edition of the *Antología toxicológica del glifosato* was published, which contains nearly 1,000 scientific and academic papers on the herbicide effects on the environment and human health (Mejía-Haro, 2025). In India, the promotion of the use of GM and GLF cotton has affected the agricultural industry (Sánchez, 2024), while the EU banned the import of Brazilian coffee due to the herbicide's toxicity, which represents significant economic losses for the sector (Afane, 2024). In Spain, the presence of nearly 106 pesticides was detected in fruits and vegetables (Rejón, 2024), so environmental associations consider that the suspension of the use of GLF in non agricultural activities would be an important step to reduce their environmental

impact (BioEco, 2025). For its part, Great Britain has been more flexible regarding tea regulations.

Some countries such as Argentina and Bolivia have documented GLF resistance of up to 90 % in at least 24 weed species (Pavot, 2024). In Mexico, GLF has been reported in maize from the United States (García, 2024a, 2024c; Valencia, 2025), as well as in water sources and aquatic species in several states (Cervantes, 2024). Some reports indicate that companies operating in these regions have exerted pressure to stop research into the use of agrochemicals (Valencia, 2024).

Recurrent criticisms point to the fact that governments favor the economic aspect, since they lack the political will to enforce existing laws regarding the use of GLF (del Valle, 2024; Pomar, 2024). This scenario benefits transnational corporations, which maintain oligopolistic practices, promote the privatization of genetic resources (Contreras, 2024; Díaz-Guillén, 2024) and create technological dependence (Mejía-Haro, 2025; Ortega, 2025) through consolidation governance structures parallel to constitutional powers (González-Díaz, 2025). Therefore, a reconversion of production models is necessary, as well as new legal instruments to regulate the use of GLF (Pérez-Vázquez et al., 2024).

Public health category

At the international level, negative effects of GLF on human health have been reported. Among the associated conditions are brain inflammation, acceleration of Alzheimer's disease (Infosalus, 2024), neurodegenerative diseases (Welle, 2024) and immunotoxic effects (de la Chica, 2024), as well as liver, kidney, reproductive (Wise & Malkan, 2024) and immune system damage. In Mexico, at least 146 cases of GLF and 2,4-D have been reported in urine samples from residents of Jalisco and Yucatán (García, 2024b; Ucán, 2024); in addition, low birth weight has been documented in rural communities in Monterrey (Martínez, 2025) and intoxication in children in the state of Veracruz (Guardian, 2024).

SEMARNAT has linked GLF to various diseases, such as encephalopathies, autism, parkinsonism, malformations and different types of cancer. Impacts on the endocrine, reproductive, immune, digestive, hepatic, renal, nervous and cardiovascular systems have also been reported (SEMARNAT, 2020a). Other effects reported include allergic reactions, antibiotic resistance, immunosuppression and nutrient loss from food (Rejón, 2024; Reuters, 2024), which coincides with international data.

A relevant aspect is the degradation time of the GLF molecule in the organism, which ranges between 50 to 300 days (González-Díaz, 2025). This persistence is related to inadequate dosing practices, application and use of

protective equipment (Benbrook, 2016; CONACYT, 2019; Ribeiro, 2024b). Therefore, several studies highlight the importance of addressing these factors (Andreotti et al., 2018; Gandhi et al., 2021).

Position of the new government (2024-2030)

Policy regarding glyphosate and GM maize has been a central issue during the last two APFs, although there have been differences among its members. In the document *100 Steps to transformation*, the current government (2024-2030) established as a central axis the rescue of national sovereignty, particularly in the agricultural sector, under the concept of a "Just and Sovereign Rural Republic" (Aguirre, 2024; Sheinbaum, 2024).

The *National Development Plan* (PND by its Spanish abbreviation) (2025-2030), which gives continuity to the agricultural policies of the previous administration (Antúnez, 2024; Flores, 2024), establishes three axes: 1) promotion of food self-sufficiency through support for producers and the development of sustainable agriculture, 2) promotion of technological innovation to improve productive efficiency, and 3) strengthening of small producers through financing and technical assistance (Fajardo, 2025). Along with the PND, *Plan Mexico* aims to position the country among the top 10 global economies, produce 50 % of national consumption and be one of the top five tourist destinations worldwide (BBC News Mundo, 2025). However, opposition sectors argue the concentration of power and authoritarianism, and consider these plans to be unrealistic (Cámara de Diputados, 2025).

With respect to GLF, the government proposes to continue with the policy of reducing imports and use, in addition to reviewing another 200 products that are prohibited in other countries (Agencia Reforma, 2024b; Benítez, 2024; Miranda, 2024; Salgado, 2024). After the deadline to comply with the T-MEC trade dispute expired (Barragán, 2025; Romero, 2025), in early February 2023 decree, previously postponed by the previous APF, was published (DOF, 2025; Soriano, 2025a). Nevertheless, the government has publicly stated its commitment to maintain such policy, regardless of the T-MEC panel, and has even announced its intention to elevate it to constitutional status. To this end, it is proposed to modify articles 4th (right to a healthy and sustainable environment) and 27th (land ownership) of the Constitución Política de los Estados Unidos Mexicanos (Alegría, 2024; González, 2024; Méndez, 2024), in order to avoid future trade disputes (Vela, 2025). This strategy is legally viable, as the panel's ruling does not prevent Mexico from amending its constitution (Forbes, 2025; George, 2025).

In March 2025, the constitutional reform on the conservation of native maize was published (DOF, 2025), and in April a new decree was announced aimed at banning various

pesticides classified as highly toxic, already banned in other countries (del Ángel, 2025; Olivares & Urrutia, 2025).

Conclusions

In Mexico, current public information about glyphosate considers it to be a herbicide that is harmful to health and the environment, but its use is justified from a productive and commercial perspective. The federal government lost the trade litigation and repealed its decrees, but the policy on its use will be raised to constitutional rank, so the discussion and controversy will go on both public and scientific-academic spheres.

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English version

Establishment and Monitoring of a *Pinus greggii* Engelm. ex Parl. Plantation as a Climate Change Mitigation Strategy in Huehuetla, Hidalgo

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Abstract

Climate change, as a global phenomenon, has affected the planet in multiple ways, altering weather patterns and prompting the search for alternatives and strategies for mitigation and adaptation. Forest plantations have emerged as an environmental option due to their potential for CO₂ capture, making it essential to identify tree species that can contribute to this effort. One method for evaluating a species' adaptability to specific climatic conditions is climatic matching. In this study, *Pinus greggii* Engelm. ex Parl. was evaluated for its potential to adapt to the subtropical conditions of Huehuetla, Hidalgo—a region with high potential for restoring degraded lands and supporting fast-growing species. Climatic parameters considered included cardinal temperatures, precipitation range, and altitude, along with tree measurement characteristics: height and basal diameter. The species showed a 50 % adaptation rate to the natural conditions, primarily attributed to altitude, given a 300-meter difference between the planting site and the lower distribution limit of the species. Growth was deemed acceptable, as after 24 months of monitoring and evaluation, the trees reached an average height of 94.6 cm and a basal diameter of 1.6 cm. Individual trees exhibited a diameter at breast height of 1.14 cm, which translated to an average volume of 147.5 cm³ per tree, equivalent to 61.81 g of stored carbon.

► **Keywords:** Climate homologation, *Pinus greggii*, Engelm. Ex Parl., environmental services.

Introduction

In recent years, forest plantations in Mexico have increased due to the opportunity they represent for enhancing timber production. These plantations are defined as the establishment and management of species on land designated primarily for forest use. As a result, the total area covered by commercial forest plantations (CFP) nationwide has reached 373 127.17 ha, of which timber species account for 85.2%. These plantations are primarily distributed across the states of Veracruz, Tabasco, and Campeche (CONAFOR, 2024; Diario Oficial de la Federación, 2024). In addition, forest plantations play a crucial role in mitigating the effects of climate change. In southern and southeastern Mexico, fast-growing species such as white teak (*Gmelina arborea*

Roxb.), teak (*Tectona grandis* L.f.), and eucalyptus (*Eucalyptus* spp.) have been widely used for timber production (Martínez-Zurimendi, 2015). In central Mexico, species from the *Pinus* genus are commonly planted, with *Pinus greggii* Engelm. ex Parl. being one of the most frequently used. This species is particularly valued for its ability to restore eroded soils, as well as its high potential for carbon sequestration, drought tolerance, adaptability to temperature fluctuations, and resistance to certain pests (López et al., 2017; Martínez-Sifuentes et al., 2020; Ortiz et al., 2021; Villegas-Jiménez et al., 2013). According to Carrillo-Castañeda et al. (2024), *P. greggii*, along with *P. pseudostrobus* and *P. devoniana*, demonstrated a high capacity for carbon sequestration when established for the restoration of severely degraded soils in Michoacán.

Moreover, the increase in atmospheric CO₂ has triggered extreme weather events—such as floods, intense hurricanes, and others—resulting in both human and economic losses worldwide. In response, the Kyoto Protocol of 1997 requires developed countries to reduce their greenhouse gas emissions. It also offers flexible mechanisms, such as the Clean Development Mechanism (CDM), which promotes carbon capture and CO₂ sequestration through forest biomass (IPCC, 2014; Gómez-Guerrero, et al., 2021).

In the municipality of Huehuetla, there have been some attempts to establish small-scale plantations on private land, primarily to improve shade for coffee plantations and to obtain firewood. Arteaga and Castelán (2008) established an agroforestry plantation combining white teak (*Gmelina arborea* Roxb.), teak (*Tectona grandis* L.f.), and pink cedar (*Acrocarpus fraxinifolius* Wight & Arn.) with coffee. Their findings indicated that pink cedar and white teak were the species that adapted best to the site conditions. Huehuetla's altitudinal range spans from 400 to 1 300 meters above sea level and includes three different climate types, providing an opportunity to test various species with different temperature tolerances. According to Medina-Pérez et al. (2023), the Sierra Otomí-Tepohua—which includes the municipalities of Huehuetla, San Bartolo Tutotepec, and Tenango de Doria—is highly vulnerable to extreme hydrometeorological events. These are largely driven by deforestation and the conversion of forest land to agricultural and livestock uses, with landslides caused by heavy rainfall identified as one of the main regional hazards.

In light of these considerations, the current approach to forest plantations should be strategic, aiming to mitigate the effects of climate change—particularly through CO₂ capture, which is part of the payment for environmental services scheme (FAO, 2022; UNDP, 2023). This represents a local action with global impact. Consequently, there is a clear need to establish forest plantations and conserve natural forests. In the case of Huehuetla, Hidalgo, *Pinus greggii* appears to be a promising species, as it is well-suited to the municipality's natural conditions due to its subtropical characteristics (Perry, 1991; Ramírez-Herrera et al., 2005; Martínez-Sifuentes et al., 2020). Furthermore, Ortiz et al. (2021) recommend using *P. greggii* provenances from northern and central Mexico, where the species is endemic. Its genetic plasticity allows it to thrive on eroded soils, making it highly valuable for watershed protection and the restoration of degraded areas. Additionally, it exhibits rapid growth in such environments.

This implies the use of methodologies that address the degree of species adaptation to environments different from their natural distribution, such as climatic matching. In this sense, species distribution is limited by physiological, ecological, and biogeographic factors, which make it difficult to accurately determine a species' distribution area, as it is a dynamic phenomenon (Maciel-Mata, 2015). Climatic matching aims to expand the adaptation range

of species from a different place of origin than the one where they are intended to be established, taking into account climatic parameters, with the most relevant being extreme temperatures, absolute minimum temperature, and the seasonal distribution and annual volume of precipitation (Golfari, 1963). Gómez (2021) discusses the concept of ecological matching, which is more complex, as it considers lithological, physiographic, climatic, and soil characteristics that influence the degree of adaptation. Therefore, considering that *P. greggii* is distributed along the mountain range of the Sierra Madre Oriental, where the municipality of Huehuetla, Hidalgo is located, the use of climatic matching will allow for the extension of the local distribution range of this subtropical conifer species.

Thus, the objective of this study was to establish and monitor an experimental plantation of *Pinus greggii* Engelm. ex Parl. in the municipality of Huehuetla, Hidalgo to evaluate initial growth, adaptation potential, and carbon capture.

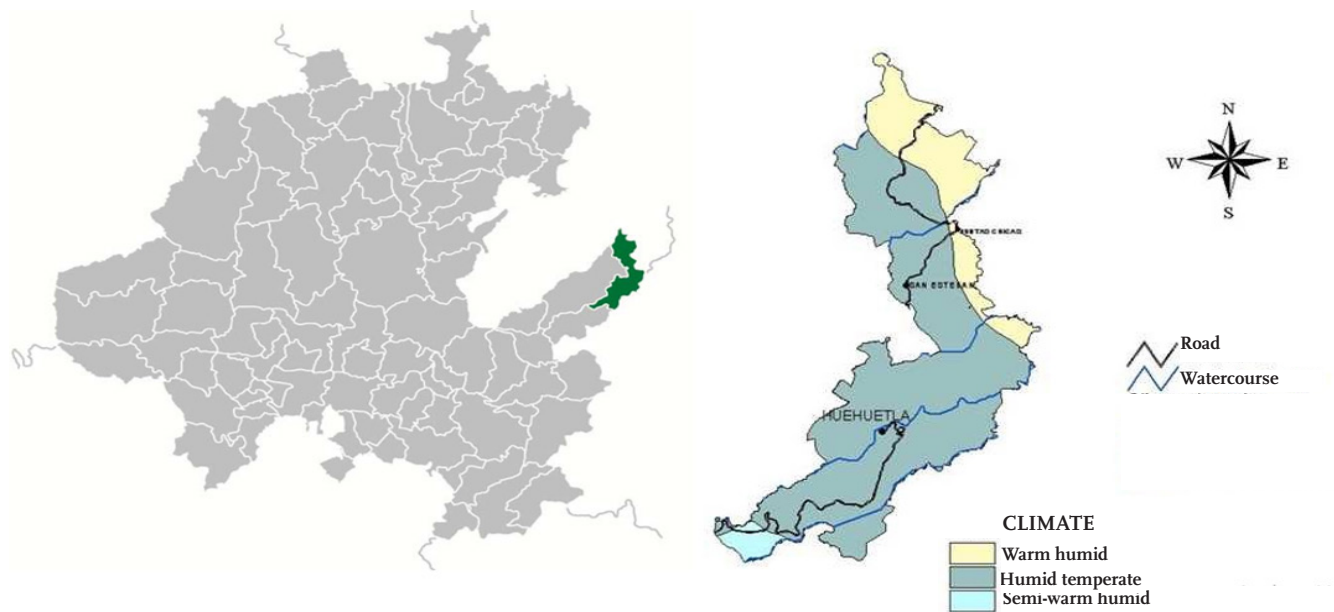
Materials and Methods

Study Area Description

The municipality of Huehuetla is located between 20° 23' and 20° 41' N and 97° 59' to 98° 10' W, situated in the northwestern and eastern part of the state of Hidalgo. It is part of the Tuxpan-Nautla region, within the coffee-growing area known as Otomí-Tepohua. According to García (2004), Huehuetla has three climate types: humid warm (Am) with summer rainfall, characterized by a short dry season during the cooler half of the year, yet with sufficient total annual precipitation to keep the soil moist year-round. This climate is intermediate between Af and Aw, resembling the former in total rainfall and the latter in annual distribution. The highest precipitation occurs in summer and part of autumn, when tropical cyclones affecting Mexico are most frequent, significantly increasing rainfall in this region. Other climate types present include humid sub-warm (ACf) with year-round rainfall, and humid temperate (Cm) with heavy rainfall during the summer. The average annual temperature ranges from 16 °C to 22 °C, with an average annual precipitation of 2 400 mm and elevation ranging from 400 to 1 300 meters above sea level. According to INEGI (2010), the predominant soil types are Umbrisol (56.95 %), Luvisol (36.2 %), Leptosol (4.32 %), and Phaeozem (2.33 %), with vegetation consisting of tropical rainforest and cloud forest. The plantation was established on Umbrisol soils, which, according to FAO (2025), are characterized by a dark layer rich in organic matter but with acidic pH (below 5.5) and low fertility. Figure 1 shows the location map of the municipality and the distribution of climate types.

Plantation Establishment

A total of 200 pine seedlings were planted, with an average height of 85 cm and a basal diameter of 8 mm, after



Source: Government of the State of Hidalgo (2010). Compilation based on INEGI data.

Figure 1. Location of the municipality of Huehuetla, Hidalgo, and climate.

having been grown in a nursery for two years. The site was prepared manually using the common pit system, since the slope of the terrain did not allow mechanization. Pits measuring 40 x 40 x 40 cm were dug to create favorable conditions for root establishment and to increase survival chances. The spacing between plants was 2.5 x 2.5 m in a square grid design. The plantation was established in June 2022 under a humid sub-warm climate. The seedlings originated from Estado de México.

Climatic matching

As a tool to assess adaptability to specific regions with particular climatic conditions, climatic matching was carried out using the FAO (1981) methodology, which considers the climatic variables of a species' place of origin in relation to the site where it will be introduced. Initially, the thermal profile of *Pinus greggii* was obtained. Then, the temperature data for the municipality of Huehuetla, reported by the National Meteorological Service, were evaluated and adjusted using the Regional Thermal Gradient for the planting site. The following climatic parameters were considered: minimum, optimal, and maximum temperatures, precipitation range, and altitude—for both the species and the plantation site.

The climatic requirements used for *P. greggii* included an altitudinal range of 1 400 to 2 600 meters above sea level, vital cardinal temperatures from 12 to 20 °C, with an optimal range of 16.8 to 17.5 °C, and extreme or lethal cardinal temperatures of 45 °C and -9 °C. Precipitation range between 640 and 1 370 mm, although it may extend from 500 to 2 900 mm (Dvorak et al., 1996; Ramírez-Herrera et

al., 2005; Muñoz et al., 2012; Rodríguez et al., 2013). However, Martínez-Sifuentes et al. (2020) reported an average annual temperature of 25.4 °C in the distribution area in the Sierra Madre Oriental.

For the case of the municipality of Huehuetla, the thermal range considered was 14.0 to 32.5 °C, with an annual precipitation of 2 400 mm. The specific elevation of the planting site was 1 100 meters above sea level.

Evaluation and Monitoring

A total of four evaluations of survival and growth were conducted following the planting date, one every six months (December 2022, June 2023, December 2023, and June 2024). Tree measurement characteristics considered were height, basal diameter, and diameter at breast height (DBH). In the fourth evaluation, some trees reached or exceeded a height of 1.30 meters; for these, volume was calculated using the following formula:

$$V = \frac{\pi}{4} * d^2 * A * Cm$$

Where:

V = total tree volumen in cm³

d = diameter at breast height (cm)

A = total tree height (cm)

Cm = form factor or morphic coefficient. This coefficient is used to correct the overestimation of tree volume that

results from calculating it as a perfect cylinder. A value of 0.65 was used for conifers.

Initial Estimation of Carbon Content in Biomass

The amount of biomass in tree stands, expressed in terms of carbon content, is estimated to be approximately 50 %, regardless of ecosystem type, species, or region (Díaz et al., 2007; Acosta et al., 2009; Ronquillo-Gorgúa, et al., 2022). Similarly, the IPCC (2005) states that the carbon content in a tree is equivalent to 50 % of its biomass. Therefore, the carbon content for young trees is estimated using the following equation:

$$\text{Biomass} \times \text{Carbon content} = \text{Carbon stock.}$$

Several methods are used to estimate carbon stock; however, all are subject to a certain degree of approximation. According to Portillo et al. (2023), the margin of error in biomass estimation—and therefore in carbon stock—ranges from 53 % to 80 %, regardless of the method used. Consequently, this study employed three estimation methods, including:

1) the method recommended by CONAFOR (Comisión Nacional Forestal), expressed by the following formula:

$$C = V * FD * CC * BEF$$

Where:

C: amount of carbon stored per tree

V: volume in m³

FD: wood density factor; for conifers, the value is 0.48

CC: carbon content by species; for *P. greggii*, it is 51 %

BEF: biomass expansion factor; the value is 1.3

2) The Acosta et al. (2009) method, expressed with the allometric equation for the determination of biomass obtained for *Pinus patula* Engelm. Ex Parl. and other pine species given by:

$$B = 0.0948 * DN^{2.4079}$$

Where:

B: biomass (kg)

DBH: diameter at breast height (cm)

Multiplying the biomass by 0.50, which means that 50 % of the tree biomass is C.

3) The method proposed by the IPCC (2005) recommends multiplying the estimated tree volume by the carbon fraction, which is equivalent to 0.5.

Results and Discussion

The thermal table for *P. greggii* is presented in Table 1. This table made it possible to determine the species' adaptation percentage to the environmental conditions of Huehuetla, specifically at the plantation site, after subtracting the temperature differential (3.4 °C) resulting from the Regional Thermal Gradient.

An adaptation rate of 50 % to the local conditions was observed, which may be attributed to altitude as a natural limiting factor, given the 300-meter difference between the plantation site and the lower altitudinal limit of the species' natural distribution. However, as noted by Gómez (2021), although altitude is part of the physiographic characterization, it may also be influenced by factors such as lithology and soil properties. Similarly, Romahn-Hernández et al. (2020), in a study on the natural distribution of sacred fir (*Abies religiosa* (Kunth) Schldtl. et Cham.), reported high mortality rates among mature trees at the lowest altitudinal range. They also observed lower vigor in both mature and young trees, concluding that altitude is a key factor in the survival of sacred fir and suggesting an upward altitudinal shift in response to climate change.

In terms of temperature, the evaluated area falls within the thermal thresholds reported by Ramírez-Herrera et al. (2005), whose optimal range is between 16 and 18 °C. Regarding precipitation, the annual 2 300 mm recorded in Huehuetla falls within two of the reported ranges for *P. greggii*: 500 to 2 900 mm and 750 to 2 300 mm. Based on these favorable conditions, planting was carried out on June 26 and 27, 2022, when the soil was sufficiently moist following the onset of the rainy season. Initial measurements of the specimens—specifically, height and basal diameter—were taken on June 28, 2022, after having spent two years in the nursery.

The growth performance of the individuals is considered acceptable. After 24 months of monitoring and evaluation, 85 % of the 104 surviving trees exceeded 1.30 m in height. The average height was 1.85 m, with a maximum of 3.45 m (Figure 2). The average diameter at breast height was 1.14 cm, which allowed for an initial volume estimate of 147.5 cm³. Additionally, the mean basal diameter exceeded 20 mm, with the largest reaching over 50 mm (Figure 3). Table 2 presents the estimated statistics, showing the percentage increase in both height and basal diameter two years after planting, relative to initial measurements.

Regarding survival, a gradual mortality of individuals was observed, with evaluation results showing survival rates of 85.5%, 79.5%, 67.5%, and 51% in the final assessment. Among the main factors contributing to mortality was pest damage, primarily by the stem borer (*Oncideres* spp.), which caused girdling of the stem and restricted sap flow in affected individuals. This was observed only during the first two evaluations. Additionally, mortality was attributed

Table 1. Thermal table generated for *P. greggii* Engelm. ex Parl., based on its cardinal temperatures.

Temperature range (°C)	Value
Above 25.2	0
23.5	25.1
21.8	23.4
20.1	21.7
18.4	20.0
16.7	17.5
15.0	16.6
13.3	14.9
11.6	13.2
9.9	11.5
Below 9.8	0

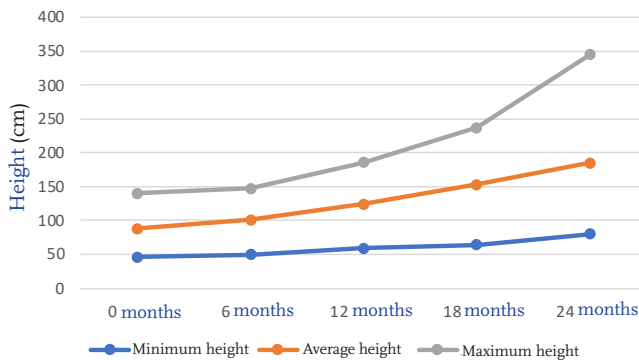


Figure 2. Height growth of *P. greggii* Engelm. ex Parl. in Huehuetla, Hidalgo.

to the exceptional and extreme drought that occurred in 2024, particularly during March, April, and May. This drought was driven by anticyclonic systems that caused heatwaves, resulting in hot to very hot conditions and a lack of rainfall. The drought affected 32 municipalities in the state of Hidalgo and nearly the entire national territory (CONAGUA-SMN, 2024). Despite these challenges, the survival percentage remained higher than the national average for reforestation efforts (36 %), although it fell short of the 80 % threshold required by the Comisión Nacional Forestal during the first year (Vázquez-Cisneros et al., 2018).

Table 2. Estimated statistics for height and basal diameter variables.

Parameter	Variables					
	Initial height (cm)	Initial basal diameter (mm)	Height at 24 months (cm)	Basal diameter at 24 months (mm)	Height increase (%)	Basal diameter increase (%)
Minimum value	46	3.6	80	9	53.96	85.71
Mean value	90.1	6.9	184.7	22.7	68.85	106.75
Maximum value	140	11.3	345	51	84.53	127.45
Range	94	7.7	265	42	95.26	138.03
Standard deviation	19.689	1.612	52.134	8.046		

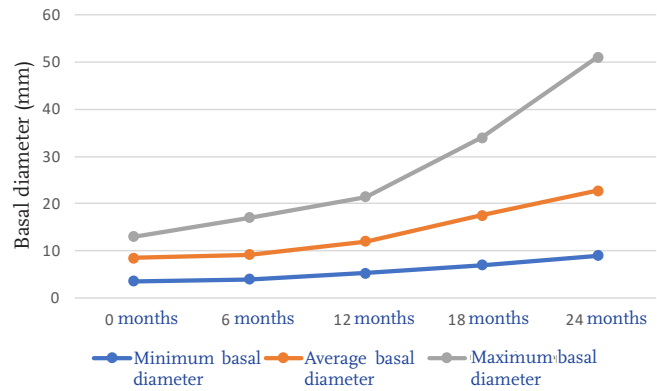


Figure 3. Basal diameter growth of *P. greggii* Engelm. ex Parl. in Huehuetla, Hidalgo.

Ortiz et al. (2021) tested 13 provenances from northern and central Mexico on eroded soils in the Mixteca region of Oaxaca, finding high adaptability. Based on these results, the provenance from Estado de México was selected. Similarly, Flores et al. (2014) recommend the use of any provenance due to their strong performance, particularly as *P. greggii* is native to the Sierra Madre Oriental, including the state of Hidalgo (Domínguez-Calleros et al., 2017). Hernández-Martínez et al. (2007) also note that *P. greggii* Engelm. var. *australis* grows naturally in the municipality of Tenango de Doria, Hidalgo, which borders Huehuetla, thus potentially extending the species' adaptation range.

P. greggii has been used for its rapid growth and potential in programs aimed at the restoration of degraded soils and carbon sequestration. López et al. (2017) estimated the amount of carbon stored in soils associated with *P. greggii* and *P. oaxacana*, finding high levels of carbon both in the soil and leaf litter, and recommended their use on soils under extreme erosion conditions. Similarly, Muñoz et al. (2012) evaluated the survival and growth of *P. greggii* during the initial growth stage, finding that after six years, individuals exceeded five meters in height with a survival rate of 60 %.

In this context, the initial merchantable volume of pines was $1.475 \times 10^4 \cdot m^3$ obtained after 24 months of monitoring. When expressed as carbon, this corresponds to 46.94 g per tree using the CONAFOR method. Using

the method by Acosta et al. (2009), the estimated carbon content was 64.98 g, and according to the IPCC (2005) method, 73.5 g were obtained. From these results, an average of 61.81 g of carbon stored in the aboveground biomass of each tree was calculated, representing 226.84 g of CO₂ removed from the atmosphere. This conversion considers the molecular weight ratio between CO₂ (44) and carbon (12) (Jiménez et al., 2013; Robledo, 2015; Jáuregui et al., 2022). Furthermore, Pacheco et al. (2007) highlighted the importance of *P. greggii* for carbon capture, reporting a storage potential of 17.9 t·ha⁻¹ during the first six years, equivalent to 65 t·ha⁻¹ of accumulated carbon dioxide. These findings support the classification of this species as having high carbon sequestration potential. The variations in carbon storage estimates are attributed to the use of indirect methods, which calculate tree biomass using allometric equations or mathematical models based on regression analyses involving tree variables such as diameter at breast height (measured at 1.30 m), commercial (hc) and total height (ht), diameter growth, basal area, and wood specific gravity. Alternatively, estimates can be derived from stem volume, dry weight, and an expansion factor to calculate total tree weight. The “average tree” technique is also used, which assumes that the average-sized tree contains an average quantity of biomass (Fonseca et al., 2013; Fonseca-González, 2017).

Moreover, tropical species have also been used due to their important environmental role, as tropical forests can capture more than 40 t·ha⁻¹ of CO₂ per year, equivalent to 2.3 to 10 t of carbon (Robledo, 2015). Aguirre et al. (2018) estimated that in an Andean forest in Ecuador, one hectare can capture 42.29 t·ha⁻¹ of carbon accumulated in the tree, shrub, herbaceous, and necromass strata; when considering only the tree stratum, the value was 26.56 t·ha⁻¹. Similarly, Seppänen (2002) reported that in southeastern Mexico, high-yield eucalyptus plantations (40 m³·ha·yr) can sequester between 320 and 610 t·ha⁻¹ of CO₂ over a seven-year period, equivalent to 91 to 175 t·ha⁻¹ of carbon. As early as 2007, the Ibero-American Network on Environmental Physics and Chemistry published several works on carbon capture in terrestrial ecosystems across Ibero-America, highlighting the growing need to evaluate this environmental service in economic terms (Gallardo, 2007).

The underlying principle is that forest ecosystems can capture significant amounts of greenhouse gases (GHGs), especially CO₂. For this reason, there has been increasing interest in recent decades in enhancing carbon stocks in vegetation through forest conservation, reforestation, and related strategies. Many studies have demonstrated the capacity of forest species to store carbon in their biomass (Rodríguez et al., 2016; Ronquillo-Gorgúa et al., 2022). This capacity has led to the development of payment schemes for environmental services related to carbon capture, contributing to forest conservation, soil recovery in deforested areas, and the restoration of degraded ecosystems.

Conclusions

After 24 months of plantation establishment, the increase in height has been greater than 90 cm, while basal diameter has increased more than 1.5 cm, and diameter at breast height exceeded 1 cm. After 12 months, 28% of the trees had surpassed the minimum height of 1.30 m required to measure diameter at breast height (DBH), reaching 85% by the final measurement. This growth has resulted in an initial volume that, when quantified as carbon stored per tree, exceeds 60 g.

The contribution of this species to mitigating climate change may be significant, as reforestation and afforestation efforts are a priority strategy for capturing excess atmospheric carbon dioxide while simultaneously promoting the conservation of native forests.

Local climate matchin is expected to expand the distribution range of *P. greggii*, since regional temperatures fall within the species' thermal tolerance. This makes it a promising candidate for reforesting unproductive or degraded lands requiring conversion to forest use.

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English version

Popcorn from native maize with normal endosperm produced using a cannon puffing machine

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Abstract

Maize is the most widely produced cereal in the world and is the basis for many processed products, including snacks and breakfast cereals. One of the main technologies used to process whole grains is the cannon puffing machine; however, its application in maize popping has been scarcely studied. The objective of this study was to evaluate the puffed quality of native maize varieties with different endosperm hardness levels using a cannon puffing machine, to validate the performance of a semi-industrial device. Native popping maize and maize with normal endosperm were evaluated under different processing conditions. Expansion volume (EV), percentage of unpuffed (UPK) and partially puffed kernels, expansion shape, moisture content, and grain hardness were determined. The best processing conditions were achieved using grains without moisture conditioning and an operating pressure of 1.03 MPa in the cannon puffing machine. Under these conditions, no unpuffed kernels (UPK) were observed, and an average EV of $11.8 \text{ cm}^3 \cdot \text{g}^{-1}$ was obtained, with a mushroom shape and low moisture content. Puffed maize is a healthy snack alternative that adds value to native maize varieties, regardless of their endosperm hardness.

► **Keywords:** shape of puffed maize, floatation index, operating pressure, expansion volume.

Introduction

Maize is the most widely produced cereal in the world and is the basis for a wide variety of food products. In Mexico, approximately 600 food and beverage items made from maize have been reported (Pérez-Ruiz et al., 2024). In the snack and breakfast cereal industries, maize plays a key role as the primary raw material (Fast et al., 2020; Serna-Saldívar & Chuck-Hernandez, 2019). One of the most used technologies for processing maize in these industries is extrusion, which involves a mixture of corn grits with water (15-18 %) and various flavoring ingredients. This mixture is passed through an extruder, where it is subjected to high pressure and short residence times (Sharifi et al., 2021).

An alternative method for processing maize kernels is the use of a cannon puffing machine (Lee et al., 2019), which allows for the processing of whole grains such as rice, wheat, barley, and oats. In this method, grains are first conditioned to a moisture content between 13 and 15 % (Lee et al., 2019; Mariotti et al., 2006) and then introduced into the cannon chamber, where they are heated and subjected to pressures ranging from 0.5 to 1.5 MPa (Jia et al., 2021; Lee et al., 2019). Subsequently, the sudden release of the chamber causes rapid decompression, which leads to grain expansion.

The principle behind this technology lies in the generation of superheated steam within the grain, where the pericarp acts as a micro-pressure chamber that traps the vapor.

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Intense heating induces a glass transition in the grain matrix, making it more extensible and allowing internal diffusion of the vapor. Upon the opening of the expansion chamber, the steam escapes abruptly, expanding the grain and forming a new internal structure (Mounir et al., 2023).

The use of a puffing cannon machine for maize processing has received limited attention in scientific literature. Although studies by Mrad et al. (2014) and Rajha et al. (2021) have examined Peruvian maize, they do not provide information on grain characteristics. Therefore, the aim of this study was to assess the puffed quality of native maize varieties with varying endosperm hardness using a puffing cannon machine to validate the performance of a semi-industrial device developed by the Centro Nacional de Estandarización de Maquinaria Agrícola, at the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Mexico.

Materials and methods

A total of three experiments were conducted to identify the optimal conditions for processing maize using a lab-scale cannon puffing machine (JCK-Baomihua, JVSUFUIK, China). In the first experiment, three landrace maize (Palomero Toluqueño, Chapalote, and Arrocillo) were used. These landraces are known for their high kernel hardness and their ability to pop using traditional methods such as pan, microwave, and hot air. Two moisture levels (13 and 14 %) were tested, based on previous reports indicating that these conditions are optimal for achieving higher expansion volumes (Sweley et al., 2013). The operating pressure in the cannon puffing machine was set at 0.98 MPa (Jia et al., 2021).

The second experiment aimed to determine whether puffed maize could be produced from varieties with lower

endosperm hardness. Therefore, native maize samples were selected and classified into three categories based on endosperm hardness: hard (H), intermediate (I), and soft (S). All samples were conditioned to 14 % moisture content (the optimal level identified in the first experiment) and processed at 0.98 MPa using the cannon puffing machine. In the third experiment, unconditioned maize samples with moisture contents between 11 and 13 % were used, and the operating pressure was increased to 1.18 MPa. Maize types included hard (H), intermediate (I), soft (S), and very soft (VS) endosperm categories. Maize conditioned to 14 % moisture was not used in this experiment, as it was observed that, under this pressure, the grains lost their mushroom shape and instead developed a butterfly-like form (Figure 1).

In all three experiments, a lab-scale cannon puffing machine with an 80 mL capacity was used. Each sample consisted of 70 g. Once the processing conditions were identified in the laboratory, they were scaled up to a semi-industrial cannon puffing machine. (Figure 2). At this stage, native maize samples without moisture conditioning were processed at a pressure of 1.03 MPa (Oscoco-Quispe, 2013), using a sample size of 1 kg. All measurements in both the lab-scale and semi-industrial cannon puffing machines were performed in duplicate.

Grain characterization and puffed quality

Moisture content (MC), flotation index (FI), and 100-kernel weight (HKW) were determined in the grains (Vázquez-Carrillo et al., 2023). Regarding FI, values from 0 to 12 correspond to very hard maize grains, 13 to 37 to hard maize grains, 38 to 62 to intermediate maize grains, 63 to 87 to soft maize grains, and >88 to very soft maize grains. Meanwhile, HKW values greater than 38 g indicate large maize grains, 33 to 38 g medium maize grains, and less

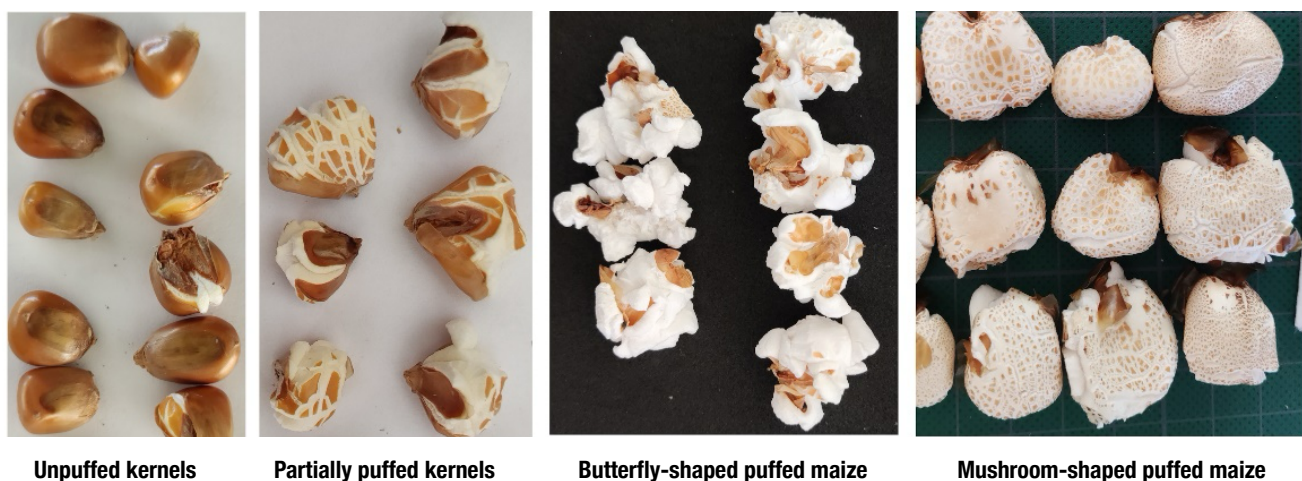


Figure 1. Shapes of normal-endosperm maize kernels obtained through processing with a cannon puffing machine.

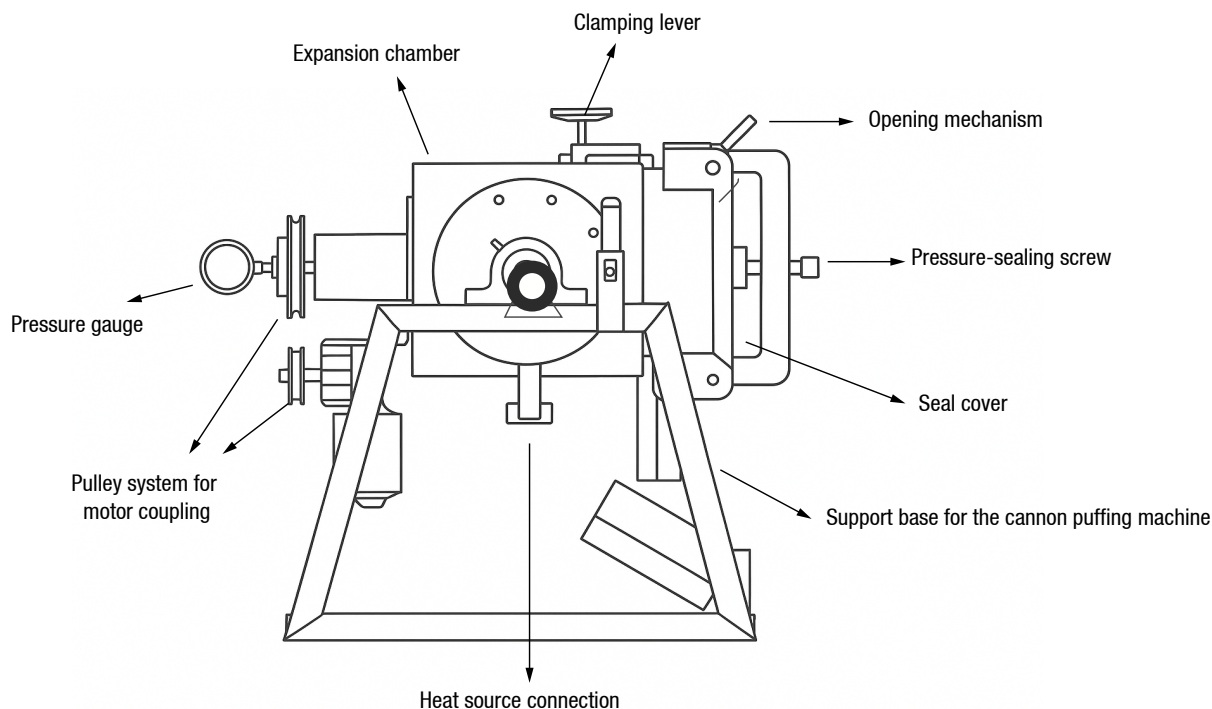


Figure 2. Diagram of the cannon puffing machine manufactured by the *Centro Nacional de Estandarización de Maquinaria Agrícola*.

than 33 g small maize grains. To evaluate puffed quality, expansion volume (EV; $\text{cm}^3 \cdot \text{g}^{-1}$) was estimated based on the total volume of puffed kernels in a 1000 mL measuring cylinder (García-Pinilla et al., 2019). Additionally, unpuffed kernels (UPK) and partially puffed kernels (PPK) were counted (Figure 1) to calculate their percentages (Sweley et al., 2013). The expanded kernels were separated according to their shape (mushroom or butterfly), and their percentages were estimated. The moisture content of the puffed maize was determined using method 44-15.02 of the American Association of Cereal Chemists (AACC, 2020). To evaluate texture, 10 kernels were randomly selected and analyzed using a texture analyzer (model CT3, Brookfield, USA) with a 25.4 mm diameter spherical probe. The test speed was $1 \text{ mm} \cdot \text{s}^{-1}$, and the target deformation was 10 mm. Results were expressed in Newtons (N).

Data analysis

In experiment one, a factorial design nested within a completely randomized design was used, with two sources of variation: genotype (three levels) and conditioning moisture (two levels). In the subsequent experiments, a completely randomized design with a single factor (genotype) was implemented. In all cases, an ANOVA was performed, followed by Tukey's multiple comparison test at a 5 % significance level. Data was analyzed using the SAS software (SAS Institute Inc., 2002).

Results and discussion

Native popping maize

The Palomero Toluqueño, Arrocillo, and Chapalote landraces are ancient indigenous maize known for their ability to pop (Wellhausen et al., 1951). However, this trait has been gradually lost as their germplasm mixed with other types of maize (Bautista-Ramírez et al., 2020). These varieties are also at risk of extinction due to their low productivity (Gámez-Vázquez et al., 2014).

The analysis of the genotype factor showed that processing with a cannon puffing machine produced higher expansion volumes (Table 1) than previously reported for grains of the same landraces (Bautista-Ramírez et al., 2020; Vázquez-Carrillo et al., 2019), with low percentages of unpuffed kernels and a predominance of the butterfly shape. Final moisture values were similar to those reported for popcorn processed by microwave or hot air methods (Ranathunga et al., 2016). Significant differences ($p < 0.05$) observed in the puffed quality variables can be attributed to the inherent characteristics of each landrace, although factors such as soil conditions, harvest and postharvest practices, and grain composition also play a role (Sweley et al., 2013).

Conditioning moisture had a significant effect ($p < 0.05$) on VE, UPK, and the moisture content of the product.

Table 1. Comparison of mean values for puffed quality variables in conditioned native maize processed with a cannon puffing machine.

Genotype	EV (cm ³ ·g ⁻¹)	UPK (%)	MS (%)	BS (%)	Moisture (%)
Toluqueño	19.45 b	2.85 b	8.64 c	79.6 a	2.97 b
Arrocillo	21.14 a	0.36 b	26.86 a	69.5 b	5.07 a
Chapalote	17.43 c	19.44 a	18.13 b	57.2 c	5.0 a
HSD	1.35	2.9	7.73	8.36	0.32
Conditioning					
13 %	18.56 b	9.26 a	17.09 a	68.98 a	4.55 a
14 %	20.12 a	5.83 b	18.66 a	68.6 a	4.15 b
HSD	0.878	1.89	5.04	5.44	0.21

EV: expansion volume; UPK: unpuffed kernels; MS: mushroom shape; BS: butterfly shape; HSD: honestly significant difference. Different letters within each column and each source of variation indicate significant differences (Tukey, $p < 0.05$).

An initial kernel moisture of 14 % resulted in the highest VE, as well as the lowest UPK and moisture content in the puffed kernels (Table 1). Several studies with popcorn maize hybrids have reported that conditioning moisture is a critical factor for achieving optimal popping characteristics (high VE and low UPK), as moisture levels below 12 % or above 15 % reduce VE (Cañizares et al., 2024; Gökmen, 2004). Additionally, this parameter also affects the shape of the puffed kernel (García-Pinilla et al., 2019).

Furthermore, it was demonstrated that maize with very hard endosperm (VH, FI 0 to 9 %) can also be puffed using a cannon puffing machine. The best results, in terms of higher VE and fewer UPK, were achieved at 14 % conditioning moisture. Consequently, this condition was applied in the second experiment.

Native maize with different endosperm hardness conditioned

Puffed maize using a cannon puffing machine has been scarcely studied. In this regard, Oscco-Quispe (2013) worked with hard yellow maize, while Rajha et al. (2021) evaluated different Peruvian maize samples (Morado, Chulpe, and Cancha). In both studies, the kernels were conditioned to 25–30 % moisture. However, maize exhibits considerable variability in shape, size, and endosperm hardness (López-Morales et al., 2023). Therefore, this stage aimed to evaluate the feasibility of obtaining puffed maize from kernels with different endosperm hardness.

The results showed that it is possible to pop dent maize classified as H, I, and S, with a flotation index between 28.5 and 75 % and varying 100-kernel weights (Table 2). In all cases, the proportion of semi-puffed kernels (PPK) was higher than that of unpuffed kernels (UPK) (Table 2). Additionally, high expansion volumes (VE) were obtained (19.4 to 21.89 cm³·g⁻¹), and no direct relationship was observed between endosperm hardness and VE. This contrasts with the hypothesis that harder maize produces higher VE, while softer maize generates lower

expansion volumes. No significant differences ($p > 0.05$) were found in UPK percentages or in kernel shape, with the butterfly form predominating. The moisture content of the puffed maize ranged from 2.57 to 5.28 %, with significant differences.

From this stage, it can be concluded that using a cannon puffing machine makes it possible to pop maize of different sizes and endosperm hardness, which would not pop with conventional methods (pan, microwave, and hot air). However, the mushroom shape, typical of puffed maize, did not predominate.

Native maize without conditioning

The results of this test demonstrated that it is possible to obtain puffed maize from kernels with varying endosperm hardness (Table 3), ranging from very hard (VH) to very soft (VS), with a FI of 5 to 95.5 %. However, in tests conducted with highly floury maize (FI > 98 %), such as the Cacahuacintle and Conical landraces, it was observed that the kernels did not expand and were only toasted (data not shown).

The EV ranged from 11.07 to 25.72 cm³·g⁻¹, which is consistent with values reported for some popcorn maize hybrids (Zulkadir & Idikut, 2021). Similarly, low PPK and UPK values were obtained (Table 3), even lower than those reported using the pan-popping method, one of the most efficient for heat transfer to induce popping (Gökmen, 2004). No direct relationship was found between endosperm hardness and HWK with EV. However, the shape adopted by the kernel upon expansion (mushroom or butterfly) could influence EV. This is because butterfly-shaped puffed maize kernels have appendages that prevent compactness in the measuring cylinder, leaving empty spaces, whereas mushroom-shaped kernels are more compact (Figure 1) resulting in fewer empty spaces.

García-Pinilla et al. (2019) mention that in mushroom-shaped puffed maize kernels, the starch granule undergoes minimal expansion, maintains its structure, and retains the

Table 2. Comparison of grain characteristics and puffed quality variables in native maize with different endosperm hardness using a cannon puffing machine.

Hardness	FI (%)	HKW (g)	EV (cm ³ ·g ⁻¹)	PPK (%)	UPK (%)	MS (%)	BS (%)	MPM (%)
H1	28.5 e	33.85 ab	20.81 ab	0.24 b	1.01 a	34.56 a	64.4 a	3.18 cd
H2	33.5 de	26.31 b	19.88 ab	1.79 ab	0.45 a	19.22 ab	79.02 a	2.57 d
I1	56.5 bc	30.64 b	17.42 c	5.55 ^a	1.32 a	26.45 ab	71.75 a	3.5 c
I2	47.0 cd	30.40 b	20.08 ab	3.26 ab	0.0 a	15.83 b	81.75 a	5.28 a
S1	75.0 a	33.89 ab	19.4 bc	1.04 b	0.69 a	21.21 ab	76.52 a	4.98 ab
S2	67.0 ab	45.22 a	21.89 a	0.66 b	0.33 a	19.09 ^{ab}	77.67 a	4.35 b
HSD	15.54	11.52	2.36	4.46	3.09	15.78	27.26	0.72

H: hard; I: intermediate; S: soft; FI: flotation index; HKW: 100-kernel weight; EV: expansion volume; PPK: partially puffed kernels; UPK: unpuffed kernels; MS: mushroom shape; BS: butterfly shape; MPM: moisture content in puffed maize; 1 and 2: correspond to the analyzed population; HSD: honestly significant difference. Different letters within each column and each source of variation indicate significant differences (Tukey, $p < 0.05$).

Table 3. Comparison of mean values of kernel characteristics and popping quality variables in native maize with different endosperm hardness, without moisture conditioning, and processed in a cannon puffing machine.

	FI (%)	HKW (g)	EV (cm ³ ·g ⁻¹)	PPK (%)	UPK (%)	MS (%)	BS (%)	MPM (%)	HPM (N)
VH1	5.0 g	27.19 h	22.93 ab	0.31 a	1.5 a	66.12 cd	33.88 ab	4.23 a	21.5 e
VH2	9.0 g	29.43 g	19.86 bc	0.0 a	0.0 c	84.98 ab	15.02 cd	3.53 ab	47.61 bc
H1	13.5 fg	31.26 f	25.72 a	0.0 a	0.0 c	65.95 cd	34.06 ab	3.54 ab	62.90 a
H2	29.0 ef	21.45 i	14.11 de	1.3 a	0.45 bc	69.27 bcd	30.73 abc	2.55 cde	65.61 a
I1	55.0 d	38.95 e	16.27 cd	0.0 ^a	0.96 abc	77.74 abc	22.27 bcd	2.11 de	33.52 d
I2	41.0 de	56.74 c	11.07 e	0.84 a	0.42 bc	91.68 a	8.32 d	2.96bcd	50.38 b
S1	73.5 c	42.28 d	25.0 a	0.0 a	0.0 c	72.10 bc	21.91 bc	3.22 bc	47.53 bc
S2	75.5 bc	62.55 b	21.72 ab	0.93 a	0.0 c	64.41 cd	35.60 ab	3.38 abc	43.87 c
VS1	95.5 a	81.72 a	18.42 bcd	0.0 a	0.12 c	69.33 bcd	30.67 abc	3.38 abc	43.67 c
VS2	91.5 ab	42.45 d	15.50 cde	0.0 a	1.41 ab	54.60 d	45.41 a	1.82 e	37.26 d
HSD	17.98	0.53	4.58	2.31	1.0004	17.12	17.12	0.94	4.31

VH: very hard; H: hard; I: intermediate; S: soft; VS: very soft; FI: Flotation index; HKW: 100-kernel weight; EV: expansion volume; PPK: partially puffed kernels; UPK: unpuffed kernels; MS: mushroom shape; BS: butterfly shape; MPM: moisture content in puffed maize; HPM: hardness of puffed maize; 1 and 2: correspond to the population studied. HSD: honestly significant difference. Different letters within each column and each source of variation indicate significant differences (Tukey, $p < 0.05$).

hilum, which served as the pathway for water migration. In contrast, butterfly-shaped puffed maize kernels with more than one appendage likely form due to the presence of two main vapor release sites, which also generate larger cavities.

In the unconditioned kernels and at higher working pressure, the mushroom shape predominated (Table 3), which is typical of puffed products (Mariotti et al., 2006). The moisture content of the puffed maize (MPM) was slightly lower compared to that obtained from kernels conditioned to 14 % moisture (Tables 2 and 3), with a hardness ranging from 21.5 to 65.61 N, indicating a porous and crunchy texture.

Native maize processed in a semi-industrial cannon

Based on the previous results, which demonstrated the possibility of puffing kernels with different endosperm hardness (from very hard to very soft), maize with similar

hardness was selected for processing in a semi-industrial cannon (Table 4).

Under this condition, 100 % of the kernels expanded and maintained the mushroom shape (Table 4, Figure 3), with an average processing time of 11.36 min. The main difference between processing in the semi-industrial cannon and the laboratory cannon was the expanded volume, which was reduced by almost half (Tables 3 and 4). This can be attributed to the shape of the puffed kernel, as the absence of the typical appendages of the butterfly shape resulted in a smaller sample volume. The values of MPM and HPM ranged from 2.99-3.52 % and 35.86-54.21 N, respectively, similar to those obtained with the laboratory cannon (Table 3). It is important to mention that the puffed kernels were obtained from maize without moisture conditioning, which represents an advantage in processing. Several studies report the need to condition maize to high moisture levels for

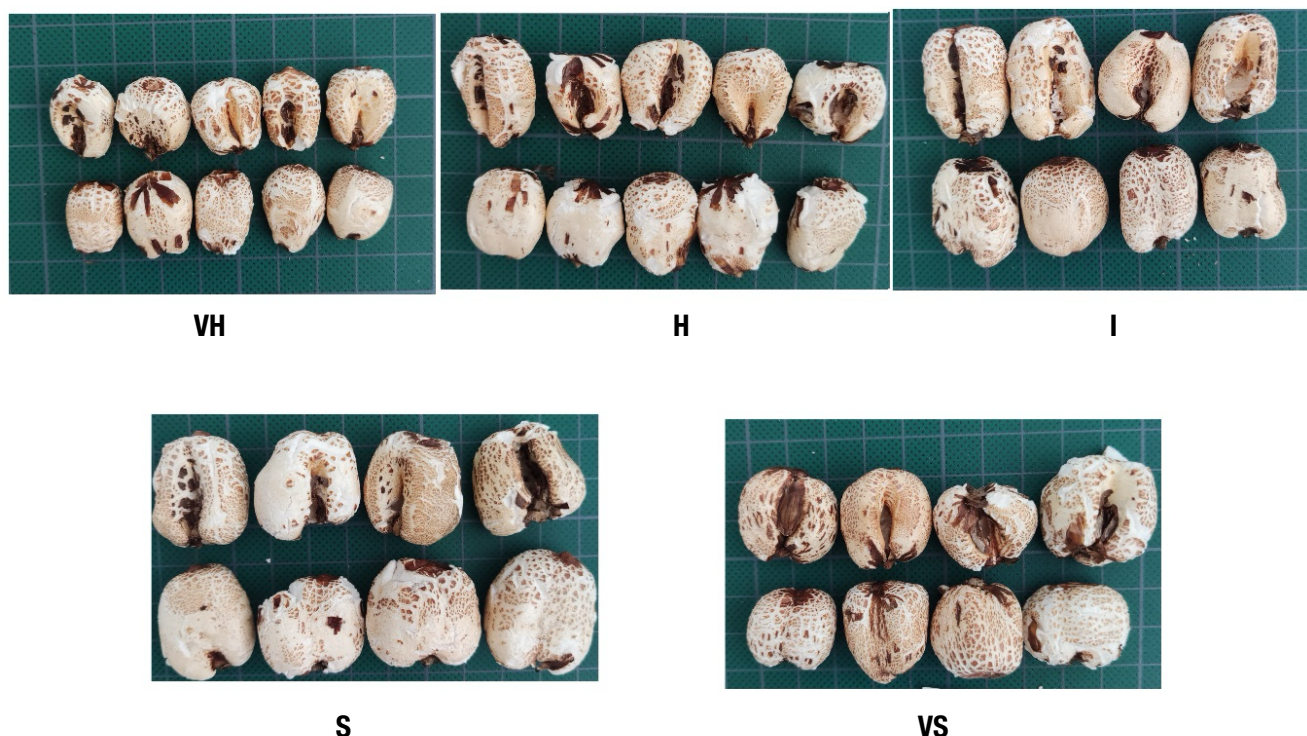


Figure 3. Native maize with different endosperm hardness processed in a semi-industrial cannon. The grid represents 1 cm². VH: very hard; H: hard; I: intermediate; S: soft; VS: very soft.

Table 4. Comparison of mean values of kernel characteristics and puffed quality variables in native maize with different endosperm hardness, without moisture conditioning, and processed in a semi-industrial cannon puffing machine.

	FI (%)	HKW (g)	KM (%)	Processing time (min)	EV (cm ³ ·g ⁻¹)	MS (%)	MPM (%)	HPM (N)
VH	9.0 e	26.13 e	13.15 a	11.19 a	11.58 a	100	3.08 b	47.84 b
H	36.0 d	30.97 d	12.15 bc	11.28 a	11.98 a	100	3.52 a	35.86 d
I	63.0 c	54.40 c	11.75 c	11.5 a	11.93 a	100	2.99 b	54.21 a
S	75.5 b	62.56 a	12.15 bc	11.36 a	12.0 a	100	3.09 b	41.49 c
VS	93.5 a	58.54 b	12.4 b	11.48 a	11.8 a	100	3.31 ab	38.12 cd
HSD	7.39	3.71	0.51	0.51	0.52		0.39	3.41

VH: very hard; H: hard; I: intermediate; S: soft; VS: very soft; FI: Flotation index; HKW: 100-kernel weight; KM: kernel moisture; EV: expansion volume; MS: mushroom shape; MPM: moisture content in puffed maize; HPM: hardness of puffed maize. HSD: honestly significant difference. Different letters within each column and each source of variation indicate significant differences (Tukey, $p < 0.05$).

processing in expansion cannons (Mrad et al., 2014; Oscco-Quispe, 2013; Rajha et al., 2021). This requirement adds additional steps to the process and requires greater control, as increased moisture can raise the risk of fungal contamination.

Conclusions

Using the cannon puffing method, it is possible to obtain popcorn with low percentages of unpuffed kernels and a butterfly shape from ancient indigenous maize varieties that have lost their popping ability.

Conditioning the kernels to 14 % moisture allowed for high expansion volumes (19.4-21.9 cm³·g⁻¹) and butterfly-shaped puffed kernels. In contrast, higher operating pressure without moisture conditioning favored the formation of mushroom-shaped kernels, typical of puffed maize.

The expansion of native dent maize, with endosperm classified from very hard to very soft (floating indices from 9.0 to 93.5 %) and varying kernel sizes, is achievable using a semi-industrial cannon puffing machine operated at 1.03 MPa. The resulting product exhibits low moisture, along with desirable porosity and a crunchy texture.

In the context of new public policies promoting the reduction of junk food in schools, puffed maize represents a viable option as a healthy snack. Its production from native maize without a specific prior use allows for added value through a fast process that generates a ready-to-eat food product.

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Versión en español

Factors influencing avocado and coffee production in Huatusco, Veracruz

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Abstract

Avocado and coffee production sustain the agricultural economy of Huatusco, Veracruz; however, it faces significant challenges such as coffee leaf rust and other pathogens. Therefore, the objective of this research is to develop a biophysical and descriptive characterization to analyze the environmental conditions, agricultural practices, and socioeconomic factors influencing the production of both crops. This approach will enable the development of strategies to optimize production, promote sustainability, and strengthen the resilience of local producers. The methodology consisted of a biophysical characterization based on cartographic analysis, complemented by a descriptive characterization using both local and technical knowledge related to crop management, challenges faced, and actions taken to mitigate impacts. The results indicate that shaded coffee production accounts for 69% of the agricultural land, while avocado covers 28 %. Avocado production shows good development, although it is affected by pathogens and market fluctuations. The coffee sector continues to struggle with persistent issues such as coffee leaf rust and a shortage of labor, which has led to increased production costs. Based on these findings, the implementation of specific management strategies is recommended: for avocado, improving soil management and the adoption of drip irrigation systems are necessary; for coffee, the Colombia and Costa Rica varieties remain productive and rust-resistant, although drought has adversely affected the flowering stage. Enhanced soil and water management practices are needed, along with efforts to address labor shortages that are contributing to rising production costs.

► **Keywords:** Agricultural practices, irrigation, phytosanitary control, *Persea americana* var. Hass, *Coffea arabica* var. Colombia and Costa Rica.

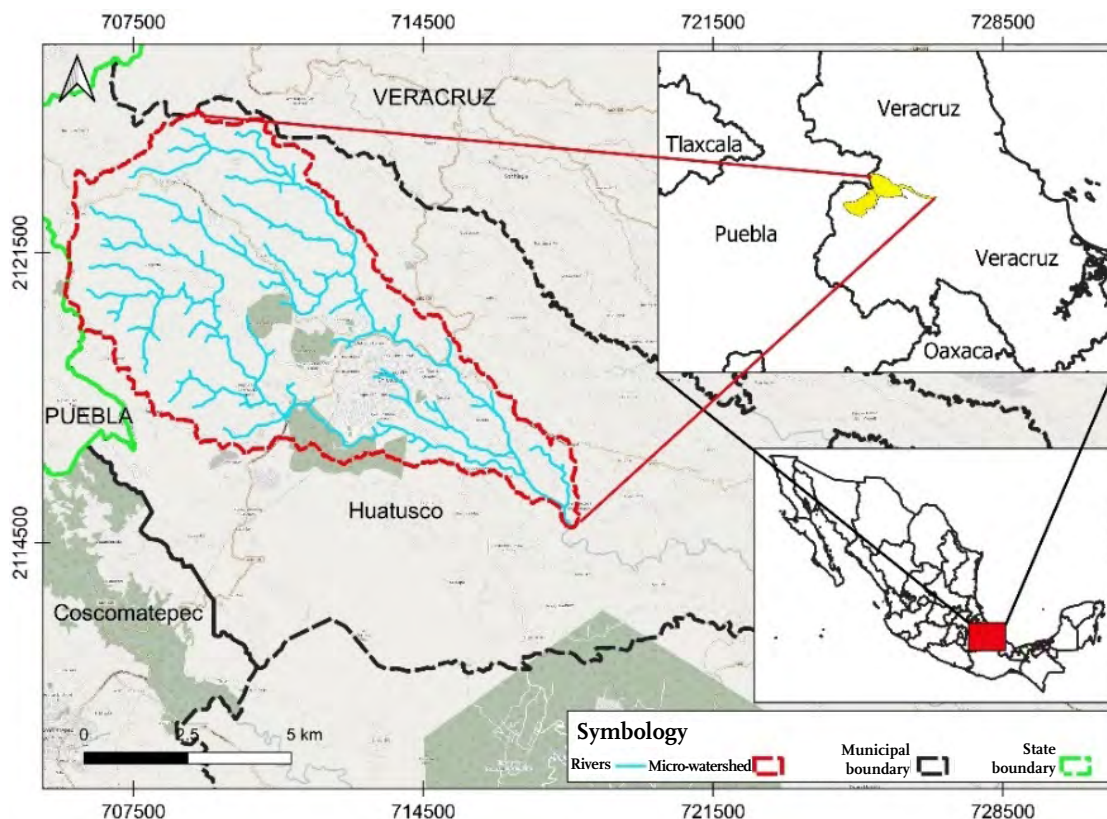
Introduction

The biophysical and descriptive characterization of crops is an important aspect for understanding and optimizing agricultural practices, especially in regions with diverse crop systems such as the central micro-watershed of Huatusco, Veracruz. Biophysical characterization involves the analysis of physical factors such as soil, climate, topography, and water availability (Nkiaka et al., 2024). Evaluating the biophysical aspects of agricultural systems through their physiographic and climatic characteristics

provides communities with specific information that is useful for decision-making in production management (Ruiz-García et al., 2020). Descriptive characterization, on the other hand, involves the identification of crop varieties, the agricultural practices employed, and their adaptation to different climatic conditions, soil types, water requirements, pests, and diseases, as well as certain socioeconomic factors influencing production. This characterization is essential for informed decision-making related to the management and optimization of agricultural yields (Malagón & Prager, 2001).

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Figure 1. Geographic spaces of the study area.



Source: Compiled by the authors using information from INEGI and CONABIO.

In this context, the central micro-watershed of Huatusco shows optimal biophysical characteristics for the establishment of permanent agricultural systems: primarily shaded coffee (Sc) and avocado (Av), which are essentially the main pillars sustaining the local economy (García et al., 2023). However, the production of these crops is commonly affected by crop-specific pests and diseases, climatic variability, inadequate soil and water resource management, and the influence of market dynamics. These factors have a direct impact on the economic stability of producers (Ploetz, 2013; Bunn et al., 2015).

As previously mentioned, one of the most significant crops in this micro-watershed is coffee (*Coffea arabica*), as Mexico ranks 11th among global green coffee producers and 1st in organic coffee production (Centro de Estudios para el Desarrollo Sustentable y Soberanía Alimentaria, 2020). Approximately 80 % of national production is geared toward export, while the remaining 20 % is destined for the domestic market (Fideicomisos Instituidos en Relación con la Agricultura, 2019). This industry accounts for 0.66 % of the national agricultural GDP, with Veracruz contributing 24 % (Gobierno del Estado de Veracruz, 2019), making it a significant source of foreign exchange and employment (Martínez-López et al., 2022).

In addition to coffee, avocado cultivation (*Persea americana* var. Hass) has increased in the region, driven by a significant rise in international demand over the past decade. In response, Mexico has positioned itself as the global leader in avocado production and export, contributing 32 % of the total export value worldwide (García et al., 2023). Although the state of Veracruz accounts for only 0.29 % of national production (García et al., 2023), the establishment of this crop—particularly the Hass variety—has expanded notably over the past 20 years. This growth has had a positive impact on the regional economy by generating multiple sources of employment (DANE, 2016).

Considering the economic significance of these crops, it is essential to sustain their productivity and long-term viability. This underscores the need for in-depth knowledge of the factors influencing their establishment and performance. The aim of this characterization is to deliver a comprehensive overview of the biophysical conditions, management practices, existing challenges, and both corrective and preventive strategies that affect the development of coffee and avocado cultivation within the micro-watershed. The resulting information is intended to support decision-makers and producers in optimizing productivity, minimizing risks associated with climatic

and soil-related factors, and ensuring the resilience and continuity of local agricultural systems.

Materials and Methods

Study area

The study area is the central micro-watershed of the municipality of Huatusco, Veracruz, located between parallel 19° 01' N and meridian 97° 03' W (Figure 1). This micro-watershed is part of the Jamapa river basin, which belongs to the Central Gulf Administrative Hydrological Region. The area has an average annual temperature of 17.5 °C and average annual precipitation of 1 750 mm. The micro-watershed was selected as it presents a variety of biophysical conditions suitable for agricultural systems, specifically coffee and avocado, which allow us to find out which conditions are best for crop growth.

This research was conducted following these stages: i) review of official cartographic information and creation of reference maps; ii) field reconnaissance of the study area and establishment of control points to develop soil type, land use, and vegetation maps; iii) preparation of specific cartography for the study area based on field data; iv) biophysical characterization of the reference crops; and v) description of crop varieties, distribution, planting density, agricultural practices employed, and their adaptation to varying climatic conditions, soil types, water requirements, pests, and diseases, and socioeconomic factors influencing production.

Biophysical characterization

This characterization was conducted through the analysis of cartographic data related to the following biophysical aspects: climate, relief, slope, altitudinal range, soil type, and vegetation.

Data and information sources

Slope and altitudinal range data were obtained from the Continuo de Elevaciones Mexicano 3.0 (CEM 3.0) by INEGI (2023). To generate the soil type map, targeted sampling was carried out in agricultural production areas. Sample collection was supported by local knowledge from producers and the edaphological vector dataset at a scale of 1:250,000, Series II (INEGI, 2014). Land use and vegetation cartography was generated through supervised classification based on 2023 SENTINEL satellite images provided by the United States Geological Survey (USGS). Meanwhile, climatic data were extracted from the climate, precipitation, and average annual temperature layers provided by CONABIO.

Land use and vegetation mapping

A supervised classification was performed based on the preprocessing of SENTINEL satellite images, using the

Semi-Automatic Classification Plugin (SCP) in QGIS (Congedo, 2021). A false-color band composite (bands 4, 3, and 2) was used to define six land cover classes: urban, temperate forest, shaded coffee, avocado, lemon, and grassland. This approach enabled the identification and quantification of surface areas, distribution, and the biophysical conditions in which the reference agricultural systems are established.

Soil type mapping

This map was developed based on data reported by INEGI and was supplemented with soil sampling conducted in agricultural areas. The sampling was carried out with the support of local knowledge, following the methodology proposed by Ortiz et al. (1990), which involves conducting field surveys with farmers to generate control points and establish the boundaries of different soil types. The sampling intensity depended on the frequency of crop cultivation in the study area. A total of 15 composite samples were collected throughout the micro-watershed and were distributed across the upper, middle, and lower sections of the plots.

Subsequently, laboratory analysis of the samples was conducted to determine the values and parameters of the following physical and chemical properties: color, texture, permanent wilting point (PWP), field capacity (FC), electrical conductivity (EC), porosity (P), pH, and bulk density (BD). Based on the report provided by INEGI, the laboratory data, and the methodology of Ortiz Solorio, a new taxonomic classification of the region's soils, was developed according to the World Reference Base for Soil Resources (FAO, 2014). Finally, the soil type map was produced using this updated taxonomic classification.

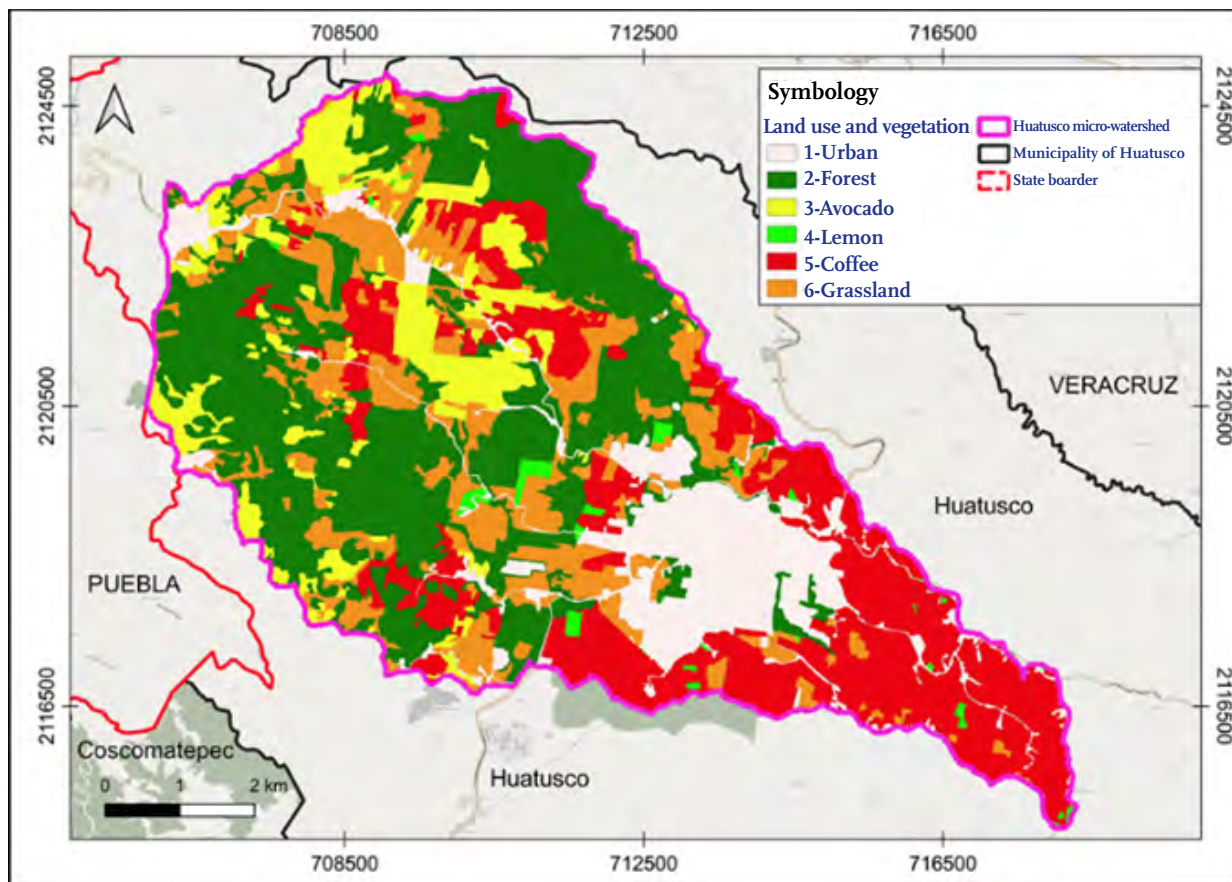
Slope and altitudinal range mapping

The slope and altitudinal range maps were generated by processing data from the CEM using the QGIS interface. The initial slope values were reclassified to produce a new classification expressed in percentage ranges: 0-2, 2-4, 4-8 and de 8-15 %. The altitudinal range was derived by generating a contour layer, which was used to define elevation intervals of 250 meters.

Characterization of agricultural crops

The description of the agricultural crops was developed through on-site participation with the producers. Field visits were carried out with 15 informants per crop, following the methodology of Ortiz-Solorio & Gutiérrez-Castorena (1999), which involves complementing local knowledge with technical expertise to provide a detailed characterization of the agricultural crops. The informants were selected for being members of the community with extensive knowledge of crop management, specifically coffee and avocado. The producers also contributed information to describe the activities required to achieve optimal

Figure 2. Distribution and area covered by the reference crops: avocado and coffee.



Source: Compiled by the authors.

production, the environmental and socioeconomic challenges they face, and the actions they take to mitigate these impacts.

Results and Discussion

Distribution and area covered by the reference crops

According to the supervised classification, the micro-watershed evaluated covers a total area of 5 939.5 hectares, where agricultural land use predominates with 35.4 % (2 104.3 ha), followed by temperate pine forest at 34.2 % (2 031.2 ha), and areas designated for livestock at 17.2 %.

Shade-grown coffee is the main crop in the agricultural area, covering 1 457 hectares (69.2 %) and mostly situated in the lower part of the micro-watershed. In contrast, avocado is the second most prominent crop, occupying 592.4 hectares (28.2 %) and primarily located in the upper part of the micro-watershed (Figure 2).

Influence of biophysical factors on coffee and avocado production

Climatic conditions

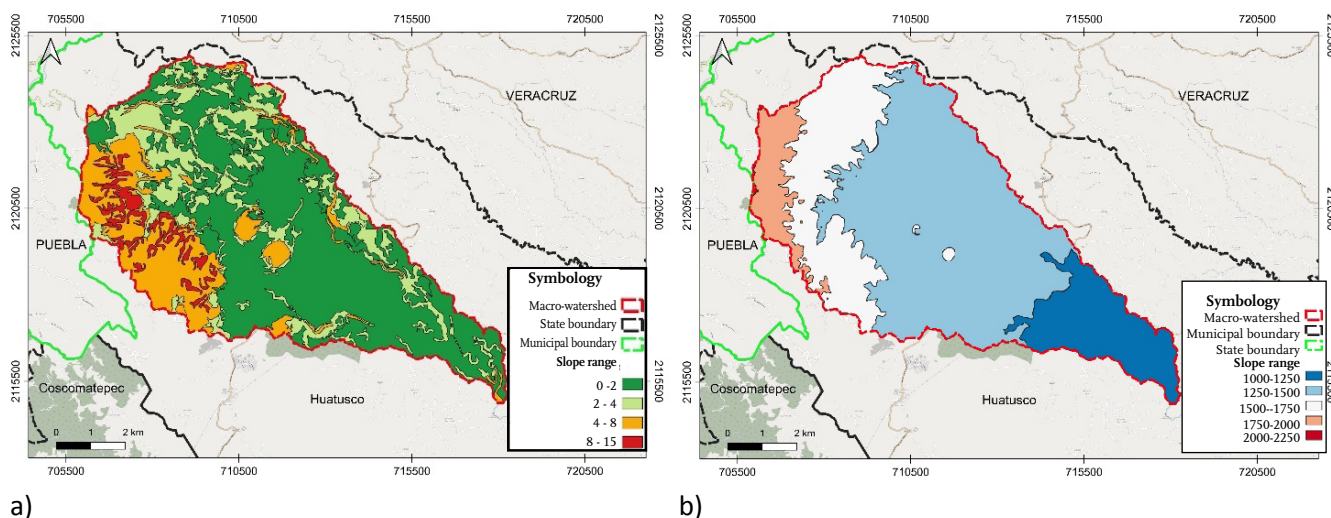
Within the micro-watershed, four distinct climates were identified according to García's classification (2024) (Table 1). The

predominant climate is warm sub-humid, covering 48.7 % of the total area, followed by humid temperate at 20.9 %.

Avocado

According to different studies, the environmental factors that have the greatest impact during the development and production stages of avocado cultivation are temperature, wind, and precipitation (Campos et al., 2015). The optimal temperatures for this variety range between 5 and 17 °C (DANE, 2016), and it requires between 1 200-1 600 mm of well-distributed rainfall throughout the year (Bernal-Estrada et al., 2020). The characterization results indicate that 70.9 % of the crop has been established in a humid-temperate climate. It is also observed that 74.3 % is located within an average annual temperature range of 16-18 °C, while 87.7 % has been planted in areas receiving approximately 1 900 mm of precipitation. These climatic conditions are favorable for maintaining optimal crop yields, as avocado adapts well to humid and sub-humid climates (DANE, 2016). However, excessive rainfall can cause damage due to pathogens (Cruz et al., 2020). Intense rains prolong moisture on the foliage, increasing anthracnose infection during flowering and fruiting stages, which results in greater fruit drop and reduced economic yield (DANE, 2016). Temperatures outside the optimal range

Figure 3. Slope percentage (a) and elevation range (b) of the micro-watershed.



can drastically reduce crop yields, especially when they coincide with the reproductive stage (DANE, 2016).

Coffee

According to different studies, temperature and precipitation significantly affect coffee yield (Gabriel-Hernández & Barradas, 2024). The optimal environmental conditions for the yield of *Coffea arabica* are as follows: ideal temperature of 18.7 °C, within a range of 17 to 21 °C; regarding precipitation, an average annual rainfall of 1 770 mm is required, with a range between 1 000 and 3 000 mm (Gabriel-Hernández & Barradas, 2024; Jiménez et al., 2023). The characterization results indicate that 77.2 % of the crop is established in a humid warm climate, 69.8 % of the crop is located in areas with an average temperature of 19 °C, and 85.4 % is planted in areas receiving around 1 900 mm of precipitation, ranges that fall within the ideal conditions to maintain good crop yields.

Slopes and Altitudinal Range

Table 1 shows the distribution of reclassified slopes. In the study area, slopes between 0-2 (59.2 %) and 2-4 (20.1 %) predominate (Figure 3a). The altitude range covering the largest area is 1 250-1 500 meters above sea level, with 55.8 %, followed by 1 500-1 750 meters above sea level with 22.2 % (Figure 3b).

Avocado

In terms of elevation, avocado crops are established between 1 250 and 2 250 m.a.s.l. However, 81.9 % (480.2 ha) of the crops are located between 1 250 and 1 750 m.a.s.l., with 66.6 % specifically found between 1 500 and 1 750 m.a.s.l. (Figure 4). Elevation has a direct influence on fruit morphology; avocados grown in lower areas (<1 300 m.a.s.l.) tend to be rounder and with rougher skin compared to

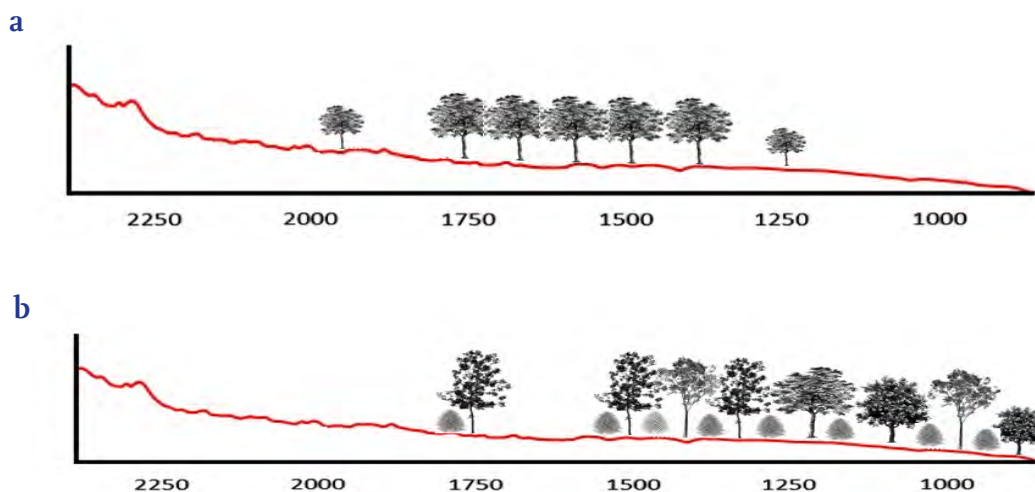
those grown under cooler climates at higher elevations, such as 2 400 m.a.s.l., where the fruit is more elongated with smoother skin (Bernal-Estrada et al., 2020). Therefore, the relationship between temperature and elevation suggests that, within the study area, the fruits typically display an oval shape with rough skin, traits that align well with current market demands. According to Bernal-Estrada et al. (2020), elevation also affects the flowering-to-harvest period. The elevations present in the study area support an intermediate harvest period ranging from 8 to 9 months, which is considered ideal for the Hass variety, thereby enabling optimal yields. Regarding slope, 97.8 % of the avocado plantations are established on slopes ranging from 0 to 8 %, which facilitates management activities. This agrees with the that reported by DANE (2016), which states that the ideal terrain slope for avocado crops should be less than 30 %, preferably with undulating topography, because this favors agronomic management.

Coffee

According to Gabriel-Hernández and Barradas (2024), coffee grown in Veracruz is found at elevations ranging from 300 to 3 000 meters above sea level (m.a.s.l.). The lower temperatures associated with higher elevations influence the coffee maturation period (Escamilla et al., 2016). In the study area, 94.4 % of the coffee plantations grow between 1 000 and 1 500 m.a.s.l., an optimal elevation range that promotes ideal maturation conditions and supports high yield potential (Figure 4). Additionally, elevation within the study area has a positive effect on the chemical composition of the coffee (flavor, body, and quality), allowing to produce high-quality specialty coffee for export (Bertrand et al., 2006).

Steep slopes are generally preferred for coffee cultivation, because they often overlap with areas of high biological diversity (Jiménez et al., 2023). As noted by Classen et al.

Figure 4. Semi-realistic profile of the distribution of reference crops by elevation range: (a) avocado (*Persea americana* var. Hass), (b) coffee (*Coffea arabica* var. Colombia and Costa Rica)



(2014), regions with greater plant diversity tend to support a richer community of pollinators, which can enhance agricultural productivity, particularly in coffee systems. Despite this, in the micro-watershed, 84.5 % of the coffee plantations are established on gentle slopes ranging from 0 to 4 %. This slope range does not negatively affect optimal coffee yields, as the crop exhibits high adaptability to the region's topographic conditions (Gabriel-Hernández & Barradas, 2024).

Type of soil

According to soil analysis, two additional soil types, distinct from those reported by INEGI, were identified: Cambic Andosol (AnCa) and Plinthic Acrisol (AcPl) (Figure 5). The most extensive soil type is Humic Andosol (AnHu), covering 71.7 % of the area, followed by Plinthic Acrisol with 12.5 % (Table 1). Orthic Acrisol (AcOr) is present to a lesser extent. These findings provide greater precision regarding soil distribution, which is essential for improving agricultural management and enabling comprehensive planning.

The values in Table 1 provide a comparative overview of the predominant biophysical conditions in areas cultivated with coffee and avocado. This integrated analysis allows for establishing a direct relationship between the evaluated variables to determine how they collectively influence crop growth and yield. For example, the combination of acidic soil, humid climate, and gentle slopes may create favorable conditions for pathogen development. Conversely, avocado achieves its greatest stability and productivity when grown on Humic Andosol soils, with slopes between 0 and 4 %, under a humid temperate climate.

Avocado

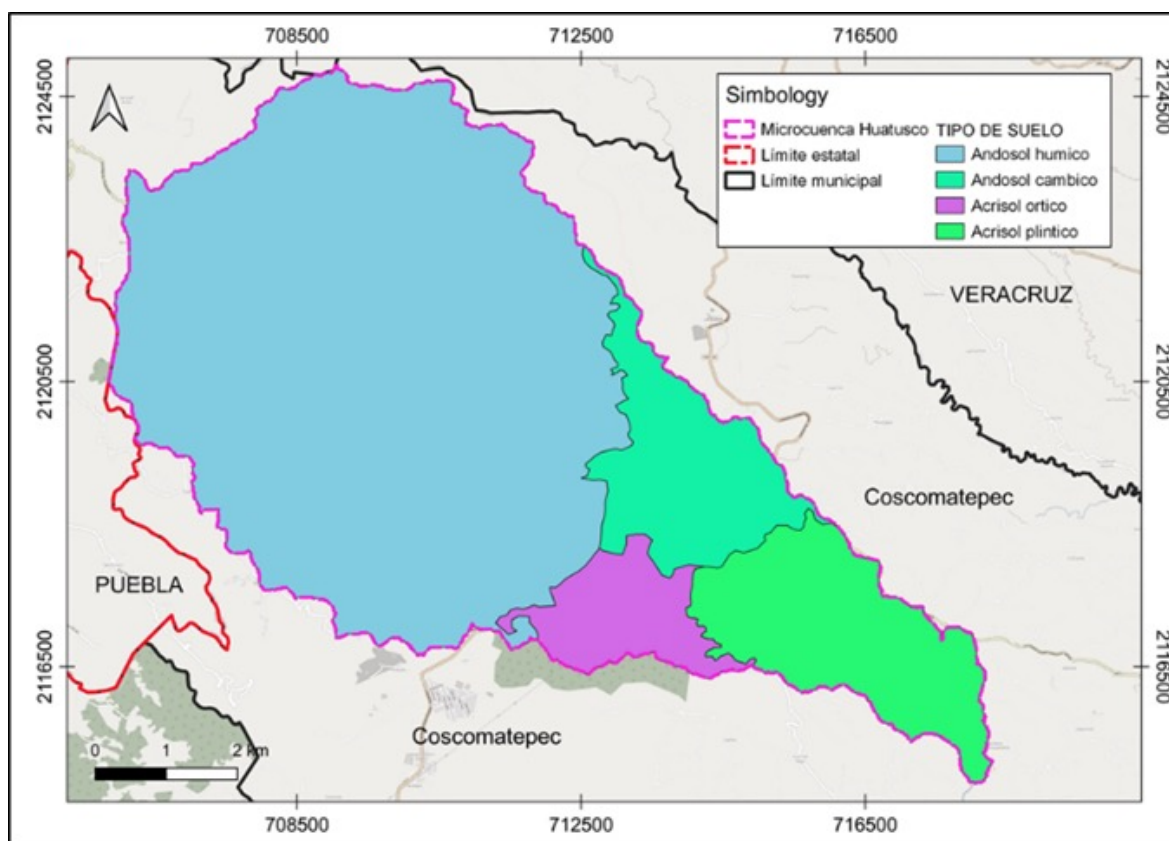
The crop is predominantly established (99.6 %) in humic Andosol soils characterized by a pH of 5.2, a

level that may limit nutrient availability and negatively affect yield. Despite this, the loamy-sand texture of the soil supports favorable water and nutrient retention (Table 2). Avocado trees are known to tolerate a wide range of soil types, from sandy to clay soils, although the latter are the most suitable. Optimal nutrient uptake typically occurs at a soil pH between 5.5 and 6.5 (Garrido-Ramírez, 2013; DANE, 2016). However, Salazar-García et al. (2009) observed pH values ranging from 4.7 to 6.4 in avocado orchards. Their research demonstrated that pH correction through lime application enhanced the absorption of key nutrients such as nitrogen, phosphorus, potassium, zinc, and boron, resulting in improved Hass avocado yields in Nayarit, even in non-irrigated systems.

Coffee

The predominant soils in coffee-growing areas are classified as Andosols with a pH ranging from 5.0 to 6.5 (Valencia & Rodríguez, 2016). However, according to the results, 53 % of coffee plantations are established on Acrisol soils, which showed more acidic pH levels than recommended (4.2-4.7), and are characterized by a predominantly clayey texture, low organic matter content, poor fertility, and drainage that ranges from moderate to poor. These conditions are reflected in a general decline in coffee productivity (Table 2). This finding is consistent with Sadeghian et al. (2019), who reported that high soil acidity reduces the availability of nitrogen, phosphorus, potassium, calcium, and magnesium, while increasing aluminum levels, which is toxic to plant roots and directly affect nutrient uptake. To compensate for these deficiencies, farmers apply both organic and chemical fertilizers, which contributes to increased production costs. Larios-González et al. (2014) highlighted that agroecological practices played a key role in stabilizing soil pH and mitigating active acidity, leading to improved soil fertility.

Figure 5. Soil types identified in the study area.



Source: Compiled by the authors.

Table 1. Area covered by reference crops in relation to biophysical variables.

Elevation (m.a.s.l.)		1000-1250	1250-1500	1500-1750	1750-2000	2000-2250
Area %	Ag	0	49.2	32.6	17.9	0.3
	Ca	47.1	47.2	5.6	0	0
Slope (%)	Ag	0-2	2-4	4-8	8-15	-
	Ca	43.4	34.4	20	2.2	-
Area %	Ag	60.1	29.4	9.5	1	-
	Ca	60.1	29.4	9.5	1	-
Soil	Ag	AnHu	AnCa	AcOr	AcPl	-
	Ca	99.6	0.4	0	0	-
Area %	Ag	34.6	12.5	12.5	40.5	-
	Ca	34.6	12.5	12.5	40.5	-
Climate	Ag	Humid temperate	Warm semi-humid	Warm semi-humid	Humid temperate	-
	Ca	Humid temperate	Warm semi-humid	Warm semi-humid	Humid temperate	-
Area %	Ag	44.3	14.9	14.2	26.6	-
	Ca	8.6	77.2	9	5.3	-

Note: Av: avocado; Co: coffee; AnHu: Humic andosol; AnCa: Cambic andosol; AcOr: Orthic Acrisol; AcPl Plinthic Acrisol

Based on the general analysis of the soil's chemical properties, the following can be determined: The EC values indicate that there are no salinity issues. The BD data suggests that the soils are organic and volcanic. Salamanca et al. (2005) highlight that and increase in BD leads to soil compaction, which gener-

ates unfavorable physical conditions that negatively affect coffee development during its early stages. The soil texture type is related to the amount of water retained at FC and PWP (Kramer & Boyer, 1996), as well as to the volume of water available for plants (Lambers et al., 1998).

Based on the above, it can be determined that humic andosol with loamy sand texture shows the highest field capacity (58.7 %) but also a relatively high permanent wilting point (34.8 %), resulting in 23.9 % plant-available water. Although its texture is coarser, the high porosity (61.3 %) and low bulk density (0.91 g·cm³) indicate a good structure, typical of volcanic soils rich in organic matter. Plinthic Acrisols, with a clayey texture, have a field capacity of 43.9 % and a permanent wilting point of 31.4 %, representing 12.5 % plant-available water. The cambic andosol has a loam texture, resulting in 18.9 % available water. Its bulk density is relatively high (0.99), which may limit aeration but remains favorable for root development. Lastly, the orthic Acrisol, with a clay loam texture, has only 14.5 % available water. Although it has a finer texture, its BD=1 suggests soil compaction, which may reduce the total volume of usable water.

Characterization of agricultural crops

Avocado crop

Within the study area, only *Persea americana* var. Hass is grown as a commercial crop, because it has the highest demand in both national and international markets (Montealegre, 2014). Although avocado trees can reach up to 20 m in height (Bravo et al., 2009), in the study area, trees are kept at a height of 3 to 5 meters. The tree canopy is typically pruned into semicircular, semi-elliptical, or irregular shapes, with an average canopy diameter ranging from 4 to 6 m. The first harvest in this region generally occurs between 3 and 5 years after planting. However, this depends on the management practices applied during the plant's development (Campos et al., 2015) and on the origin of the planting material: grafted or seed-grown (Cruz-López et al., 2020).

Regarding orchard management, the following practices are primarily being implemented: pruning (for shaping, maintenance, and production), fertilization aimed at correcting soil acidity, irrigation to reduce water stress during the dry season, pest and disease control, weed management, and proper harvesting techniques.

The main issues affecting avocado production are caused by the seed borer (*Copturus aguacatae*), which significantly damages the fruit, leading to premature fruit drop or the development of deformities. Additionally, physical damage from hail is common, as well as size reduction and fruit abortion due to drought. Furthermore, producers face challenges related to price fluctuations. According to local growers, the avocado market is highly volatile. This crop is currently experiencing an economic crisis, as prices are no longer rising as they did in the past, while production costs continue to increase, placing the profitability of avocado crops at risk (Diaz, 2024).

Regarding climatic impacts, strong winds, heavy rainfall, and frost can lead to direct post-harvest fruit loss due to scarring. The increase in pathogenic organisms such as fungi and

insects, mainly associated with excessive rainfall, especially during flowering stage, can result in diseases like anthracnose (Cruz et al., 2020), which directly affect crop yield.

To address the aforementioned issues, the implementation of an integrated pest management strategy is recommended, particularly for controlling the seed borer. One control method involves collecting and destroying infested fruit through burial or incineration. In addition, pruning host trees is necessary to reduce shading and promote proper aeration. For biological control, the use of entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* is advised. These fungi should be applied directly to the soil or tree stem (SENACICA, 2016). Application of these methods should be carried out during the critical period from July to October, when adult borers are active and fruits are developing (SAGARPA, 2005).

The use of chitosan, a natural fungicide derived from chitin, is recommended as a preventive method to combat anthracnose. It forms a protective film that inhibits fungal growth. It is recommended to apply it during flowering and fruit development, every 15 days throughout the rainy season, using foliar spraying (Munhuweyi et al., 2020) and to avoid excessive humidity within the plantations.

The adoption of localized irrigation techniques, particularly drip irrigation, is recommended as a strategy following the principles of Regulated Deficit Irrigation (RDI). This approach involves applying water supply below the crop's full potential requirements by restricting irrigation during specific phenological stages, without significantly affecting yield or product quality (Yang et al., 2022). For avocado and coffee crops, RDI can be implemented by limiting irrigation during the vegetative growth phase, when the crop can tolerate moderate water stress, and concentrating water supply during flowering of fruit filling stages. This approach helps maintain fruit quality while reducing total water consumption, without negatively impacting production. Gasque et al. (2016), demonstrated that the application of RDI in orange crops was able to save between 50 % and 55 % of water without affecting productivity. Additionally, it helped reduce fruit development issues and improve the commercial quality of citrus fruits.

Furthermore, protective measures should be implemented against wind, hail and frost. Lahak et al. (2024), found that covering young Hass avocado trees with 60 % shade netting during the winter season mitigates frost damage and improves tree performance. To prevent damage from hail and strong wind, Tapia et al. (2011) recommended establishing windbreaks, live fences, or planting companion trees to mitigate these impacts.

Coffee crop

Specifically, within the micro-watershed, only the species *Coffea arabica* is grown, predominantly established with

the Colombia and Costa Rica varieties, which replaced the *Typica* variety starting in 2013. This change was due to the impact of coffee leaf rust (*Hemileia vastatrix*), leading producers to increase the presence of rust-resistant varieties (Escamilla et al., 2016).

Coffee crop in the studied area is classified as commercial, characterized by the use of native or introduced species with high commercial value that provide shade for the coffee plants (García et al., 2020). The tree species commonly associated with the coffee plantations include chalahuite or vainillo (*Inga vera*), cedar (*Cedrela odorata*), oak (*Quercus* sp.), avocado (*Persea americana*), ash (*Fraxinus uhdei*), macadamia (*Macadamia integrifolia*) and mango (*Mangifera indica*). It is important to note that fruit trees are harvested exclusively for self-consumption.

Coffee is a perennial shrub of tropical origin; however, it has successfully adapted to different ecosystems such as pine-oak forests and cloud forests (Toledo & Moguel, 1996). The Colombia and Costa Rica varieties show similar structures, both averaging heights of 2 to 2.5 meters and crown diameter of 1.5 to 2 meters. They are usually planted at a density of 3 500 plants · ha⁻¹, arranged in staggered or square patterns with spacing of 1.5 to 2 meters between plants.

In relation to crop management, one of the main activities is the use of fertilizers: manures derived from livestock waste. However, it is recommended that these wastes be composted beforehand, as this process increases the content of Molybdenum, Nitrogen, Phosphorus, Potassium and Magnesium, thereby producing a fertilizer with greater nutritional value (Pérez, 2008). As part of traditional practices, producers apply waste from coffee harvests, however, the study by Blagodatskaya & Kuzyakov (2013) indicates that its direct application is detrimental due to the high concentration of phenols and polyphenols in the coffee pulp, which are toxic substances for most arthropod species and microorganisms. Therefore, it is recommended to apply only 25 % coffee pulp in relation to the total compost volume (Cervantes Beyra et al., 2015). Other common management practices, according to producers, include weed control and tree pruning to regulate shade. Despite previous outbreaks of coffee leaf rust, 93 % of producers fail to carry out preventive pest or diseases control measures. The management of coffee farms is based solely on traditional techniques, with little to no use of agricultural technology, mainly due to a lack of financial resources and the prevailing landform (Bautista et al., 2018).

One of the main challenges faced by coffee crops is the gradual decline in available labor, primarily due to the migration of young people and the average age of producers, which is around 60 years. This situation represents a significant risk to the sustainability of coffee cultivation in the region, because the transmission of valuable local knowledge related to production is gradually being lost.

This highlights the need to encourage younger generations to remain in rural areas through programs aimed at generational renewal. Additionally, actions should be taken to emphasize local traditions and cultural heritage so that coffee production can be sustained over time (Jiménez Barbosa et al., 2018).

On the other hand, coffee leaf rust remains a persistent phytosanitary threat that could lead to a further decline in grain production. This disease primarily affects old plantations that are nutrient-deficient and weakened by nematodes or other fungi (Hernández-Martínez & Velázquez-Premio, 2016). It is recommended to continue growing the Colombia and Costa Rica varieties, because they show resistance to coffee leaf rust (Escamilla et al., 2016).

According to Escamilla et al. (2005), additional damage can result from improper pruning of coffee plants and poor shade regulation. Furthermore, this year experienced a drought period that exceeded historically recorded ranges. Producers reported that the lack of rainfall during March and April affected the flowering cycle. In many cases, flowering was delayed until May, because it depends on the onset of the first rains to initiate the process. This delay caused a reduction in the number of flowers, which will likely be reflected in decreased grain production during the current harvest season. Therefore, it is recommended to establish the San Román variety, because it is drought-resistant. Additionally, it is proposed to complement this with moisture conservation techniques such as organic mulching and water harvesting structures, aligned with the principles of RDI (Yang et al., 2022). These measures aim to reduce production costs and increase resilience against the impacts of climate variability.

Overall diagnosis of coffee and avocado agricultural systems

By integrating biophysical variables and management practices, the following diagnosis is proposed for the evaluated crops: soil conditions are favorable for the development of coffee and avocado; however, there are key deficiencies that limit the yield and sustainability of these systems. These limitations are primarily related to: i) high soil acidity, especially in coffee-growing areas established on Acrisols, which reduces nutrient availability; ii) limited use of technologies for efficient water management, particularly during dry periods; iii) lack of preventive phytosanitary management, which increases production costs and vulnerability to diseases such as rust and anthracnose; and iv) low levels of mechanization, limited adoption of agroecological practices, and the progressive reduction of qualified labor.

Conclusions

The biophysical and descriptive characterization of shaded coffee and avocado crops allowed for the identification of

environmental factors, agricultural practices, and socio-economic factors that are directly influencing their production. Although these crops are fundamental to the regional economy, they continuously face challenges such as pests, diseases, climate variability, and inadequate resource management, among others, which could compromise their viability and productivity.

The Hass avocado variety has significant potential to expand its planted area within the micro-watershed, because the biophysical conditions are favorable for its establishment. The crop is grown on humic andosols; a soil type possesses suitable characteristic for water retention and availability. However, the crop faces challenges that impact productivity, mainly related to soil acidity, which limits nutrient availability, and phytosanitary issues such as the seed borer and anthracnose, that become more severe in the presence of excess moisture.

To counteract these challenges, it is recommended to implement management practices aimed at correcting soil pH through the application of inputs accessible to producers, such as lime and compost. The use of natural preparations, including chitosan and entomopathogenic fungi, is also advised. Additionally, establishing localized irrigation based on the principles of RDI, and physically protecting the crop from environmental stresses such as hail, wind and frost through windbreaks and shade net, are recommended. These measures will help optimize productivity, reduce production costs and secure the long-term sustainability of the crop in response to environmental and economic variability.

While 94.4% of coffee is cultivated in regions with ideal elevations, slopes, and climates for optimal growth, soil type remains a major limiting factor. Over half of all coffee crops are grown on acrisols, soils that are acidic, clayey in texture, low in fertility, and poorly drained. These conditions reduce nutrient availability and increase exchangeable aluminum levels, which negatively impact the root system and nutrient uptake. As a result, farmers are compelled to use corrective input, leading to higher production costs.

Therefore, it is necessary to apply pre-composted organic fertilizers and include only 25 % coffee pulp in the total volume to correct acidity issues and improve soil fertility. It is important to continue establishing rust-resistant varieties such as Colombia and Costa Rica. The progressive loss of labor, which threatens the sustainability of coffee cultivation, can be addressed through government programs or private initiatives aimed at generational renewal. One strategy to combat drought is the introduction of the San Román variety and the implementation of moisture conservation techniques under the RDI approach, to reduce the impact of climate variability and thereby improve profitability, productivity, and the long-term sustainability of coffee production.

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English version

Mistletoe in the 21st Century: A bibliometric analysis from a phytosanitary perspective

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Abstract

In recent years, the study of mistletoe has increased notably, with a particular focus on its medicinal properties. However, this has left a gap in the phytosanitary impact of the plant on forest ecosystems. In this study, different bibliometric parameters were analyzed to characterize the research published on mistletoe worldwide between 2004 and 2025. This period was established due to the technological advances and climatic changes that have taken place in the last decade. Two databases were used: one consisting of 253 articles from Web of Science and another database of 104 articles related to forestry topics obtained from Scopus, PubMed, Lens.org, and Google Scholar. The results show that the most studied mistletoe species were *Viscum*, *Phoradendron*, and *Arceuthobium*, typical of temperate ecosystems, while the most frequently referenced host was *Pinus sylvestris* L. The countries with the greatest scientific production were the United States of America, Mexico, and Brazil. Of the analyzed studies, 48.9 % focused on plant sciences, 22.8 % on forestry sciences, 4.3 % on pharmacology, and 4.1 % on integrative and complementary medicine. It is concluded that research on mistletoe in forest ecosystems remains limited and that new technologies should be integrated into future research. Furthermore, existing studies focus on genera of temperate zones genera, leaving species from subtropical or arid ecosystems underexplored. This represents an area of opportunity area for future research.

► **Keywords:** Pests, hemiparasites, forest ecosystem, bibliometrics, ecology.

Introduction

Forests are vital to the functioning of the Earth, both for the provisioning, regulating, and supporting ecosystem services they provide (Pan et al., 2011) and for their important role in interspecific ecological dynamics (Watson et al., 2018). However, in recent years, factors such as climate change have intensified their vulnerability (Bell et al., 2020), altering their ecological balance (Ndagurwa et al., 2014) and significantly reducing individual vigor (Allen et al., 2015). In turn, they become more susceptible to attack by biotic factors such as insects, pathogens, and hemiparasitic plants

(Camarero et al., 2025). Under this scenario, one of the most notable agents due to its wide distribution and notable impact are mistletoes (Ayres & Lombardero, 2000), whose proliferation has increased alarmingly in recent decades globally (Sturrock et al., 2011).

Mistletoe is the common name for hemiparasitic plants that infest trees and shrubs in order to obtain the necessary elements to survive (Endara-Agramont et al., 2022). There are three families of mistletoe species of forest importance: i) *Santalaceae* which includes around 550 species from 40 genera among which some of great importance stand out,

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such as *Viscum* (European mistletoe) (González de Andrés et al., 2024), *Phoradendron* (American mistletoe) (Dawson et al., 1990), and *Arceuthobium* (dwarf mistletoe) (Tinnin et al., 1999) these genera mainly affect woody species of the *Pinus*, *Juniperus*, *Arbutus*, *Populus*, *Abies*, which are typical of temperate ecosystems (Der & Nickrent, 2008). ii) *Loranthaceae*, a family that includes around 73 genera and 1,000 species, is considered one of the most diverse groups of hemiparasitic plants. *Cladocolea* (Díaz-Limón et al., 2016), *Psittacanthus* (Silva et al., 2021) and *Struthanthus* (González & Morales, 2004) are three important genera in this family and are commonly known as grafts, a term that, although it is not a graft in the traditional horticultural sense, has acquired that name due to the similar way in which it joins another tree (Coria Ávalos et al., 2018). They are mainly distributed in tropical and subtropical areas, although they can also be found in temperate zones, and their main hosts belong to the genera *Quercus*, *Alnus*, *Cedrela* and *Ficus* (Grajales et al., 2022). Finally, iii) the *Misodendraceae* family that only has species of *Misodendrum* genus (Henríquez-Velásquez et al., 2012) which are mistletoes confined to South America since they only parasitize individuals of the genus *Nothofagus* (Leadlay & Jury, 2006) (See Supplementary Material 1).

In response to this problem, some recent studies have explored how the dynamics of phytosanitary-important mistletoes alter the physiology of their hosts (Das et al., 2016). Specifically, it has been suggested that their uncontrolled expansion, in addition to diminishing forest health, compromises the resilience of forests, which are already vulnerable to climatic conditions in adverse environments (Rigling et al., 2010).

Despite the ecological importance of these parasitic organisms in forest dynamics (Press & Phoenix, 2005), the study of their impact on forest health is fragmented (Lira-Noriega et al., 2013), both conceptually and geographically (Mellado & Zamora, 2017). This dispersion arises from the diversity of approaches used in the study of mistletoe, many of which transcend the strictly forestry (e.g., medicine, fauna, biology, taxonomy) (Mathiasen et al., 2008). Therefore, the importance of conducting a critical analysis that not only integrates relevant findings on the phytosanitary impact of mistletoe in forests, but incorporates cutting-edge detection and monitoring techniques at the global level is highlighted (Zhang et al., 2020). This comprehensive approach will enrich the amount of available knowledge and facilitate its transfer among affected geographic regions. At the same time it will guide the development of a state-of-the-art approach that provides the community with a broader perspective, enabling the identification of gaps and trends that can guide the development of future research.

Thus, this bibliometric analysis integrates multidisciplinary evidence on mistletoe in forest systems. The main objective of this study is to compile a document that serves

as a state of the art of global research on this hemiparasitic plant and its phytosanitary impact. It is hypothesized that existing research has focused primarily on descriptive and geographic distribution approaches (Bilgili et al., 2020), and that it focuses on certain mistletoe genera, thus creating a gap in the study of other genera of phytosanitary importance, revealing areas of opportunity for future research.

Materials and Methods

Compilation of publications

Publications in scientific journals (scientific articles) were considered, excluding gray literature. The purpose was to systematize the state of the art of the study of mistletoe under the current global perspective and conditions, considering publications in the last 21 years (from January 2004 to January 2025) to include relevant scientific studies aligned with the contemporary era. The determination of this period (21 years) is based on the important technological advances developed at the beginning of the XXI century, these advances refer to an increase in the use of remote sensing, programming, etc. for urban, agricultural, and environmental monitoring purposes (Cheng et al., 2024). In addition, climate change in the last decade has fostered the development and spread of pests and diseases worldwide (Sturrock et al., 2011). The research was carried out through different scientific search engines such as Web of Science, Scopus, PubMed, Lens.Org, and Google Scholar.

An extensive search was conducted on all platforms using the following keywords: “mistletoe”, “forest”, “*Viscum*”, “*Arceuthobium*”, “*Phoradendron*”, “parasitic plant”, “pest”, “tree”, “forest health” and “canopy”.

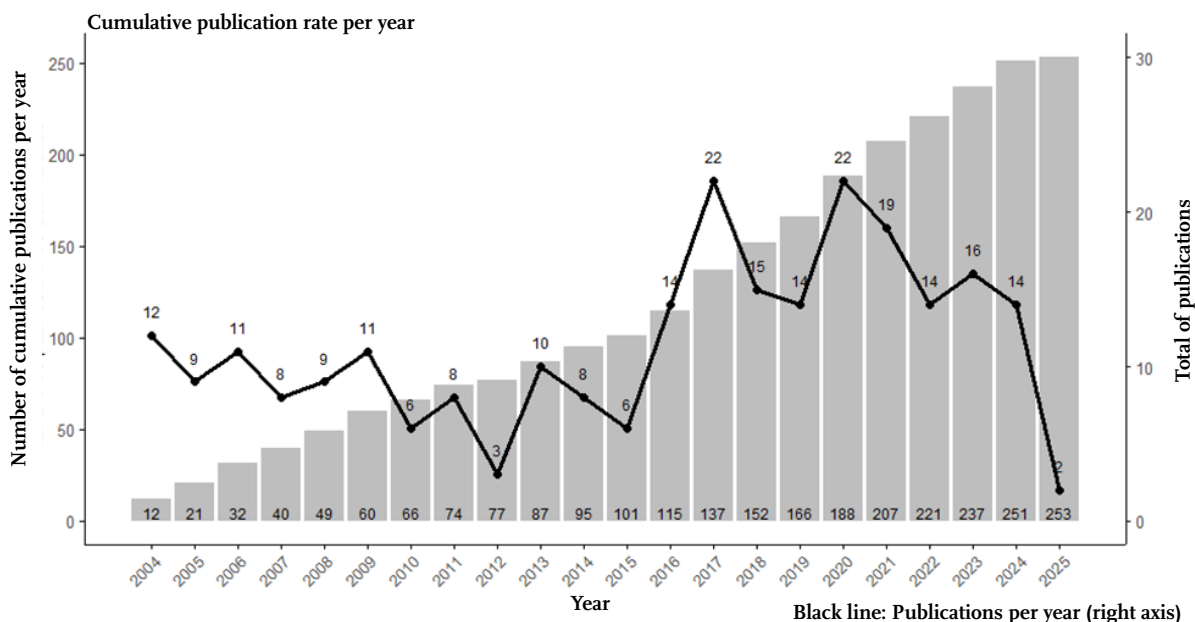
The collected articles were organized into a database (See Supplementary Material 2) in Microsoft Excel®. Within this database, important data were captured for each article found: DOI, bibliographic citations, title, authors, journal of publication, journal impact factor, study objectives, country of origin, tree species studied, mistletoe species studied, methodology used in the study, software and inputs used, drone used (when applicable) and study results. This database consisted of 104 articles.

As a complement to the previous database, a database obtained from the Web of Science platform with the keyword “mistletoe” was used. This database included all publications from 2004 to 2025 in several research areas, yielding a total of 253 articles, this database included more articles than the previous one because it is not limited to forestry studies.

Data analysis

A bibliometric analysis was performed using the Web of Science database (253 articles) using VosViewer software

Figure 1. Cumulative publication rate vs. absolute publication rate for the scientific contribution of mistletoe research for the period 2004-2024. N = 253.



version 1.6.20 (van Eck & Waltman, 2010), where bibliometric networks were created using the “visualization of similarities” analysis algorithm for different parameters (co-authorship, main countries, bibliographic coupling). One of these analyses was the keyword occurrence analysis, to visualize the most notable topics in the consulted literature. This was done for those keywords with more than 5 occurrences. For this same database, RStudio scripts (*R: The R Project for Statistical Computing*, 2010) and such as “ggplot2” (Wickham, 2016) were used to perform the statistical analysis of bibliographically important metrics such as: i) temporal publication rate, the annual and absolute count of the number of articles published was performed. This count helps determine which are the most libraries important years in terms of scientific contributions, ii) research areas in the general study of mistletoe, articles from the Web of Science database were classified within a research area in order to put into perspective which are those topics that capture the most attention within the scientific community and which topics present an area of opportunity for future research, the result was 7 research areas 4 of which are the most notable. iii) publication distribution map, to know the geographic distribution of the scientific contribution to the study of mistletoe and to determine which are the countries that have made the most publications in the studied period in this analysis. iv) main publication journals, for the purpose of getting to know in which articles on this topic are published during the period of the bibliometric analysis. RStudio scripts were also used for the second database of mistletoe studies related to the forestry field (104 articles) in order to obtain graphs of metrics related to: i) research subtopics related to the forestry area; which were classified according to the study objectives and methodology, resulting in the

following categories: Ecology, detection and monitoring, impact on plant growth, interaction with climatic effects, biochemical analysis and influence on mortality. And ii) mistletoe genera studied in the forestry field, which were: *Viscum*, *Phoradendron*, *Arceuthobium*, *Amyema*, *Cladocolea*, *Loranthus*, *Misodendrum*, among others unspecified. This grouping and classification of the data eased the preparation of comparative tables and figures which helped meet the objective of this study

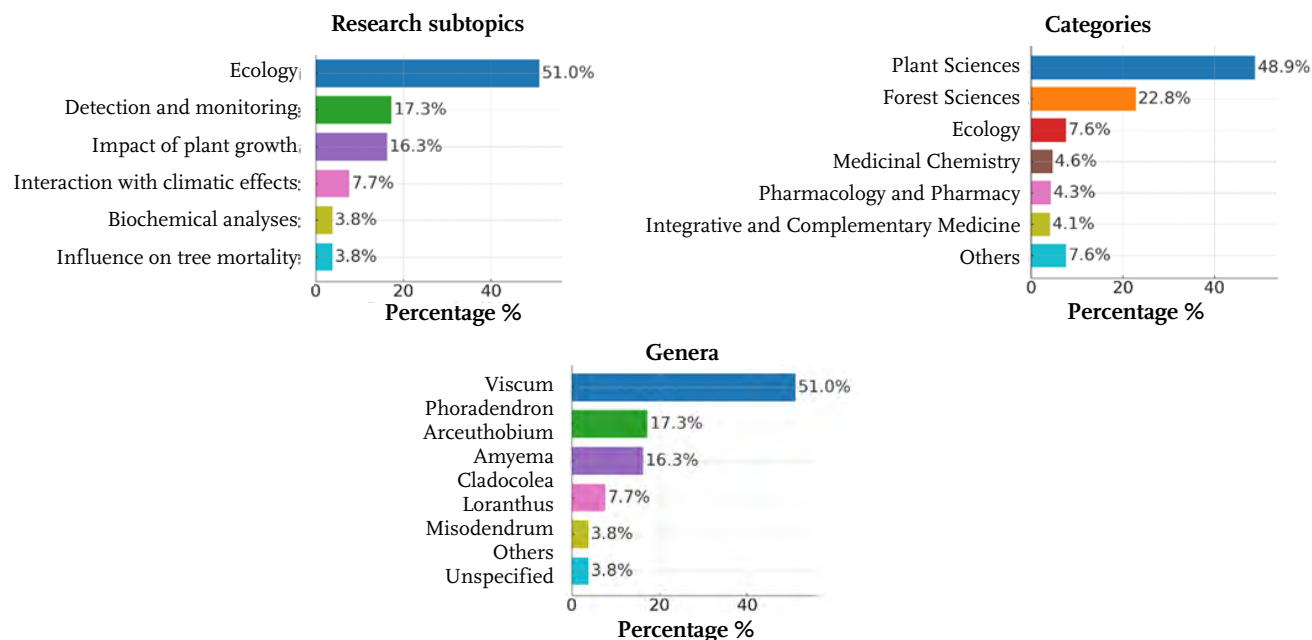
Results

Publications per year and research area

Setting the scientific contribution made over the last 21 years into perspective, cumulative and absolute publication rates were compared (Figure 1). In recent decades the number of publications per year has remained constant with slight increases and decreases. Highlighting 2017 and 2020 as the years with the highest number of publications to date (22 articles).

The publications were classified into four main areas: i) “Plant Sciences” (48.9%), which includes biological studies mostly with descriptive, taxonomic, geographical distribution and population dynamics analyses; ii) “Pharmacology and Pharmacy” (4.3%), with studies focused on the pharmacological properties of the bioactive compounds present in some mistletoe species and which analyzes their effects on the human body; iii) “Integrative and Complementary Medicine” (4.1%), which includes articles that study the use of mistletoe in complementary or alternative medical practices that are combined with conventional medicine, this category is broader since it can cover not only the phar-

Figure 2. Research areas, research subtopics, and most studied mistletoe genera in phytosanitary terms.



macological effects of mistletoe, but also some cultural and spiritual effects of its medicinal use; finally iv) “Forest Sciences,” focused on the study of the interaction of these hemiparasitic plants with forest ecosystems, representing 22.8% of all publications found during the period studied. This analysis of the classification of research areas allowed us to identify trends in the studies, revealing an area of opportunity for research into the impacts of mistletoes on forests, since this topic represents less than a quarter of the articles found.

Forest subtopics and main mistletoe genera

To contextualize the state of the art regarding the study of mistletoe in forest ecosystems, articles in this area were classified into research subtopics, with the purpose of visualizing the applications developed in recent years.

The area of ecology is the most studied, hosting works related to the dynamics of mistletoes in ecosystems, their taxonomic description, distribution, interaction with other plant species, etc. Secondly, there is the area of “Detection and Monitoring” which involves studies that have used emerging technologies such as Unmanned Aerial Vehicles (UAVs), satellite images and LiDAR to detect and monitor the presence of mistletoe in forests, highlighting the potential of these technologies which has yet to be fully explored. Finally, the category “Impact on plant growth” includes studies that evaluated the effect of mistletoe on the growth of its host (Figure 2).

Figure 2 also shows the mistletoe genera that have been studied in forestry publications. The three most important genera were: *Viscum* (29.1%), *Phoradendron* (26.2%), and *Arceuthobium* (23.3%). These genera are representative of

temperate ecosystems, whose hosts are coniferous and broadleaf species that inhabit these ecosystems. The most frequently mentioned genus in the literature is *Pinus*, likewise, the main pine species that has been studied for its interaction with mistletoe (of the genus *Viscum*) is *Pinus sylvestris* L., both characteristic of northern European forests. This explains why much of the research has been conducted in this part of the world. In the same way, the genera *Quercus* (particularly the species *Q. douglasii* Hook. & Arn., *Q. lobata* Née and *Q. agrifolia* Née), *Eucalyptus* (*E. fibrosa* F. Muell. and *E. moluccana* Roxb.), *Abies* (*A. alba* (Aiton) Michx., *A. nordmanniana* (Steven) Spach and *A. concolor* (Gordon & Glend.) Lindl. ex Hildebr.), as well as *Juniperus deppeana* Steud. are mentioned. Most of these species inhabit temperate forests and are characteristic of ecosystems in the northern hemisphere of the planet, mainly in Europe, some parts of Asia and to a lesser extent in regions of America. This indicates a geographical fragmentation in the study of mistletoe in forests and how this field has developed more in these regions of the world. Finally little-studied mistletoe genera such as *Amyema* (2.9%), *Cladocolea* (1.9%), *Loranthus* (1.9%), and *Misodendrum* (1.9%) were found, which are characteristic of other ecosystems such as tropical and subtropical ones. Such is the case of *Misodendrum* which is limited only to ecosystems in the southern hemisphere, affecting only individuals of the genus *Nothofagus*.

Occurrence of keywords in research

This analysis allowed the generation of a thematic network composed of five clusters that were representative within the field of study (Figure 3).

- Red cluster, with 28 elements, housed the most relevant element in the database, mistletoe (An element with 199

Figure 4. Publications by country regarding the study of mistletoe in forests in the period 2004 to 2025. N = 253.

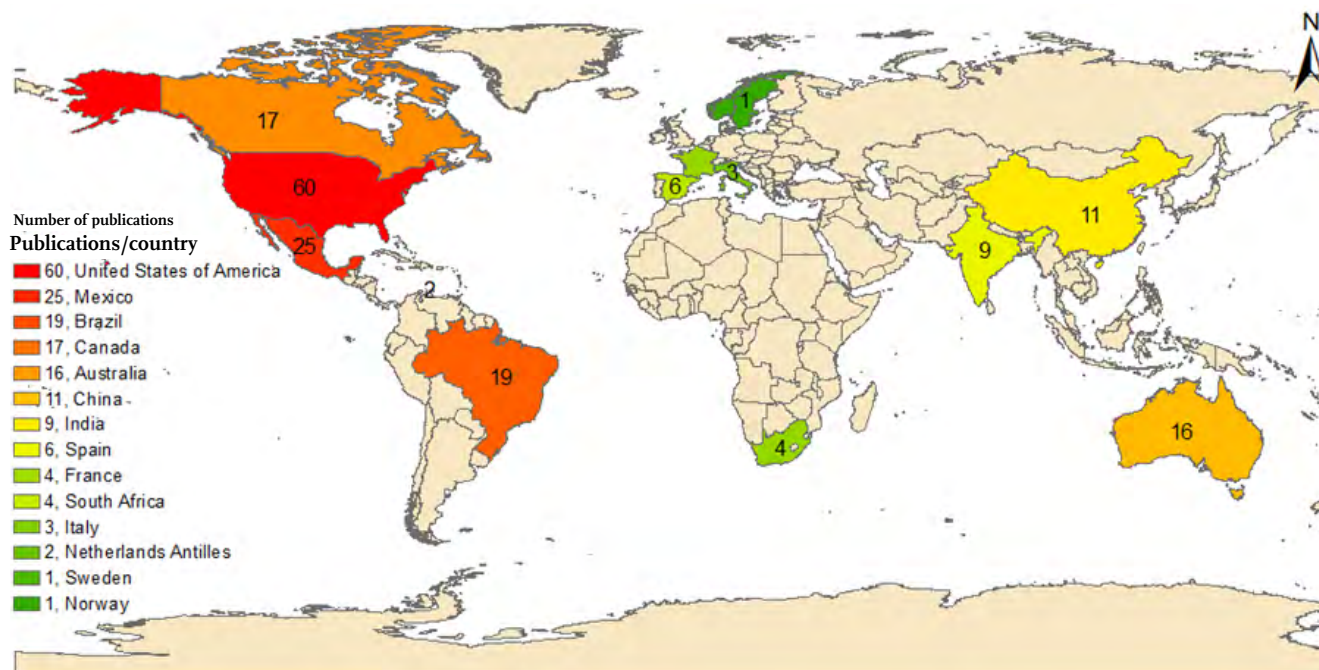


Figure 5 shows the most published journals on mistletoe during the period 2004 to 2025. The analysis included multidisciplinary articles; in medicine, ecology, biology, forestry and zoology, with the aim of identifying the areas where the study of this hemiparasitic plant has been prioritized. The grouping highlights that the journals “Botany”, “Plant Basal” and “Forest Ecology and Management” were the main ones in the ecological areas (18 publications), biological (12 publications) and forestry (9 publications) fields. However, the total number of articles in these areas is low, indicating limited scientific production related to these areas of study compared to the 253 publications contained in the database.

Discussion

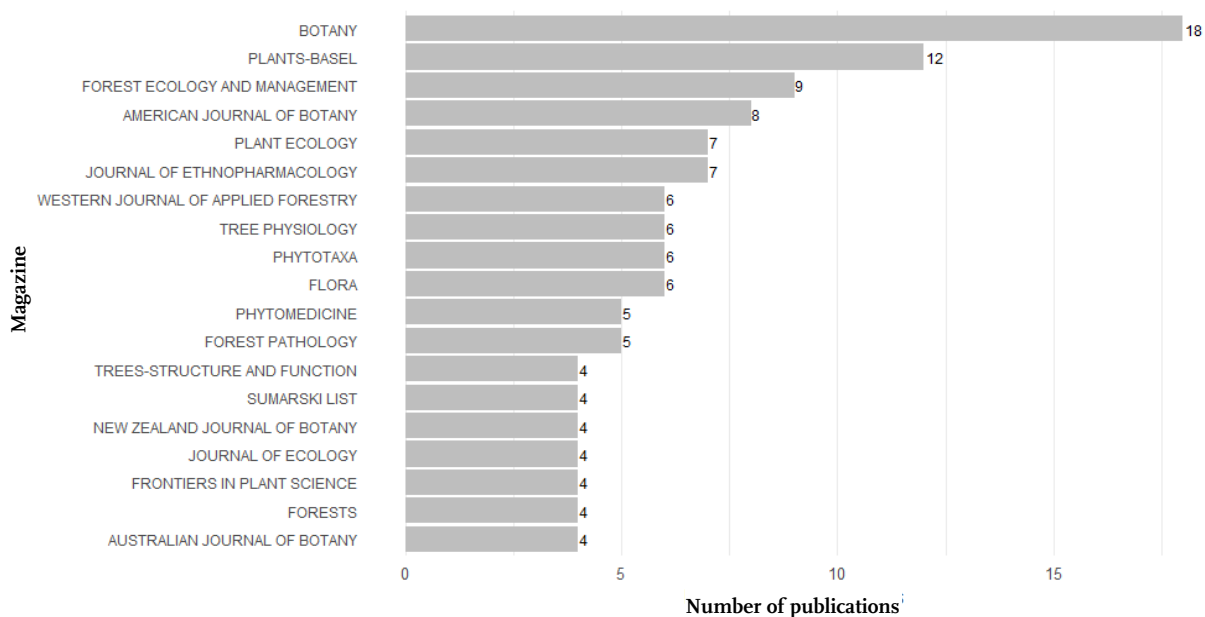
This bibliometric analysis revealed a steady growth in scientific interest in the study of mistletoe since 2016, when a period of increased scientific productivity began. This may have been due to technological advances in that year, the trend in the use of new technologies such as UAVs or drones, satellite images, geographic information systems, etc. (Shahi et al., 2023). The scientific community could have been provided with a new line of research in the study of mistletoe beyond the taxonomic description of the species or its geographic distribution. The peaks of scientific contributions (22 publications for each) were found in 2017 and 2020. However, starting in 2020 a decrease in the number of publications per year is observed. This may reveal an area of opportunity for future research seeking to integrate emerging technologies such as artificial intelligence and machine learning algorithms in the study of mistletoe, coupled with the

constant climate change that the planet has faced in recent years and which could be directly affecting the ecological development of these hemiparasitic plants. Knowing how scientific contributions to mistletoe research have changed over time, helps to identify trends coupled with other factors such as technological advancement or global climatic conditions that explain the increase in publications.

To our knowledge, this bibliometric analysis is the first to describe the current perspective of mistletoe studies and their limitations in the forestry field. Studies carried out by (Szmidla et al., 2019) and (Shaw & Agne, 2017) focused on a single species of mistletoe and its impact on forest ecosystems, leaving behind the entire group of genera of phytosanitary importance.

A gap was also identified in studies using cutting-edge monitoring and detection tools. As explained in (Missarov et al., 2024), the use of remote sensing for the study of mistletoe, is emerging with great potential; however, it still requires further research due to technical limitations such as the difficulty of implementing diverse methodologies in dense forests and adverse climatic conditions, including its high operating cost. These conditions limit the ability to replicate these techniques globally, isolating this type of study to a limited number of countries that invest sufficient funds in research in this discipline. Despite this, the study focused on systematizing the mistletoe genera of phytosanitary importance that have been studied, uncovering an area of opportunity for future research delving deeper into the interaction of mistletoes with their corresponding ecosystems.

Figure 5. Number of articles on mistletoe, grouped according to the journal where they were published from 2004 to 2025. N = 253.



The author Těšitel, (2016) mentions how mistletoe plays certain important roles within ecosystems such as providing habitat for bird species and acting as a biological control. This undermines the interest in studying the impact of excessive populations of this hemiparasite in forests.

The co-occurrence analysis of keywords provided an overview of the most important topics in the study of mistletoe worldwide. The links between keywords provide insight into which elements tend to appear together in the scientific publications found and which are the most researched subtopics of study with regard to mistletoe. For example, the close connection between the element “mistletoe” and “cancer” indicated a large number of studies analyzing how the medicinal properties of this plant help in the treatment of this disease. In forestry terms, we found a connection between “dwarf mistletoe” and “forests” this means that a large part of the studies published regarding the impact of mistletoe in forests, were those of the genus *Arceuthobium* (commonly known as dwarf mistletoes), which represented a greater degree of distribution and impact on forest ecosystems, as mentioned by Stanton (2006). Another case of similar connection is that of the element “european mistletoe” which has strong connections with “forest”, “growth”, “scots pine”, suggesting that another large part of the studies in this field are linked to mistletoes of the genus *Viscum*, commonly known as european mistletoes (Kollas et al., 2018). Finally, it is possible to see that the impact studies on mistletoe that have been published worldwide between 2004 and 2025 were mostly on the genera *Arceuthobium* and *Phoradendron*.

The analysis of the countries which have contributed the most to the study of mistletoe allows to identify geogra-

phical regions such as: United States and Mexico, this explains why *Arceuthobium* and *Phoradendron* are among the most frequently mentioned mistletoe genera in scientific literature, these genera are widely distributed in both countries and represent a potential threat to the forests in this region (Sabrina et al., 2020). The distribution of studies in Europe and Oceania explains why *Viscum* is the most studied genus of mistletoe worldwide and affects a large forest area in european countries (Castagneri et al., 2015). The lack of publications in South American countries indicates a lack of study of *Misodendron* another genus of mistletoe of phytosanitary importance confined to these regions of the world (Tercero-Bucardo & Kitzberger, 2004).

Conclusion

This bibliometric analysis confirmed that global research on mistletoe, during the period 2004–2025, has been dominated by descriptive and ecological approaches, with limited scientific output focused on its phytosanitary impact on forest ecosystems. Furthermore, a significant geographic and taxonomic bias was identified: studies focus on a few mistletoe genera (*Viscum*, *Phoradendron*, and *Arceuthobium*) and on temperate ecosystems in the northern hemisphere, particularly European and North American coniferous forests.

Results of this bibliometric analysis highlight the need to direct efforts toward mistletoe research in Mexican forest ecosystems, where genera such as *Phoradendron* and *Arceuthobium* represent a red flag of phytosanitary risk, yet their study remains limited compared to their actual impact. This requires the integration of emerging technologies such as remote sensing and artificial intelligence,

among others. These findings highlight critical gaps that represent an immediate opportunity for future research, especially in tropical, subtropical and arid regions, and for the development of modern monitoring methodologies. Thus, this study significantly contributes to redefining priorities in mistletoe research, promoting a more comprehensive and contextualized view of its role as a forest pest in the XXI century.

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APPENDIX 1

Ecological description of genera

Viscum: It is a genus of 70 to 100 species of mistletoes. They are native to temperate and tropical regions of Europe, Africa, Asia and Australia. They belong to the family *Santalaceae*. They are hemiparasitic plants with branches between 15 and 80 cm long, the foliage is dichotomous, with opposite pairs of green leaves that photosynthesize. The flowers are inconspicuous, greenish-yellow, 1 to 3 mm in diameter. The fruit is a white, yellow, orange or red berry when ripe. It has several seeds inside the sticky pulp of the fruit. (Szmidla et al., 2019c).

Phoradendron: includes evergreen shrubs, monoecious or dioecious, with simple, decussate paired leaves of variable shape ranging from falcate to liguliform or lanceolate to narrowly elliptic. Inflorescence of 1 or several axillary spikes, each one with 1 or several fertile articles and each article with 2 or more rows of flowers. Unisexual, sessile, green to yellowish flowers, the staminate ones with 3 or more valvated petals, 3 or 4 bilocular anthers and a rudimentary pistil in the center; the carpellate ones with a unilocular ovary, a straight style originating from a small annular disc and an undifferentiated stigma. The fruit is a whitish, ovoid to globose berry with 1 seed surrounded by a viscid layer. (Gómez-Sánchez et al., 2011).

3. *Arceuthobium*. It is a genus of 42 plant species, belonging to the Santalaceae family. They parasitize members of the Pinaceae and Cupressaceae families. Commonly known as “dwarf mistletoe,” they are small plants (less than 20 cm) with foliage that varies in color from yellow to brown, black, and red. The leaves of this genus of plants are so small that it is sometimes described as a leafless plant, however, the leaves are distributed in the form of scales that begin at the periphery of the shoot apex through periclinal divisions in the subsurface layer (Hawksworth, 1996)

4. *Psittacanthus*: It is a genus of 42 plant species belonging to the Santalaceae family. They parasitize members of the Pinaceae and Cupressaceae families. Commonly known as “dwarf mistletoes,” they are small plants (less than 20 cm) with foliage color that varies between yellow, brown, black,

and red. The leaves of this genus of plants are so small that it is sometimes described as a leafless plant; however, these are distributed in the form of scales that begin at the periphery of the shoot apex through periclinal divisions in the subsurface layer (Hawksworth, 1996).

5. *Misodendrum*. It is a hemiparasitic plant, commonly called Chinese lantern or ñire flower, it is a dioecious species, belonging to a monogeneric family endemic to the forests of southern Argentina and Chile. The Misodendraceae family comprises eight species that specifically infect species of the *Nothofagus* genus throughout most of their geographical range. The fruits of *Misodendrum* are small (2 mm) dry achenes with three setae up to 1 cm long that allow them to fly and anchor themselves passively, mainly on small branches and stems of the host. The stems grow sympodially and have reduced photosynthetic tissue with small scale-like leaves. (Tercero-Bucardo & Rovere, 2010).

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English version

Zeolite modifies the development of lettuce (*Lactuca sativa* L.) grown in substrate

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Abstract

Zeolites are crystalline, nanoporous minerals with a high capacity for water adsorption and nutrient retention, which can be gradually released into the root zone. These properties make them promising materials for improving nutrient availability and crop productivity. The objective of the present study is to evaluate the effect of zeolite incorporation into the substrate on the development of lettuce (*Lactuca sativa* L. cv. Maximus) plants. Seeds were sown in polystyrene foam trays and irrigated with Universal Steiner nutrient solution at 25 and 50 % concentrations. After thirty days of sowing, the seedlings were transplanted into 3 L polyethylene bags containing a mixture of tezontle (particles > 0.5 cm) and zeolite at 0, 2, 4 and 6 % (v/v). The addition of zeolite to the substrate reduced plant height and leaf number at the tested doses; additionally, the 6 % treatment decreased the length of the largest leaf compared with the control. The addition of zeolite to the substrate affects lettuce plant development.

► **Keywords:** Minerals, vegetables, sustainable agriculture, yield.

Introduction

Agriculture, an essential global activity, currently faces two major challenges: increasing productivity to meet growing market demand, and implementing sustainable management practices that enable more efficient use of resources. These challenges arise from the exponential growth of the population and the impacts of phenomena such as climate change, global warming, and ecosystem degradation (Sangeetha & Baskar, 2016).

Consequently, the use of clay-based mineral inputs has gained prominence as a strategy to counteract various factors that limit agricultural production. In this context, zeolite stands out as a material with high potential to enhance the productivity and sustainability of cropping systems (Lahori et al., 2020). Zeolites are crystalline, nanoporous minerals characterized by a high capacity for water

and nutrient absorption, which can be gradually released (Zeinalipour & Saadati, 2024), improving nutrient use efficiency in plants and thereby contributing to enhanced yield, soil properties, and overall agricultural productivity (Hassan et al., 2024).

Currently, zeolite is mixed with the soil for its ability to improve their physical and chemical properties, as well as to promote plant growth. Several studies have shown that the addition of this mineral increases the content of exchangeable nitrogen (N), potassium (K), and calcium (Ca), while also preventing their leaching (Ahmed et al., 2010). Furthermore, zeolite application helps reduce the uptake of heavy metals such as lead (Pb), cadmium (Cd), copper (Cu), and zinc (Zn) in cabbage (*Brassica chinensis* L.), maize (*Zea mays*) and rice (*Oryza sativa*) grown in soils contaminated with these elements (Lahori et al., 2020; Rahmany-Samani et al., 2023).

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Similarly, zeolite is recognized for its high-water absorption and retention capacity, which increases water availability in the soil and enhances its uptake and use efficiency in plants, thereby allowing higher yields even under drought conditions (Hazrati et al., 2017; Hassan et al., 2024; Zeinalipour & Saadati, 2024). Additionally, studies have highlighted that zeolite promotes a slower release of herbicides (Tanaka et al., 2023), thereby extending their effectiveness in crops.

Regarding the use of zeolite as a fertilizer, it has been shown that its addition to the soil enhances the uptake and use efficiency of nitrogen (N), phosphorus (P), and potassium (K) in various cultivated species, including maize (*Zea mays* L.), tomato (*Solanum lycopersicum* L.), beans (*Phaseolus vulgaris* L.), spinach (*Spinacia oleracea* L.), lettuce (*Lactuca sativa* L.), canola (*Brassica napus* L.), rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and aloe (*Aloe vera* L.) (Ahmed et al., 2010; Bybordi & Ebrahimian, 2013; Li et al., 2013; Ozbahce et al., 2014; Cabrera-Fajardo et al., 2018; Hazrati et al., 2017; Hazrati et al., 2022; Hassan et al., 2024). This improvement translates into increased growth and yield, which is further enhanced when zeolite is combined with inorganic nitrogen and phosphate fertilizers, as it functions as a reservoir that provides a gradual nutrient release, thereby improving plant nutrient use efficiency (Ahmed et al., 2010).

Lettuce (*Lactuca sativa* L.) is a vegetable of high commercial and nutritional value, rich in vitamin A, vitamin K and beta-carotene (Kim et al., 2016). Soilless production systems represent a sustainable alternative that optimizes water use and addresses current challenges related to water availability (Maestre-Valero et al., 2018).

However, few studies have addressed the effect of zeolite when incorporated into growing substrates, as well as its influence on the growth of leafy vegetables. The purpose of this research was to assess the effect of adding zeolite to the substrate on lettuce plant growth.

Materials and Methods

Plant material

For seeding production, lyophilized lettuce seeds (*Lactuca sativa* L.) were sown in polystyrene trays with a capacity of 100 cavities. The seedlings were watered with water only in the first week, and from the second to the fourth week, Steiner's (1984) universal nutrient solution was applied, with a composition of 12.0, 1.0, 7.0, 7.0, 9.0 and 4.0 meq·L⁻¹ of nitrate (NO₃⁻), phosphate (H₂PO₄⁻), sulfate (SO₄²⁻), potassium (K⁺), calcium (Ca²⁺) and magnesium (Mg²⁺), respectively, at concentrations of 25 and 75 %. Transplanting was performed thirty days after sowing, selecting plants with at least three true leaves and consistent height (Sharma et al., 2009).

Substrate, Treatments and Experimental Units

Zeolite was obtained from Minerales Lozano. Transplanting was carried out in 3-L polyethylene bags filled

with a mixture of tezontle (particles larger than 0.5 cm) and zeolite at doses of 0, 2, 4 and 6 % (v/v), corresponding to the treatments under evaluation. Each treatment consisted of four replicates, resulting in a total of 24 experimental units.

Measurement of variables

The response variables were recorded fourteen days after transplanting (DAT), including plant height (cm), number of leaves, length and width of the largest leaf (cm), stem diameter (cm), and canopy coverage (%). Plant height was measured from the base of the stem to the tip of the longest leaf using a tape measure. Only fully expanded leaves were included in the leaf count.

The length and width of the largest leaf were determined by first selecting the leaf with these characteristics. A ruler was used to measure the distance from the base to the apex for length and at the widest point for width. Stem diameter was measured with a caliper at the midpoint of the stem. Canopy coverage was estimated using the Canopy app, which calculates the leaf canopy area from overhead photographs taken under controlled lighting and distance conditions (Govindasamy et al., 2022).

Data Analysis

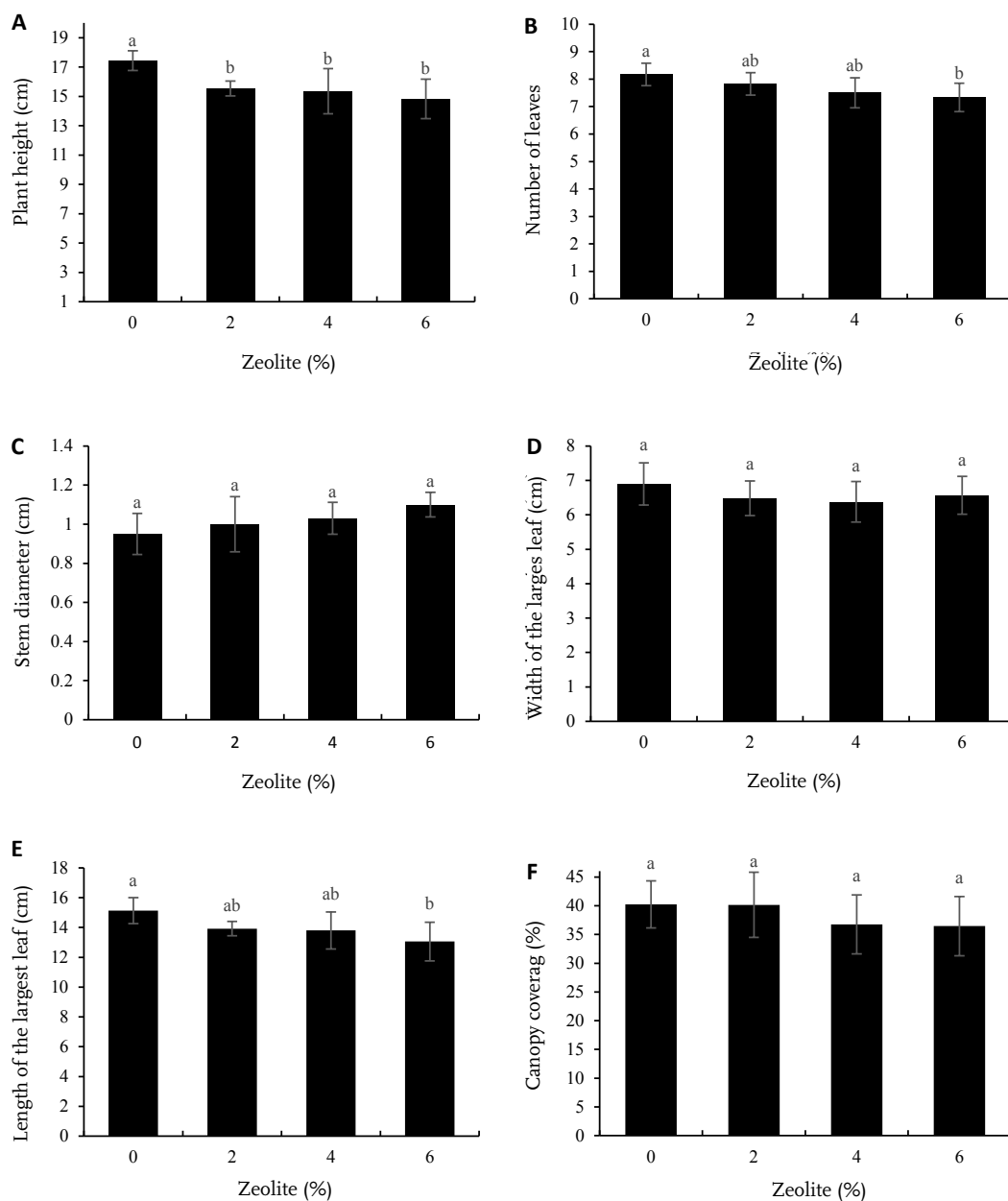
The data for each variable was processed using the R statistical software. The ANOVA was conducted following a completely randomized experimental design, and mean comparisons were performed using Tukey's test ($P \leq 0.05$).

Results and Discussion

Plant height decreased with the evaluated zeolite doses (Figure 1A), while the number of leaves (Figure 1B) and the length of the largest leaf (Figure 1E) were significantly reduced by the 6 % zeolite application compared to the control treatment. In contrast, no significant differences were observed in stem diameter, leaf width, or canopy coverage (Figures 1C, D, F). These results suggest that zeolite may negatively affect variables related to elongation and leaf development during the early growth stages of lettuce.

Although several authors have reported positive effects of zeolite on crop growth and yield such as maize, wheat, aloe vera, coriander (*Coriandrum sativum*) and strawberry (*Fragaria × ananassa*) (Ahmed et al., 2010; Hassan et al., 2024; Hazrati et al., 2017; Mahmoud et al., 2023; Zeinalipour & Saadati, 2024), these benefits are generally observed under abiotic stress conditions or when zeolite is applied in combination with synthetic chemical fertilizers. In the present study, conducted under optimal environmental and nutritional conditions, the addition of zeolite had no effect on lettuce growth, possibly due to its high adsorption and cation exchange capacity, which may temporarily

Figure 1. Plant height (A), number of leaves (B), stem diameter (C), width of the largest leaf (D), length of the largest leaf (E) and canopy coverage (F) of lettuce (*Lactuca sativa* L.) plants grown in substrate 0, 2, 4 and 6 % zeolite. Means with different letters indicate statistically significant differences among treatments (Tukey, $P \leq 0.05$)



retain nutrients and decrease their immediate availability to the plant (Szatanik-Kloc et al., 2021).

Moreover, it has been reported that zeolite can retain up to 50 % of its volume in water, but part of this is stored in pores and cannot be taken by roots, limiting effective water availability (Szatanik-Kloc et al., 2021). Similar results were described by Smedt et al. (2017) and Cabrera-Fajardo et al. (2018), who reported either no effects or reductions in the growth of vegetables grown in substrates containing zeolite. These findings suggest that the impact of zeolite

depends on the species, environmental conditions, and application method, with more favorable effects observed under stress conditions or during advanced stages of plant development.

Conclusions

The addition of zeolite to the substrate had no effect on the early growth stages of lettuce cv. Maximus. In contrast, its use reduced plant height, leaf number, and leaf length, without significantly affecting stem diameter or leaf width.

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English version

Nutritional and techno-functional properties of maguey white worm flour for food product development

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Abstract

The present study evaluates the nutritional and techno-functional potential of *Aegiale hesperiaris* (maguey white worm) flour, an endemic species traditionally consumed in Mexico. Larvae were manually collected, lyophilized, and processed to obtain both defatted and non-defatted flours. Proximate composition was determined on a dry weight basis using a nitrogen-to-protein conversion factor of 4.76 to prevent overestimation. Functional properties such as water- and oil-retention capacities, emulsifying capacity and stability, foaming capacity and stability, and protein solubility as a function of pH were also evaluated. Defatted flour showed higher water retention ($2.82 \text{ g} \cdot \text{g}^{-1}$) and oil retention capacities ($2.82 \text{ g} \cdot \text{g}^{-1}$), as well as superior emulsifying properties (EC: 60.83 %, ES: 89.36 %). In contrast, the non-defatted flour showed greater foaming capacity. Protein solubility was higher in the defatted flour across the entire pH range evaluated, reaching a maximum value of 95.82 % at pH 12. These results suggest that defatting enhances the functional properties of the flour, improving its potential for use in processed foods. Therefore, *A. hesperiaris* flour presents an adequate nutritional profile and versatile functional properties, supporting its potential as an alternative ingredient for the development of innovative and sustainable food products.

► **Keywords:** *Aegiale hesperiaris*, edible insects, flour, proteins, techno-functionality.

Introduction

The growing global demand for sustainable protein sources is driven by the rapid increase in population. According to FAO projections, by 2050 the world population is expected to reach approximately 9.7 billion people, placing significant pressure on current food systems (FAO, 2013; Van Huis, 2013). This scenario has prompted the search for alternatives that are both nutritionally efficient and environmentally responsible. In this context, edible insects have emerged as a viable option due to their high nutritional value and sustainable production (Abril et al., 2022; Wade & Hoelle, 2020). Many insects are notable for their high protein content, beneficial lipids, dietary fiber, and essential micronutrients such as zinc, iron, and calcium, making

them nutrient-dense sources (Nowakowski et al., 2022). Comparative studies have shown that some insects, such as mealworms, have nutritional profiles comparable to, or even surpassing, those of conventional meats (Costa et al., 2020). Furthermore, insect farming involves lower greenhouse gas emissions, reduced land and water use, and outstanding efficiency in converting feed into edible biomass compared to traditional livestock production. These advantages position insects as a sustainable and promising alternative for enhancing global food security (Belluco et al., 2023; Rumpold & Schlüter, 2013; Van Huis, 2013). The practice of consuming insects, known as entomophagy, has a long history across diverse cultures. It is estimated that more than 2 billion people include insects in their traditional diets (Khan et al., 2020), and over 2 100 edible species have

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been documented worldwide. Regions of Asia, Africa, and Latin America have deeply rooted entomophagy traditions; for example, in Mexico, insect consumption date back to the pre-Hispanic times and remains prevalent in many communities (Rodríguez-Ortega et al., 2020; Rostro et al., 2012). With more than 549 records species, Mexico has one of the highest diversities of edible insects. In states such as Oaxaca and Hidalgo, species like grasshoppers, *escamoles*, and agave worms are highly valued, both in traditional cuisine and contemporary gastronomy (Ronquillo-de Jesús et al., 2024).

However, in Westernized societies, the acceptance of insects as food has been limited due to cultural barriers and food neophobia (Siddiqui et al., 2022). Nevertheless, in recent years, interest in edible insects outside their traditional context has grown, driven by their nutritional and ecological benefits. Regulatory frameworks, such as those established by the European Union, have even begun to create standards legitimizing their human consumption (Lähteenmäki-Uutela et al., 2017). Currently, insects are often used in the form of flour or powders, which facilitates their use in processed foods and improves consumer acceptance by eliminating visually recognizable insect characteristics. These flours are rich in proteins, minerals, and vitamins, and can serve as ingredients in the development of food products. However, their industrial utilization requires the analysis of their physicochemical and techno-functional properties, which may vary depending on the species (Devi et al., 2022; Pincirolì, 2011).

Therefore, this study proposes to evaluate the nutritional and techno-functional properties of the white maguey worm (*Aegiale hesperiaris*) as part of the material characterization. This approach will enable a comprehensive assessment of its potential as a functional food ingredient, providing scientific evidence to support its incorporation into sustainable and innovative food systems.

Materials and Methods

Sample

A. hesperiaris (white maguey worm, WW) was collected at the larval stage directly from the leaves of its host plant, *Agave salmiana*, in the Actopan region, Hidalgo, Mexico. The larvae were euthanized by freezing and then lyophilized using a laboratory-scale freeze dryer (Triad™ Labconco, 103 USA). Subsequently, the sample was ground using a Nutribullet® food processor at 10 000 rpm for 15 s to achieve a particle size of < 0.15 mm. To prevent deterioration of the resulting flour, the sample was stored in black hermetic stand-up pouches at -20 °C until further use.

Defatted white maguey worm flour (WW-D) was prepared following the method of Kim et al., (2021) with minor modifications. Flour-solvent suspensions (hexane) were made at 20 % (w/v) in 50 mL tubes and mixed on an analog

roller mixer (Cole-Parmer SRT6D, Fisher Scientific, UK) at 60 rpm and 24 °C for 6 h. The fat-containing solvent was removed by decantation, and the extraction was repeated three times to ensure complete defatting.

Proximate Analysis and Caloric Contribution

Moisture, nitrogen, lipids, and ash contents were determined following the official methods recommended by the Association of Official Analytical Chemists (AOAC, 2012). Lipid content was measured using a Goldfish extractor (E-500 Büchi, Flawil, Switzerland) with hexane as the solvent. Total nitrogen was determined using the micro-Kjeldahl method (AOAC 945.01). To avoid overestimating the protein content of WW, a nitrogen-to-protein conversion factor of 4.76, as suggested by Janssen et al. (2017), was applied. The gross energy (kcal · 100g⁻¹) of WW flour was determined using an oxygen bomb calorimeter (model 6050, Parr Instrument Company, USA) with benzoic acid as the standard.

Techno-Functional Properties

Solubility

The protein content in the solubilized fractions of the defatted (WW-D) and non-defatted (WW-ND) flour was determined using the method described by Bradford (1976). For this purpose, 1.0 g of flour was mixed in 10 mL of 20 mM phosphate buffer. The pH of each sample was adjusted from 2 to 12 by adding 0.1 M HCl or 0.1 M NaOH. The suspensions were incubated at 37 °C for 45 min and subsequently centrifuged at 6 000 rpm for 15 min at 4 °C. The protein content in the supernatant was measured by mixing 20 µL of the sample with 200 µL of Bradford reagent. Absorbance was read at 595 nm using a UV-Vis microplate spectrophotometer (Multiskan Sky, Thermo Scientific, Singapore). Quantification was performed using a standard curve prepared with bovine serum albumin (BSA, 0-1 mg · mL⁻¹). The percentage of solubility was calculated using the following equation:

$$\text{Solubility (\%)} = \frac{\text{Protein content of the supernatant}}{\text{Total protein content in the sample}} \times 100 \quad (1)$$

Water and Oil Retention Capacity

The method for evaluating the water retention capacity (WRC) and oil retention capacity (ORC) of WW-D and WW-ND flours is based on the ability of the samples to absorb water or oil over a set period. To assess WRC, 0.2 g of flour was re-suspended in mL of distilled water. The mixture was vortexed for 60 s and then centrifuged at 3000 rpm for 20 min. The supernatant was removed by decantation and further extracted using a micropipette. WRC was expressed as the mass of water (g) per unit mass of flour (g). ORC was determined using the same procedure as WRC, substituting water with mL of canola oil. ORC was expressed as the mass of oil (g) per unit mass

of flour (g). WRC and ORC were calculated using the following equation.

$$WRC \text{ or } ORC = \frac{P_f - P_i}{P_i} \times 100 \quad (2)$$

Where: P_i is the initial mass of the sample (g) and P_f is the final mass of the sample (g).

Emulsifying Capacity and Stability

The emulsifying capacity and stability of WW-D and WW-ND flours were evaluated volumetrically, following the method described by Vanqa et al. (2022) with minor modifications. Flour-water suspensions (0.1 g in 10 mL) were prepared, and 10 mL of canola oil was added. The mixtures were homogenized using an IKA T25 Ultra-Turrax homogenizer (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 10 000 rpm for 3 min. The samples were then centrifugated at 4 000 rpm for 5 min. The volume of the emulsified layer was measured volumetrically and expressed as a percentage. Emulsion stability was assessed after allowing the samples to stand for 30 min at room temperature (22 °C), evaluating the stability of the emulsified layer. Emulsifying capacity and stability (EC and ES) were calculated using the following equation.

$$\text{Emulsifying capacity (EC)} = \frac{V_{em}}{V} \times 100 \quad (3)$$

$$\text{Emulsion stability (ES)} = \frac{V_{em30}}{V_{em}} \times 100 \quad (4)$$

Where: V_{em} is the volume of the emulsified layer, and V is the total volume; V_{em30} is the volume of the emulsified layer after 30 min.

Foaming Capacity and Stability

Foaming capacity (FC) and foam stability (FS) were determined according to Vanqa et al. (2022) with minor

modifications. Flour-water suspensions (20 % w/v) were prepared and then homogenized at 10 000 rpm for 4 min. The whipped sample was transferred to a graduated cylinder, and the foam volume was measured immediately (time 0) and after 30 min. of homogenization. Foaming capacity and foam stability (FC and FS) were calculated using the following equation:

$$\text{Foaming capacity (FC)} = \frac{V_{esp} - V}{V} \times 100 \quad (5)$$

$$\text{Emulsion stability (ES)} = \frac{V_{em30}}{V_{em}} \times 100 \quad (6)$$

Where: V_{esp} is the volume of the foam immediately after whipping, V_{esp30} is the volume of the foam after 30 min.

Statistical analysis

All experimental data are presented as mean \pm standard deviation. One-way ANOVA and significant differences (Tukey's test) were performed using Minitab 18® software (Minitab Inc., USA). Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

As mentioned previously, the insects were manually collected from the host plant of the white maguery worm (WW) in the Actopan region (20.287683, -98.912330), Hidalgo, Mexico. The process began with the visual identification of infested plants, characterized by irregular holes at the base of the leaves, typically associated with plant exudates and scorching on the leaf surface). These signs indicate that the larva has penetrated the plant tissue and is actively feeding within the leaf. Once an infested leaf was located, the tissue was carefully cut using manual tools such as knives or machetes, thereby exposing the internal galleries where the larva resides. In some cases, multiple larvae were collected from a single plant, depending on the degree of infestation (Figure 1).



Figure 1. Collection process to produce white maguery worm (*A. hesperiaris*) flour.

Table 1 shows the proximate composition of WW flour expressed on a dry matter basis. The protein content was 28.11 %, while the fat content was 32.63 % ($P \leq 0.05$). The ash content was 9.60 and the crude fiber content was 27.41 %. The nitrogen-free extract obtained by subtraction represented 27.41 % of the sample. The estimated energy value was $645.95 \text{ kcal} \cdot 100\text{g}^{-1}$.

The results of the proximate composition of WW flour were expressed on a dry matter basis, allowing for a more accurate comparison with other studies that have reported the nutritional value of edible insects processed under similar conditions. The protein content in this study was estimated from the total nitrogen content using a specific conversion factor of 4.76, instead of the conventional value of 6.25 commonly employed in bromatological analyses. This choice was based on the work of Janssen et al. (2017), who proposed this factor for insects, suggesting that the use of 6.25 tends to overestimate the actual protein content due to the presence of non-protein nitrogen sources such as chitin, nucleotides, and nitrogenous metabolites. Therefore, the value reported in this study provides a more accurate estimation of the actual protein content of WW. The protein content obtained (28.11 %) is consistent with the findings of Escamilla-Rosales et al. (2022), who reported values ranging from 27.0 to 33.5 % for dehydrated WW. Melo-Ruiz et al. (2011) reported a protein content of 30.88 % for the same species; however, it is likely that they used the conventional conversion factor of 6.25, which may have resulted in an overestimation.

The high fat content observed (32.63 %) falls within the range reported in the literature for this species (27–58 %) and contributes to the calculated energy of $645.95 \text{ kcal} \cdot 100 \text{ g}^{-1}$. The presence of lipids has also been highlighted as a distinctive characteristic of lepidopteran compared with other edible insects, such as *Tenebrio molitor*, whose fat proportion is generally lower (Martins da Silva et al., 2024). Regarding the ash content (2.25 %), this value suggests a moderate presence of minerals,

similar to the findings of Rodríguez-Ortega et al. (2020), who reported an ash content of 0.82 % in *Agathymus remingtoni* larvae, while Melo-Ruiz et al. (2011) reported values up to 1.35 % in other Latin American edible insect species. These differences may be attributed to species variation, type of pre-processing, developmental stage of the insects, and even environmental and dietary factors. Nevertheless, the value reported here for *Aegiale hesperiaris* highlights its relative mineral richness and potential as a complementary source of micronutrients in food products.

The crude fiber content (9.60 %) is relatively high for an insect, which may be attributed to the inclusion of chitin in the sample, as previously documented in whole-body insect analyses. However, according to Rostro et al. (2012), fiber content can vary significantly depending on the insect's development stage (egg, pupa or larva), with reported values ranging from 3.6 to 7.23 %. In the same study, variations in crude fiber were also documented among different species with a range between 1.1 to 9.5 %. Thus, the proximate profile obtained supports the potential of *A. hesperiaris* white worm flour as a functional ingredient in the development of enriched food products, contributing both to dietary diversification and to the utilization of endemic resources of high cultural and nutritional value.

Table 2 shows the techno-functional properties of non-defatted (WW-ND) and defatted (WW-D) white worm flours. The defatted flour (WW-D) had significantly higher values ($P \leq 0.05$) for water retention capacity (WRC) and oil retention capacity (ORC), with values of $2.82 \text{ g} \cdot \text{g}^{-1}$ in both cases. In contrast, the non-defatted flour (WW-ND) showed values of 1.75 y $1.85 \text{ g} \cdot \text{g}^{-1}$ for WRC and ORC, respectively. Regarding emulsifying capacity (EC), WW-D also demonstrated a significantly higher value (15.83 %) compared to GB-ND. Similarly, emulsion stability (ES) increased following the defatting process, reaching 89.36 % for WW-D versus 85 % for WW-ND. On the other hand, foaming capacity (FC) and foam stability (FS) were

Table 1. Proximate composition and caloric contribution of *A. hesperiaris* flour (% dry basis).

Sample	% Protein	% Fat	% Ash	% Fiber crude	% NFE	Energy (kcal·100g ⁻¹)
WW	28.11 ± 0.4 b	32.63 ± 0.2 a	2.25 ± 0.7 c	9.60 ± 0.1 d	27.41*	645.95 ± 0.1

WW: White worm; ELN: Nitrogen-free extract (obtained by difference). Values with different lowercase letters in the same row are significantly different ($P \leq 0.05$).

Table 2. Techno-functional properties of non-defatted and defatted *A. hesperiaris* flour.

Sample	WRC (g·g ⁻¹)	ORC (g·g ⁻¹)	EC (%)	ES (%)	FC (%)	FS (%)
WW-ND	1.75 ± 1.0 ^a	1.85 ± 0.2 ^a	50.00 ± 0.3 ^a	85.00 ± 0.3 ^a	5.50 ± 0.5 ^a	9.42 ± 0.5 ^b
WW-D	2.82 ± 0.7 ^b	2.82 ± 0.1 ^b	60.83 ± 2.8 ^b	89.36 ± 3.1 ^b	2.03 ± 0.2 ^b	6.49 ± 2.5 ^a

WW-ND: Non-defatted white worm flour; WW-D: Defatted White worm flour, WRC: water retention capacity, ORC: oil retention capacity, EC: Emulsifying capacity, ES: emulsion stability, FC: foaming capacity and FS: Foam stability. Values with different lowercase letters in the same column indicate a significant difference ($P \leq 0.05$).

significantly higher in the non-defatted flour (WW-ND), with values of 3.47 and 2.93 %, respectively), compared to the defatted flour (WW-D).

The techno-functional properties of WW flours evaluated in this study showed that the defatting process had a significant impact on key parameters such as water retention capacity (WRC), oil retention capacity (ORC), and emulsion capacity and stability (EC and ES), while notably reducing foaming capacity and stability.

In particular, the defatted flour showed a higher WHC (WW-D, $2.82 \text{ g} \cdot \text{g}^{-1}$) compared to the non-defatted flour (WW-ND, $1.75 \text{ g} \cdot \text{g}^{-1}$), a result with the findings of Cortazar-Moya et al. (2023) for *Arsenura armida*, where the defatted flour reached water retention values exceeding $2.75 \text{ g} \cdot \text{g}^{-1}$. This behaviour can be attributed to a greater exposure of polar groups following lipid removal, which enhances interactions with water molecules (Zhang et al., 2024). Similarly, the OHC was higher in the defatted flour ($2.82 \text{ g} \cdot \text{g}^{-1}$), consistent with studies on defatted *Gryllus bimaculatus*, where Jeong et al. (2021) reported that the use of ethanol as a solvent significantly improved both oil retention capacity and protein solubility. These findings reinforce that the partial removal of the lipid fraction increases the exposure of hydrophobic protein side chains, thereby enhancing their ability to retain lipid compounds.

In terms of emulsion capacity (EC), the defatted flour showed a significantly higher value (60.83 %) compared to the non-defatted flour (50.0 %). This is consistent with the findings of Lucas-González et al. (2019) in *Acheta domesticus* flour, where both lyophilization and defatting enhanced emulsion formation and stability. Furthermore,

Zielińska (2022) reported that defatted insect flour shows superior functional performance, emphasizing the influence of protein content and the reduced lipid interference in the formation of stable emulsions.

Conversely, the foaming capacity and stability (FC and FS) were higher in WW-ND, suggesting that the lipid fraction present in the not-defatted flour may contribute to the formation and maintenance of bubbles, thereby improving foam stability, possibly due to the formation of mixed protein-lipid films at the air-water interface. This effect has been reported by Mshayisa et al. (2022), who compared *Hermetia illucens* flours and protein concentrates and found that non-defatted flours showed greater foaming stability under certain conditions, supporting the hypothesis that residual lipids may play a structural role in foam stabilization.

Overall, these findings indicate that defatting WW flour enhances properties related to protein-water/oil interactions, which is advantageous for applications such as emulsions, dressings, or meat analogues. On the contrary, the non-defatted flour (WW-ND) may be more suitable for formulations that require some degree of foaming capacity, such as baked goods or aerated beverages. This functional differentiation is essential for defining specific technological applications for each fraction, taking into account their performance within food matrices, as well as the influence of pH conditions and protein concentration.

Figure 2 shows the influence of pH on the protein solubility of non-defatted (WW-ND) and defatted (WW-D) flours from *Aegiale hesperiaris*. In both samples, solubility showed a typical protein behavior, with an isoelectric

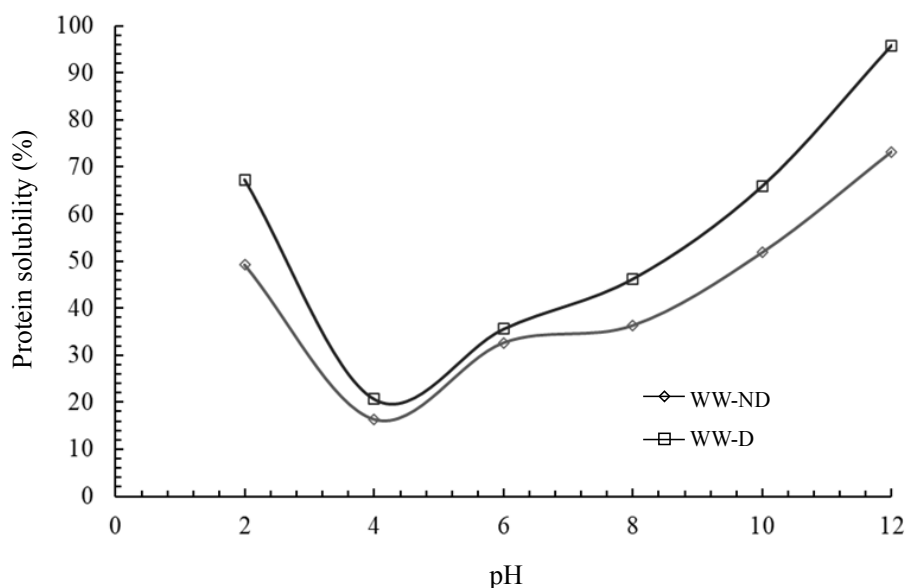


Figure 2. Effect of pH on the protein solubility of *A. hesperiaris*. WW-ND: non-defatted flour; WW-D: defatted flour.

point near pH, where the lowest solubility values were observed: 16.43 % for WW-ND and 20.68 % for WW-D. At extreme pH values (acidic and alkaline), protein solubility increased significantly. At pH 2, WW-D flour showed higher solubility (18.07 %) compared to WW-ND. This pattern persisted throughout the curve, becoming more pronounced under alkaline conditions, reaching maximum solubility values at pH 12: 95.82 % for WW-D and 73.24 % for WW-ND. This behavior can be attributed to the exposure of charged protein groups at pH values far from the isoelectric point, enhancing interaction with the aqueous medium. Additionally, the consistently higher solubility of WW-D suggested that lipid removal improves protein accessibility and dispersion, probably by reducing hydrophobic interactions that limit protein solvation.

Protein solubility as a function of pH showed the typical pattern of globular proteins, with a minimum near the isoelectric point (pH ~4) and a marked increase at extreme pH values. In this study, defatted flour (WW-D) showed higher solubility across the entire pH range, reaching a maximum of 95.82 % at pH 12, compared to 73.24 % for non-defatted flour (WW-ND). These results agree with Kim et al. (2017), who observed a significant increase in protein solubility of *Acheta domesticus* flour as pH moved away from the isoelectric point, with maximum values at extreme pH levels (60 and 120 % respectively). Similarly, Ndiritu et al. (2019), described a comparable behavior in *Acheta domesticus* protein concentrates, showing low solubility between pH 4-6 and a significant recovery of solubility above pH 8.

The higher solubility observed in WW-D flour in this study has also been reported in other species, such as *Gryllus bimaculatus* and *Tenebrio molitor*, where lipid removal improves protein accessibility to the aqueous medium and reduces internal hydrophobic interactions that limit protein dispersion in solution (Bußler et al., 2016; Jeong et al., 2021). Furthermore, the study by Villaseñor et al. (2022) highlights that defatting is a crucial step to enhance both the extraction yield and functionality of insect proteins, facilitating their application in functional foods. For *Aegiale hesperiaris*, these findings provide evidence of its high potential as a source of functional proteins, particularly when defatting is applied to improve solubility in food matrices with variable pH. This parameter is essential in the formulation of emulsified products, protein beverages, or dietary supplements, where adequate protein dispersion and stability are required.

Conclusion

White maguey worm (*A. hesperiaris*) flour showed a balanced nutritional profile and notable techno-functional properties, supporting its use as an ingredient in the development of functional foods. Defatting significantly enhanced water and oil retention capacities as well as

emulsifying properties, whereas the non-defatted flour showed superior foaming capacity. Additionally, protein solubility was higher in the defatted flour throughout the entire pH range evaluated, highlighting its versatility for various food applications. These results confirm the potential of this species as a functional protein source in sustainable food systems.

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English version

Evaluation of attractants for the black fig fly (*Silba adipata* McAlpine, 1956) under laboratory conditions

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Abstract

The black fig fly (*Silba adipata* McAlpine, 1956) is one of the main pests affecting fig (*Ficus carica* L.) production in Mexico, causing yield losses of up to 88 %. Chemical control has shown limited effectiveness against this pest; therefore, an alternative for reducing its incidence is the use of traps baited with attractants. In this context, the objective of the present study was to evaluate potential attractants for the black fig fly under laboratory conditions. Three trap designs were tested, and it was determined that designs I and II, composed of interconnected acetate cubes with different dimensions, were suitable for attractant evaluation. Six crude extracts (hexanic, methanolic, and aqueous) obtained from fig and apple fruits, as well as a control treatment (ammonium sulfate and apple), were tested in traps containing attractants. The attractant potential was assessed based on the number of flies captured. The control treatment showed the highest effectiveness, capturing 28.20 % of the flies in trap design II and 29.59 % in trap design I, followed by the methanolic extract of apple, with captured 23.17 % in design II and 26.10 % design I. The aqueous extract of fig captured 20.46 % of the flies in trap design I and 23.17 % in trap design II, making it the third most effective attractant. In contrast, both the hexanic extract of fig and the aqueous extract of apple were the least effective attractants, with 0 % captures

► **Keywords:** *Silba adipata*, attractants, trap designs.

Introduction

In Mexico, fig (*Ficus carica* L.) production has been promoted due to the growing demand for this fruit in international markets. In 2022, the Agri-Food and Fisheries Information Service (SIAP) reported the commercialization of fresh figs in 15 states across the country, with more than 11 500 t of fruit harvested. One of the main factors limiting production is the damage caused by the black fig fly (*Silba adipata* McAlpine), regarded as one of the most significant pests of fig crops worldwide.

The black fig fly is a monophagous frugivorous species native to the Mediterranean and the Middle East. It was first

described by McAlpine (1956) in Italy, Cyprus, and Greece (Abbes et al., 2021). Adults are glossy black, measuring 3.5 to 4.5 mm in length, with reddish eyes and brown legs. They primarily feed on the sweet exudate of ripe figs and on the milky sap of fig trees released from injured plant tissues (Katsoyannos, 1983).

In 2019, the first detections of *S. adipata* in commercial fig orchards were reported in the municipality of Ayala, Morelos, Mexico (SENASICA, 2021). The economic impact of this pest is because larvae feed on the tissues of the syconium, which creates internal mines. Infestation of immature figs often results in premature fruit drop (Katsoyannos, 1983), leading to losses of up to 88 % of the

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production (Flores, 2022). Because this pest was recently introduced in Mexico and there is no specific information on its chemical control, the use of traps baited with attractants represents an alternative strategy to reduce its incidence. The objective of this study was to evaluate the effect of different attractants on the black fig fly (*Silba adipata*) under laboratory conditions.

Materials and Methods

Biological material

Third-instar larvae were obtained from infested fig fruits collected from a fig orchard located in La Manzanilla, Xochiapulco, Puebla, at 19° 50' 34.5" N and 97° 38' 17.7" W, an elevation of 1 842 MASL (Figure 1).

This area belongs to the physiographic province of the Sierra Madre Oriental and has a humid temperate climate with abundant summer rainfall. Annual precipitation ranges from 1000 to 1200 mm and mean annual temperature ranges between 14 to 16 °C (INEGI, 2000).

Experimental site

The study was conducted in the Agricultural Phenology Laboratory of the Department of Crop Science at the Universidad Autónoma Chapingo, Estado de México.

Rearing medium for *Silba adipata*

Larvae were placed in glass containers with a base of absorbent paper and cotton moistened with a 50 % sucrose solution and incubated at 23 ± 0.1 °C, until adult flies emerged.

Development of Trap Desings for Attractant Evaluation

A total of three trap designs were evaluated as potential attractant devices for *S. adipata* under laboratory conditions. Six crude extracts (hexanic, methanolic and aqueous) obtained from fig and apple fruits, and a commercially available attractant used as a control, were tested.

Three trap designs were developed:

Trap Design I (TD-I) consisted of a structure made from transparent acetate sheets, which allowed direct observation of insect behavior and minimized the loss of volatile compounds emitted by the attractants. Seven acetate cubes, each measuring 22 cm³, were constructed, and different attractants were placed in each cube. The cubs were interconnected via a central rectangular prism measuring 66 × 22 × 22 cm (Figure 2). This configuration allowed free movement of the flies between the compartments, facilitating the simultaneous evaluation of different attractants.

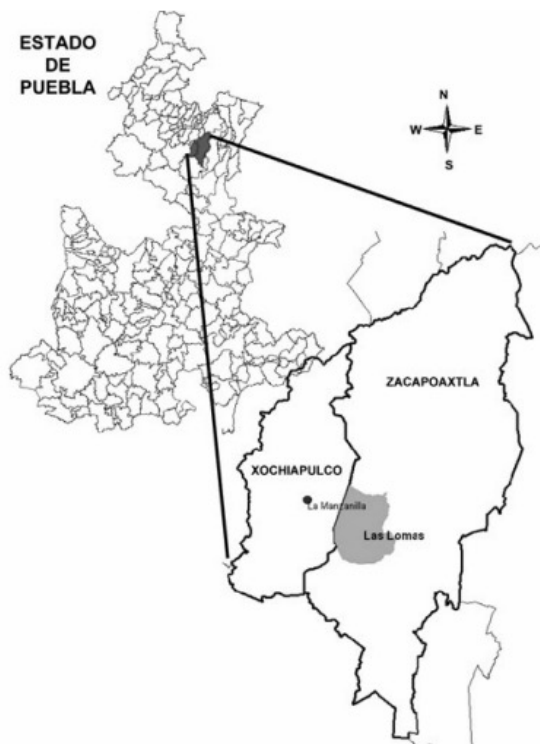


Figure 1. Geographical location of La Manzanilla, Xochiapulco, Puebla.

Source: Pérez et al., 2010.



Figure 2. Trap Design I for evaluating attractants for the black fig fly (*Silba adipata* McAlpine) under laboratory conditions.

Trap Design II (TD-II) was made using acetate sheets. Cubes measuring $10 \times 22 \times 22$ cm were used and interconnected through a rectangular prism measuring $30 \times 10 \times 10$ cm (Figure 2).

Trap Design III (TD-III) was made using seven 1-L polyethylene terephthalate (PET) bottles. Different attractants were placed in each bottle. The bottles were interconnected with $3/4$ " (19.1 mm) diameter, 30-cm-long rubber hoses leading to a central bottle with a larger capacity of 10 L (Figure 3).

Baited Trap

A baited trap was incorporated into each of the trap designs, consisting of a transparent polypropylene cup (Reyma® No. 4). Five holes, each 5 mm in diameter, were drilled around the midsection of the cup (Figure 4) to evaluate the effectiveness of the attractants used.

Plant Material Collection

Fruits of fig (*Ficus carica* L.) and apple (*Malus domestica* L.) were collected from an orchard in La Manzanilla, Xochiapulco, Puebla. The fruits were washed and cut into approximately 1 cm^3 . The resulting samples were placed in 15×22 cm non-woven fabric bags and frozen at -20°C for 24 h in a vertical freezer (frigorifère). The frozen plant material was then lyophilized using a Labconco Freezone 4.5 Liter Benchtop Freeze Dry System for 5 days until constant weight. The lyophilized samples were stored in seal bags until use.

Preparation of Crude Extracts

Aqueous extract. A total of 100 g of each lyophilized material was weighed, mixed with 150 mL of distilled water,

and boiled for 90 min. The supernatant was collected, concentrated by evaporation, and stored in amber bottles at 4°C until use.

Hexanic extract. A maceration extraction was performed using lyophilized fig and apple material. A total of 587 g of lyophilized fig and 382 g of lyophilized apple were placed in separate 5-L containers, and 3 L of hexane solvent was added to each. The mixtures were allowed to macerate at room temperature for 48 h. After maceration, the extracts were filtered and concentrated under reduced pressure using a rotary evaporator (Buchi RE120 and Buchi 461 water bath). This process was repeated four times. The concentrated crude extract from each extraction was placed in amber bottles and stored at 4°C . The final yield was 4 g of hexanic fig extract and 3 g of hexanic apple extract.

The residual plant material was air-dried at room temperature for 24 h to allow any remaining hexane to volatilize, leaving the material solvent-free. The residues from both fruits were then subjected to methanolic extraction.

Methanolic extract. The plant material residues from the hexane extraction were subjected to a second extraction using methanol as the solvent. The same procedure described for obtaining the hexane extracts of both fruits was followed. A total of four successive methanol extractions were carried out for each sample (fig and apple). As a result, 220 g of methanolic fig extract and 167 g of methanolic apple extract were obtained. The extracts were stored under refrigeration at 4°C in amber glass bottles until being used as attractants.

Control. A solution was prepared by mixing 1 L of distilled water, 20 g of ammonium sulfate, 100 g of ground apple and 1 g of Roma® soap, and the components were mixed until having a homogeneous mixture. The solution was



Figure 3. Trap Design III for evaluating attractants for the black fig fly (*Silba adipata* McAlpine, 1956) under laboratory conditions.



Figure 4. Baited trap

then placed in amber glass bottles and stored under refrigeration at 4 °C until being used as an attractant.

Statistical analysis

The attractant capacity of the extracts was evaluated based on the number of flies captured. The assays were conducted in triplicate, and the results were analyzed using the Student-Newman-Keuls multiple mean comparison test ($P \leq 0.05$).

Results

It was observed that TD-III showed no capture of flies for any of the attractants evaluated (Table 1). This result suggests that the design is not suitable for attractant evaluation under laboratory conditions. This may have been due to the shape of the design and, in particular, the material used. During experimental observations, it was noted that the rubber hoses released a strong, characteristic odor. It is likely that this odor interfered with the volatile compounds of the extracts used as attractants, reducing their

effectiveness and hindering the movement of flies toward the different traps.

No statistically significant differences were observed for TD-I and TD-II regarding the number of flies captured (Table 1), indicating that both designs were effective for fly attraction under laboratory conditions.

It is important to note that both TDs were constructed from transparent acetate, maintaining the same design but with different dimensions. TD-I was 54.55 % larger than TD-II. This suggests that the difference in dimensions between the two designs had no effect on its performance in the evaluation of attractants under laboratory conditions.

Attractant evaluation

The most effective attractants were the control treatment (ammonium sulfate and apple), which captured 29.59 % of the flies in TD-I and 28.20 % in TD-II, followed by the methanolic apple extract, with 26.10 % in TD-I and

Table 1. Multiple mean comparison of attractants in each structural design using the Student-Newman-Keuls (SNK) test.

TD	ATY	HFE	HAP	MFE	MAE	AFE	AAE	ASA
TD-I	$\sqrt{NFC-1}$	1.0000 b	1.2440 ab	1.2761 ab	1.9001 ab	1.7454 ab	1.0000 b	1.9894 a
	NFC	0.0	0.5475	0.6284	2.6104	2.0464	0.0	2.9593
	PFC (%)	0.0	5.4750	6.2840	26.104	20.464	0.0	29.593
	K	0.57139	0.69553	0.77118	0.82581	0.86851	0.90356	0.93322
TD-II	$\sqrt{NFC-1}$	1.0000 c	1.2440 bc	1.1381 c	1.8214 ab	1.8214 ab	1.0000 c	1.9546 a
	NFC	0.0	0.5475	0.2953	2.3175	2.3175	0.0	2.8205
	PFC (%)	0.0	5.4750	2.9530	23.175	23.175	0.0	28.205
	K	0.46878	0.57063	0.63269	0.67751	0.71254	0.74130	0.76563
TD-III	$\sqrt{NFC-1}$	1.0 b	1.0 b	1.0 b	1.0 b	1.0 b	1.0 b	1.0 b
	NFC	0	0	0	0	0	0	0
	PFC (%)	0	0	0	0	0	0	0
	K	0	0	0	0	0	0	0

Means with the same letter in each trap design indicate no statistically significant differences at $P \leq 0.05$; NFC: number of flies captured. K are the minimum significant differences according to the Student-Newman-Keuls test. ASA: Ammonium sulfate + apple (Control); HFE: Hexanic fig extract; HAP: Hexanic apple extract; MFE: Methanolic fig extract; MAE: Methanolic apple extract; AFE: Aqueous fig extract; AAE: Aqueous apple extract.

23.17 % in TD-II (Table 1). According to Ng et al., (2020), methanol is a solvent that facilitates the extraction of flavonoid glycosides because these compounds are polar. Monagas et al. (2003) indicate that both polyphenols and carotenoids are present in significant quantities in apple (*Malus domestica* L.), including caffeic, quinic, and p-coumaric acids, which are polar in nature and can be extracted with methanol. This suggests that the compounds may be phenolic in origin and that some terpenes may also be present, contributing to the attractant capacity of the extract. Therefore, the methanolic apple extract shows strong potential as an attractant and could be a useful tool for managing *S. adipata*.

The aqueous fig extract captured 20.46 % of *S. adipata* in TD-I and 23.17 % in TD-II, with the latter showing the highest attractant performance (Table 1). In contrast, both the hexane fig extract (HFE) and the aqueous apple extract (AAE) were the least effective attractants, resulting in 0 % capture of *Silba adipata*. These findings agree with Salih et al., (2020), who reported poor polyphenol extraction from macerated or cooked samples when using water as the solvent. This suggests that these extracts lack the compounds of active ingredients needed to function as effective attractants.

Conclusions

When comparing the three structural designs developed, it was observed that trap designs I and II are effective for evaluating attractants under laboratory conditions.

The methanolic apple extract and the aqueous fig extract can be used as attractants for the control of the black fig fly (*Silba adipata* McAlpine, 1956).

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English version

Effects of nitrogen deficiency on chlorophyll expression and synthesis in tomato

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Abstract

Chlorophyll synthesis plays an important role in maintaining photosynthetic efficiency, as well as in regulating plant development and tolerance to abiotic stress conditions such as nitrogen deficiency under nutrient management regimes. It has been demonstrated that the chlorophyll synthesis pathway maintains a mutually regulated dynamic relationship with the intracellular nitrogen assimilation pathway. This dynamic is controlled by the activity of enzymes such as uroporphyrinogen III methyltransferase (UPM1), which is key to tolerance under nitrogen-deficiency stress and to maintaining optimal levels of chlorophyll synthesis. The objective of this study was to identify the genes involved in the chlorophyll synthesis pathway and UPM1, characterize their functions, evaluate their gene expression levels *in silico*, and quantify their total chlorophyll in tomato under nitrogen-deficient conditions. The results indicate that the proteins involved in the chlorophyll synthesis pathway and their functions are conserved in tomato plants. Moreover, nitrogen deficiency reduced the gene expression levels of *SIHEMD* and *SIUPM1* (36 and 49 %, respectively), as well as total chlorophyll content by 20 %.

► **Keywords:** Comparative genomics, bioinformatics, *Solanum lycopersicum*.

Introduction

Photosynthesis is the most important biochemical mechanism for biological organisms. It is estimated that between 90-95 % of the biomass produced in plants comes directly from photosynthesis (Bag et al., 2020; Chen et al., 2023; Makino & Sage, 2007). Photosynthesis is divided into two main stages: the light-dependent reactions and the Calvin-Benson cycle (Shimizu et al., 2015). In the light-dependent reactions, the absorption of photons from solar energy induces electron transport across the thylakoid membrane within the chloroplasts. This electron gradient promotes the synthesis of energy in the form of ATP and NADPH (Pietrzykowska et al., 2014). Subsequently, this energy is used by

the reactions of the Calvin-Benson cycle for carbon dioxide fixation and the synthesis of organic compounds (Smith et al., 2024).

The maintenance of photosynthetic capacity depends on the conformation of photosystems I and II. The photosystems are composed of the reaction centers and the light-harvesting complexes (LhcA/B) (Croce & van Amerongen, 2013; Gao et al., 2018; Nelson & Yocum, 2006; van Amerongen & Croce, 2013). The light-harvesting complexes consist of a series of proteins known as Chlorophyll a/b binding Protein. These proteins serve as binding sites for the chlorophylls, which together carry out the absorption and transfer of energy to the reaction center of the photosystems, sub-

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sequently initiating the electron gradient (Croce & van Amerongen, 2013; Gao et al., 2018; Nelson & Yocum, 2006; van Amerongen & Croce, 2013).

The chlorophyll biosynthetic pathway is an essential component for the proper assembly of the photosystems and for the maintenance of photosynthetic capacity (Mao et al., 2025). This pathway is coordinated by a series of reactions that are divided into four main sub-stages (Chatterjee et al., 2015). The first stage enables the synthesis of aminolevulinic acid from glutamic acid as the precursor. The second set of reactions promotes the synthesis of uroporphyrinogen III using the 5-aminolevulinic acid generated in the previous stage as the precursor. The third stage involves the synthesis of protoporphyrin IX from uroporphyrinogen III as the precursor. Finally, the fourth stage comprises the synthesis of chlorophyll *a* from protoporphyrin IX (Wu, et al., 2019b).

Nitrogen is the most important macronutrient in crop nutrition management (Krapp, 2015). In plant nutrition, nitrogen is primarily supplied in the forms of nitrate (NO_3^-) and ammonium (NH_4^+), and to a lesser extent as amino acids and short-chain peptides (Zayed et al., 2023). Once taken up by the root systems, these compounds (NO_3^- and NH_4^+) are transported to the foliar system through the vascular tissue. In the leaves, they are metabolized via the intracellular nitrogen assimilation pathway, which involves enzymes such as nitrate reductase (NR), nitrate reductase (NiR), glutamate synthase (GOGAT), and glutamine synthetase (GS) (Gilad, et al., 2025). Factors such as the nitrogen supply rate, the $\text{NO}_3^-/\text{NH}_4^+$ ratio in plant nutrition, and the activity of the intracellular assimilation enzymes regulate the capacity for glutamic acid synthesis, thereby affecting chlorophyll synthesis and the photosynthetic capacity of crops (Roosta et al., 2025; Guo et al., 2007).

Several studies have demonstrated a reduction in chlorophyll content as well as a decrease in photosynthetic capacity in models subjected to nitrogen-deficient conditions (Cetner et al., 2017; Ferreira et al., 2016; Lu et al., 2021). Under nitrogen deficiency, a reduction in the expression levels of genes associated with enzymes of the chlorophyll synthesis pathways, such as *HEMA1*, *HEMA2*, *HEMB1*, *HEMC*, *HEMD* and *HEMF* has been observed. These results were correlated with decreased levels of 5-aminolevulinic acid and total chlorophyll, as well as with a decrease in the expression levels of genes associated with enzymes of the intracellular nitrogen assimilation pathway, including *NR*, *NiR*, *GOGAT* and *GS* (Wen et al., 2019a).

It has been shown that the dynamics of the interaction between the chlorophyll biosynthesis pathway and the intracellular nitrogen assimilation pathway may be regulated by the activity of uroporphyrinogen III methyltransferase

(UPM1) (Garai et al., 2016). UPM1 plays an important role in the synthesis of the prosthetic group siroheme. Siroheme is an integral component of nitrite reductase as a prosthetic group. This group is synthesized in the chloroplasts using uroporphyrinogen III as a precursor (Tanaka, et al., 2011). UPM1 appears to play a vital role in nitrogen metabolism through the assembly of nitrite reductase, which contributes to the regulation of glutamic acid levels in the chloroplast. This metabolic interplay, in turn, contributes to the regulation of chlorophyll synthesis and the photosynthetic capacity of crops (Garai & Tripathy, 2018).

Understanding the impact of low nitrogen supply on the expression levels of enzymes in the chlorophyll synthesis pathway and UPM1 is crucial for clarifying crop responses to nutritional stress. The objective of this study was to identify the genes involved in the chlorophyll synthesis pathway and UPM1, characterize their functions, evaluate their gene expression levels *in silico*, and assess chlorophyll synthesis capacity in tomato under nitrogen-deficient conditions.

Materials and Methods

Identification and characterization of the genes and proteins of the chlorophyll synthesis pathway and UPM1

To identify the amino acid and nucleotide sequences (CDS; coding sequence, and genomic sequence) involved in the chlorophyll synthesis pathway and UPM1 in tomato, the sequences were first identified in *Arabidopsis thaliana*, which was used as a reference model. These sequences were retrieved from the TAIR database (<https://www.arabidopsis.org>). Subsequently, a comparative genomic analysis was performed using the BLASTP tool (<https://blast.ncbi.nlm.nih.gov/>) against the complete protein collection (CDS translations+PDB+SwissProt+PIR+PRF) archived in GenBank (834,777,505 sequences; as of November 2024). The search and comparison were restricted to *Solanum lycopersicum* (taxid:4081). The following parameters were applied for the analysis: percent identity ($\geq 80\%$), percent coverage ($\geq 90\%$) and E-value ($1e^{-10}$). The accession numbers with the best results were used to retrieve the amino acid and nucleotide sequences (CDS and genomic sequences) for tomato deposited in GenBank. (<https://www.ncbi.nlm.nih.gov/>). Using the collected sequences, the following characteristics were estimated: protein molecular weight (MW-P), isoelectric point (pI), amino acid sequence length (AA-L), and protein subcellular localization through the ExPasy tool (<https://web.expasy.org/cgi-bin/protparam/protparam>), WoLF PSORT (<https://www.genscript.com/wolf-psort.html>) and Cell-PLoc 2.0 (<http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/>). The nucleotide sequences were used to determine the number of exons and introns, as well as the chromosomal location of the genes identified in *A. thaliana* and tomato (Lan et al., 2022).

Multiple sequence alignment and phylogenetic analysis of amino acid sequences

To identify intra- and interspecific homology, as well as to understand the evolutionary relationships among the protein sequence identified in *A. thaliana* and tomato, a multiple sequence alignment analysis was performed. The ClustalW method was used for this purpose, applying the default parameters in Mega XI. Subsequently, the phylogenetic tree was constructed using the Neighbor-Joining tree, using the Jones-Taylor Thornton model with 1 000 bootstrap replicates. Finally, the iTOL tool (<https://itol.embl.de/>) was used to generate a high-quality schematic representation (Lan et al., 2022).

Structural analysis of genes and proteins in the chlorophyll synthesis pathway and UPM1

To verify the functionality of the protein and genomic sequence identified in tomato, a structural analysis of these sequences was performed. Exons and introns of the genomic sequence were analyzed and identified using the Gene Structure Display Server (GSDS) (<https://gsds.gao-lab.org/>). In addition, conserved protein motifs were identified using the MEME platform (<https://meme-suite.org/meme/tools/meme>) and InterPro (<https://www.ebi.ac.uk/interpro/>). The diagrams were generated using the TBtools platform (v2.096, <https://github.com/CJ-Chen/TBtools-II>) (Lan et al., 2022).

In silico analysis of gene expression in the chlorophyll synthesis pathway and UPM1 in tomato under low-nitrogen conditions

To determine the effect of low-nitrogen conditions on the *in silico* expression levels of the genes identified in tomato, Illumina RNA-seq data were analyzed from the NCBI-GEO database (<https://www.ncbi.nlm.nih.gov/gds>). The data corresponded to two different treatments: control treatment (CT) and low-nitrogen treatment (LNT). Gene accession numbers were used to assess the effect of each treatment on their expression levels. The results were reported as FPKM (Fragments per kilobase of exon per million).

Plant material and treatments

The present study was conducted in commercial greenhouses under a hydroponic production system using coconut fiber as the substrate. The greenhouses had the following characteristics: gothic-type design, polyethylene flooring and covering, north-south orientation, and passive ventilation systems with front windows. Sixteen days after germination, the seedlings were grafted using “Maxifort” rootstocks and transplanted into the substrate in hydroponic production systems. The plants were subjected to two treatments: a control treatment (CT) and a

low-nitrogen treatment (LNT). For the control treatment, a drip irrigation system was used to supply Hoagland solution with the following composition: NO_3^- (17.54 mmol), NH_4^+ (1.91 mmol), H_2PO_4 (3.79 mmol), SO_4^{2-} (4.2 mmol), K^+ (9.65 mmol), Ca^{2+} (8.41 mmol), Mg^{2+} (2.3 mmol), B^{3+} (148.01 mmol), Cl^- (10.2 mmol), Cu^{2+} (1.58 mmol), Fe^{2+} (55.34 mmol), Mn^{2+} (13.29 mmol) and Zn^{2+} (12.54 mmol). For the LNT treatment, a drip irrigation system was also used to supply Hoagland solution with the following composition: NO_3^- (12.3 mmol), NH_4^+ (0.96 mmol), H_2PO_4 (1.34 mmol), SO_4^{2-} (3.2 mmol), K^+ (7.57 mmol), Ca^{2+} (3.87 mmol), Mg^{2+} (1.69 mmol), B^{3+} (83.26 mmol), Cl^- (4.6 mmol), Cu^{2+} (1.1 mmol), Fe^{2+} (43.34 mmol), Mn^{2+} (9.1 mmol) and Zn^{2+} (8.41 mmol). The electrical conductivity and pH of the Hoagland solution for the CT were $4.35 \text{ mS} \cdot \text{cm}^{-1}$ and 5.62, respectively, while for LNT the electrical conductivity and pH were $2.54 \text{ mS} \cdot \text{cm}^{-1}$ and 5.15, respectively. The crop production cycle extended from week 10 to week 50 of development following transplanting. During this period, 15 plants per treatment were randomly selected for sampling.

Sampling collection

Leaf samples ($n=15$) were randomly collected throughout the greenhouse. The eighth compound leaf was selected for sampling, with leaf numbering initiated from the uppermost leaf. Samples were placed in sterile bags and kept at 4°C , then flash-frozen in liquid nitrogen and stored at -81°C .

Total Chlorophyll Content

Total chlorophyll content was estimated following the methodology described by Wu et al., (2019); Xi et al., (2021) and Zhong et al., (2017). A total of 200 mg of fresh leaf tissue was weighed, and 25 mL of an alcohol-acetone extraction solution 1:1 (v/v) was added. The mixture was homogenized at 10 000 rpm for 1 minute using an Ultra-Turrax (IKA, T-25D). The homogenate was then centrifuged at 10 000 rpm for 2 minutes at 4°C . The supernatant was recovered, and absorbance was measured at 663 and 645 nm. Total chlorophyll content was expressed as $\text{mg} \cdot \text{g}^{-1}$ FW. The following equation was used:

$$\text{Total Chlorophyll Content (mg} \cdot \text{g}^{-1} \text{ PF)} = (20.2)(\text{ABS } 645 \text{ nm}) - (8.02)(\text{ABS } 663 \text{ nm})$$

Results and Discussion

Identification and physicochemical characterization of genes and proteins involved in the chlorophyll synthesis pathway and UPM1

In this study, twenty-six genes and proteins involved in the chlorophyll synthesis pathway were identified in *A. thaliana*. Additionally, a single gene responsible for

UPM1 expression was identified. In tomato, 19 genes and proteins associated with the chlorophyll synthesis pathway were identified through comparative genomic analysis using the BLASTP tool, with *A. thaliana* as the reference model, and one gene responsible for UPM1 expression was also found. The physicochemical characteristics of the identified genes and proteins, including coding sequence length (CDS length, (LS-CDS), number of exons (NE), chromosomal location, amino acid sequence length (LS-AA), protein molecular weight (kDa) (PM-P), protein isoelectric point (PI), and subcellular localization, are systematically described in Table 1. For the chlorophyll synthesis pathways genes in *A. thaliana*, LS-CDS was 938 pb (*AtHEMD*) to 2 282 pb (*AtCHLD*). In tomato, the identified genes showed LS-CDS values ranging from 908 pb (*SlHEMD*) to 2 300 pb (*SlCHLD*). Regarding chromosomal localization, in *A. thaliana*, chromosome 1 showed the highest gene density (GD) with eight genes, while chromosome 2 had the lowest GD with three genes. In tomato, chromosomes 4 and 10 showed the highest GD, each containing ten genes. The protein sequences identified in *A. thaliana* showed amino acid sequence lengths, PM-P and PI ranging from 312 aa (*AtCHLM*) – 1 381 aa (*AtCHLH*), de 33 796 kDa (*AtCHLM*) – 153 574 kDa (*AtCHLH*) and 5.26 (*AtCHLD*) - 9.49 (*AtNOL*). In tomato, the identified proteins showed LS-AA from 302 aa (*SlHEMD*) to 1 381 aa (*SlCHLH*), PM-P from 32 359 kDa

(*SlHEMD*) to 153 688 kDa (*SlCHLH*) and PI from 5.28 (*SlCHLD*) to 9.42 (*SlPORB*). Subcellular localization prediction indicated that the proteins in both species are localized to chloroplast, including specific components such as thylakoid membranes and the stroma (Table 1).

Multiple sequence alignment and phylogenetic analysis of amino acid sequences

The evolutionary relationships of the proteins identified in *A. thaliana* and tomato were evaluated both intra- and interspecifically. Phylogenetic analysis revealed a close relationship between the proteins identified in both species (Figure 1), with homologous proteins from tomato and *A. thaliana* clustering within the same clade. Similarly, UPM1 from both species was successfully grouped into a single clade. The sequences identified in tomato showed high homology relative to the proteins identified in *A. thaliana*. Multiple sequence alignment showed an average homology of approximately 78 % between the proteins of the two species. The lowest homology was observed for HEMD at 63.97 % whereas the highest was for CHLH at 86.19 %. The high degree of homology between the two species indicates the presence of orthologous genes with conserved functionality, which likely arose from speciation events during the evolutionary history of tomato and *A. thaliana* (Liu et al., 2023; Mao et al., 2022; Xi et al., 2022; Xia et al.,

Table 1. Identification of genes and proteins of the chlorophyll synthesis pathway and their physicochemical characteristics.

Species	Gene name	Protein GenBank ID	Gen GenBank ID	Protein length (aa)	Protein molecular weight (kDa)	Isoelectric point	CDS length (pb)	Number of exons	Chromosomal location	Subcellular localization	
										Cell-PLoc 2.0	WoLF PSORT
<i>A. thaliana</i>	<i>AtHEMA1</i>	NP_176125.1	NM_104609.4	543	59,515	7.95	1,631	3	1	Chloroplast	Chloroplast
<i>A. thaliana</i>	<i>AtHEMA2</i>	NP_172465.1	NM_100868.3	530	58,292	8.82	1,592	3	1	Chloroplast	Chloroplast
<i>A. thaliana</i>	<i>AtHEMA3</i>	NP_180683.1	NM_128681.3	524	58,691	8.78	1,574	3	2	Chloroplast	Chloroplast
<i>A. thaliana</i>	<i>AtGSA1</i>	NP_201162.1	NM_125752.4	474	50,370	6.42	1,424	3	5	Chloroplast	Chloroplast
<i>A. thaliana</i>	<i>AtGSA2</i>	NP_190442.1	NM_114732.5	472	50,142	7.01	1,418	4	3	Chloroplast	Chloroplast
<i>A. thaliana</i>	<i>AtHEMB1</i>	NP_177132.1	NM_105642.4	430	46,690	6.96	1,292	13	1	Chloroplast	Chloroplast
<i>A. thaliana</i>	<i>AtHEMB2</i>	NP_175085.1	NM_103545.2	406	44,878	8.39	1,221	10	1	Chloroplast	Thylakoid membrane
<i>A. thaliana</i>	<i>AtHEMC</i>	NP_196445.1	NM_120911.4	382	41,043	8.75	1,148	5	5	Chloroplast	Thylakoid membrane
<i>A. thaliana</i>	<i>AtHEMD</i>	NP_565625.1	NM_128211.4	321	34,225	7.81	965	9	2	Chloroplast	Thylakoid membrane
<i>A. thaliana</i>	<i>AtHEME1</i>	NP_850587.1	NM_180256.2	418	46,254	6.64	1,256	6	3	Chloroplast	Chloroplast
<i>A. thaliana</i>	<i>AtHEME2</i>	NP_181581.1	NM_129611.5	394	43,580	8.29	1,184	6	2	Chloroplast	Thylakoid membrane
<i>A. thaliana</i>	<i>AtHEMF</i>	NP_171847.4	NM_100230.7	386	43,796	6.24	1,160	8	1	Chloroplast	Chloroplast
<i>A. thaliana</i>	<i>AtHEMG1</i>	NP_192078.1	NM_116399.4	537	57,695	9.13	1,613	9	4	Chloroplast	Thylakoid membrane
<i>A. thaliana</i>	<i>AtCHLH</i>	NP_196867.1	NM_121366.4	1381	153,574	5.8	4,145	5	5	Chloroplast	Chloroplast
<i>A. thaliana</i>	<i>AtCHLD</i>	NP_563821.2	NM_100725.4	760	83,284	5.26	2,282	15	1	Chloroplast	Stroma
<i>A. thaliana</i>	<i>AtCHLI1</i>	NP_193583.1	NM_117962.3	424	46,270	6.08	1,274	3	4	Chloroplast	Stroma
<i>A. thaliana</i>	<i>AtCHLI2</i>	NP_199405.2	NM_123961.4	418	46,098	5.36	1,256	3	5	Chloroplast	Stroma

Table 1. Identification of genes and proteins of the chlorophyll synthesis pathway and their physicochemical characteristics. (cont.)

Species	Gene name	Protein GenBank ID	Gen GenBank ID	Protein length (aa)	Protein molecular weight (kDa)	Isoelectric point	CDS length (pb)	Number of exons	Chromosomal location	Subcellular localization	
										Cell-PLoc 2.0	WoLF PSORT
<i>A. thaliana</i>	<i>AtCHLM</i>	NP_194238.1	NM_118640.3	312	33,796	7.68	938	3	4	Chloroplast	Thylakoid membrane
<i>A. thaliana</i>	<i>AtCRD1</i>	NP_191253.1	NM_115553.4	409	47,631	8.55	1,229	5	3	Chloroplast	Thylakoid membrane
<i>A. thaliana</i>	<i>AtDVR</i>	NP_197367.1	NM_121871.3	417	45,893	7.47	1,253	1	5	Chloroplast	Thylakoid membrane /Stroma
<i>A. thaliana</i>	<i>AtPORA</i>	NP_200230.1	NM_124799.4	405	43,863	9.42	1,217	4	5	Chloroplast	Chloroplast
<i>A. thaliana</i>	<i>AtPORB</i>	NP_194474.1	NM_118879.4	401	43,359	9.23	1,205	5	4	Chloroplast	Chloroplast
<i>A. thaliana</i>	<i>AtPORC</i>	NP_171860.1	NM_100243.4	401	43,883	9.18	1,205	5	1	Chloroplast	Chloroplast
<i>A. thaliana</i>	<i>AtCAO</i>	NP_175088.1	NM_103548.5	536	60,331	7.96	1,610	8	1	Chloroplast	Chloroplast
<i>A. thaliana</i>	<i>AtNOL</i>	NP_568145.1	NM_120572.3	348	38,149	9.49	1,046	13	5	Chloroplast	Chloroplast
<i>A. thaliana</i>	<i>AtCHLG</i>	NP_190750.1	NM_115041.4	387	41,881	8.52	1,163	14	3	Chloroplast	Stroma
<i>S. lycopersicum</i>	<i>SIHEMA1</i>	XP_004238036.1	XM_004237988.4	544	59,342	8.37	1,634	3	4	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SIGSA</i>	NP_001234690.2	NM_001247761.2	482	51,500	6.53	1,448	3	4	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SIHEMB</i>	XP_004245301.1	XM_004245253.4	430	46,836	6.36	1,292	14	8	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SIHEMD</i>	XP_010320354.1	XM_010322053.4	302	32,359	8.19	908	10	4	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SIHEME</i>	XP_010327114.1	XM_010328812.4	420	45,984	6.47	1,262	6	10	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SIHEMF</i>	XP_004248412.2	XM_004248364.5	399	44,717	5.8	1,199	8	10	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SIHEMG1</i>	NP_001335308.1	NM_001348379.1	558	60,446	8.98	1,676	8	1	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SIChLH</i>	XP_004236610.1	XM_004236562.5	1381	153,688	5.93	4,145	5	4	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SICHLD</i>	XP_004236627.1	XM_004236579.5	766	83,918	5.28	2,300	15	4	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SICHLI</i>	XP_004248139.1	XM_004248091.5	427	46,434	6.07	1,283	3	10	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SICHLM</i>	XP_004235845.2	XM_004235797.5	327	35,532	6.96	983	2	3	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SICRD1</i>	NP_001332768.1	NM_001345839.1	404	47,185	8.99	1,214	5	10	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SIDVR</i>	XP_010321210.1	XM_010322908.4	420	46,086	6.41	1,262	2	1	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SIPORA</i>	XP_004251852.1	XM_004251804.5	397	42,812	9.19	1,193	5	12	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SIPORB</i>	NP_001304903.1	NM_001317974.1	395	42,611	9.42	1,187	5	10	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SIPORC</i>	NP_001296970.1	NM_001310041.1	399	43,199	9.27	1,199	5	7	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SICAO</i>	XP_004250311.1	XM_004250263.5	535	60,237	7.54	1,607	9	11	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SINOL</i>	XP_010321272.1	XM_010322970.4	346	37,925	9.22	1,040	13	5	Chloroplast	Chloroplast
<i>S. lycopersicum</i>	<i>SICHLG</i>	XP_004246318.1	XM_004246270.4	369	40,038	8.26	1,109	15	9	Chloroplast	Chloroplast

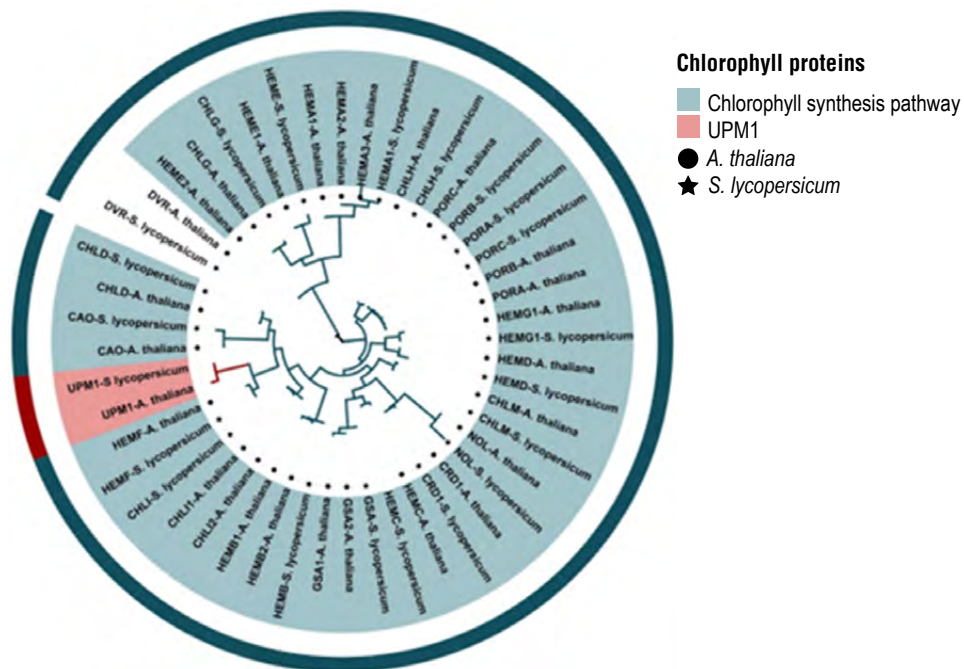
* pb; base pairs, kDa; kilodaltons, aa; amino acids

2023). In the case of *A. thaliana*, multiple sequence alignment enabled the identification of two or three isoforms for HEMA, GSA, HEMB, HEME and CHLI. For HEMA (HEMA1, HEMA2 and HEMA3), as shown in Table 1 and Figure 1, the alignment revealed high homology among the three isoforms (68-81 %). The isoform groups for GSA (GSA1 and GSA2), HEMB (HEMB1 and HEMB2), HEME (HEME1 and HEME2) and CHLI (CHLI1 and CHLI2) had homology levels of 90, 65, 51 and 83 % respectively. The POR protein family (PORA, PORB y PORC) showed high homology among its members (77-87 %). These results suggest that gene duplication events have occurred through

the evolutionary history of *A. thaliana*, leading to the generation of paralogous genes with conserved functionality (Liu et al., 2023; Mao et al., 2022; Xi et al., 2022; Xia et al., 2023). Finally, the amino acid sequences of UPM1 also had high homology (75 %).

Structural analysis of genes and proteins in the chlorophyll synthesis pathway and UPM1

The structural characteristics of the genes and proteins identified in tomato were analyzed. Exon (CDS)/Intron structural analysis revealed considerable divergence



The phylogenetic tree was constructed using the neighbor-joining method with 1000bootstrap replicates and fitted to the Jones-Taylor Thornton model.

Figure 1. Phylogenetic analysis of amino acid sequences identified in tomato and *Arabidopsis thaliana*.

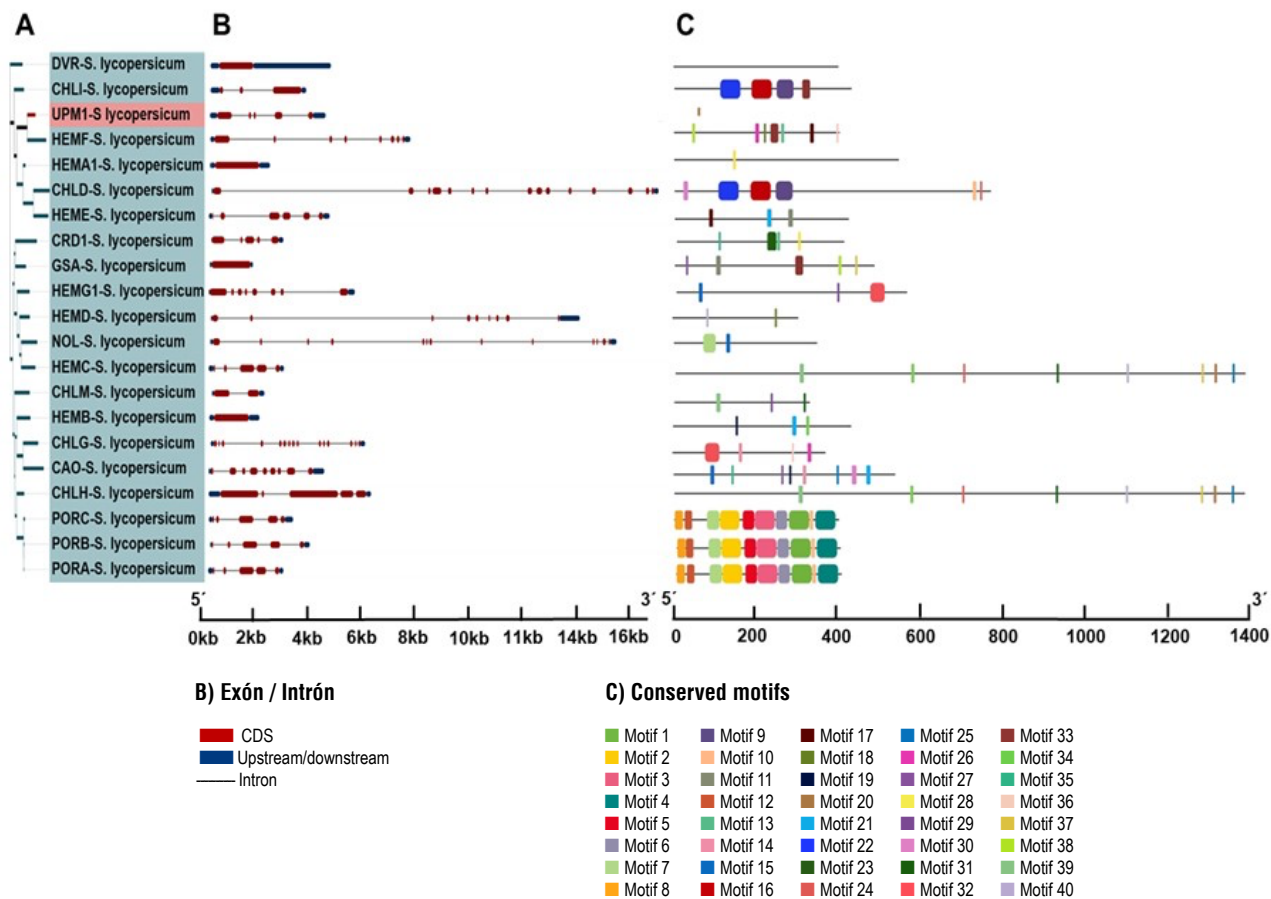
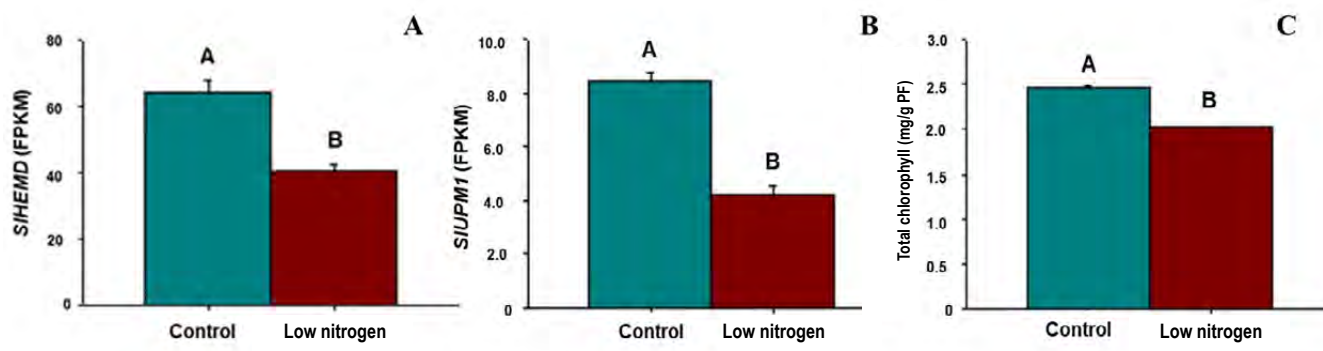


Figure 2. Phylogenetic analysis of sequences identified in tomato, generated using the Neighbor-joining method. (B) Structural genomic analysis of CDS and UTR sequences, represented by red and blue boxes, respectively, and introns represented by solid black lines. (C) Analysis of conserved protein motifs in the chlorophyll synthesis pathway and UPM1 in tomato.



* Genomic expression data was obtained from NCBI-GEO database and are expressed as FPKM (Fragments per Kilobase of transcript per Million mapped reads).

** The data is presented as mean \pm standard error ($n=15$). Bars with the same letter are not statistically different according to Tukey's test. The "Control" and "Low nitrogen" treatments are represented by green and red bars, respectively. The graphs were generated using SigmaPlot 11.

Figure 3. (A and B) Comparison of *SIHEMD* and *SIUPM1*. (c) Comparison of total chlorophyll synthesis in leaves.

among the identified genes (Table 1 and Figure 2B). In this study, the number of exons ranged from 2 (*SlDVR* and *SlCHLM*) to 15 (*SlCHLD* and *SlCHLG*). These results suggest that intron insertion and loss events have occurred throughout the evolutionary history of tomato. These structural changes may also be associated with alterations in the functionality of the proteins encoded by these genes (Liu et al., 2023). The structural analysis of the identified proteins and their corresponding genomic sequences revealed considerable structural divergence. In this study, up to 40 conserved protein motifs were identified in the amino acid sequences. In contrast, the UPM1 protein sequence showed only a single conserved motif in its structure (Figure 2C). The high number of conserved motifs is associated with the observed variability, which is also reflected in the number of introns and exons in the genomic sequences (Figure 2 B).

***In silico* expression levels of *SIHEMD*, *SIUPM1* and total chlorophyll content**

The *in silico* expression levels of *SIHEMD* and *SIUPM1* were analyzed under LNT and CT conditions. The results showed an approximately 36 % reduction in *SIHEMD* transcript level under LNT conditions (Figure 3A). For *SIUPM1*, the results also showed a \approx 49 % decrease in transcript level under LNT (Figure 3B). Total chlorophyll content (TCC) under LNT conditions showed a 20 % (Figure 3C). UPM1 is an essential component for the optimal functioning of the intracellular nitrogen assimilation pathway (Garai & Tripathy, 2018). It also promotes the synthesis of the prosthetic group siroheme, which is part of the structure of nitrite reductase (Garai & Tripathy, 2018). These findings suggest that reduced *SIUPM1* transcript levels lead to lower efficiency in intracellular nitrogen metabolism; and a decreased intracellular nitrogen metabolic rate is associated with a reduced capacity to synthesize intermediate metabolites such as glutamic acid. As the primary precursor for chlorophyll synthesis, glutamic

acid shortage contributes to the decline in *SIHEMD* transcript levels as well as the reduction in total chlorophyll synthesis. Several studies have documented the impact of nitrogen deficiency on the expression levels of enzymes in the chlorophyll synthesis pathway. Choi et al., (2016); Wen et al., (2019) and Yoneda et al., (2016) reported a decrease in the expression of *MdHEMA1*, *MdHEMA2*, *MdHEMB1*, *MdHEMD*, *SechlL* and *SechlN*. These reductions were associated with decreased synthesis of intermediate metabolites in the chlorophyll synthesis pathway, such as 5-amino levulinic acid and uroporphyrinogen III. Wen et al., (2019) reported reduced levels of *MdNiR*.

Finally, overexpression of *AtUPM1* was associated with increased expression levels of *AtNII* and *AtNIA2* (291 and 280 %, respectively). These results correlated with higher expression levels of *AtUROD*, *AtCHLH*, *AtPORC*, as well as increases of 50, 97, 79 and 25 %, respectively.

Conclusions

The results show that the proteins of the chlorophyll synthesis pathway and their functionality are conserved in tomato plants. Furthermore, a severe impact is observed under nitrogen-deficiency stress, resulting in reduced expression of *SIHEMD* and *SIUPM1* and decreased chlorophyll synthesis capacity. These findings highlight the important role of UPM1 under these conditions in regulating chlorophyll synthesis.

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English version

Edaphological and Morphometric Analysis of wild populations of common bean (*Phaseolus vulgaris* L.) in Durango, México

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Abstract

Knowledge of wild populations of common bean (*Phaseolus vulgaris* L.) and their relationship with soil quality is essential for biodiversity conservation and crop improvement. The identification of soils with high contents of nutrients such as calcium, magnesium, potassium and other trace elements can be key to the development of bean varieties more resistant to adverse conditions. In this sense, the objective was to establish the integrated diagnosis of nutrients in soils where some wild forms of common bean grow in the State of Durango and correlate it with the morphometric characteristics of pods and seeds. Soil samples were collected in five municipalities of the State of Durango (El Mezquital, Súchil, Nombre de Dios, Canatlán and Nuevo Ideal), a sample was taken from a depth of 20 cm, evaluating the amounts of organic matter, Ca, Mg, K, P, S, Zn, Cu, Fe, Mn. There is great variability in the soil samples where wild forms of common bean grow, with the municipality of Canatlán having the highest content of beneficial elements for plants. In terms of the morphometric analysis of pods and seeds, those from Nombre de Dios and El Mezquital were the longest, widest and thickest. Variations in morphological characteristics in relation to soil quality could allow strategies for the recovery and improvement of genetic resources in a context of climate change and food security.

► **Keywords:** Soil nutrients, Morphometric variability, X-ray fluorescence, wild *Phaseolus* species.

Introduction

Wild species represent a genetic resource that can be used as a source of variability to improve the quality of current cultivars. Their adaptive characteristics, such as resistance to adverse conditions like extreme temperatures, droughts, and insect attacks, are crucial for plant development and the quality of harvested seed (Andelković *et al.*, 2020; Arroyo-Peña *et al.*, 2015); In addition, its potential to improve the nutritional quality of domesticated variants has been recognized. (Salgotra *et al.*, 2021). This potential benefit justifies the need for detailed studies to support the use of wild beans and their vast genetic diversity, thereby allowing for the defi-

inition and evaluation of the stability of their phenological, morphological, physiological, biochemical, and biophysical characteristics in relation to agronomic, culinary, and nutritional quality. (Tomlekova, 2012).

In the area of soil analysis, near-infrared spectroscopy (NIRS) has proven highly effective in simultaneously analyzing various physical, chemical, and biological properties of soil. This method is utilized in the wavelength range of 400 to 2500 nm (600–4000 cm^{-1}) and allows for the estimation of properties such as total carbon, nitrogen, cation exchange capacity (CEC), as well as the presence of calcium,

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magnesium, and other elements that are fundamental to understanding soil health and productivity (Shin *et al.*, 2025; Mukherjee & Laskar, 2019; Nocita *et al.*, 2015).

On the other hand, X-ray fluorescence (XRF) is an analytical technique for determining the elemental composition of various materials, including soils (Jenkins *et al.*, 2024). In recent years, the XRF has many pedological, environmental and agronomic applications, mainly after the appearance of portable equipment (pXRF) (Jenkins *et al.*, 2024). This technique has been recently adopted and used successfully for the characterization of soils throughout the world, especially in soils of developing countries. The characterization of the soil includes the complete determination of the elemental composition (nutrients, trace elements and rare earths) and allows estimation of some physical and chemical properties of the soil (Hart & Siebecker, 2024). The objective of this study was to analyze the morphological variations of the pods and seeds of wild forms of common bean in relation to soil quality.

Materials and methods

Study area, soil samples and processing

The soils where the wild common bean grows, as well as pods, were collected in six locations, these localities are representative of the southern, central and northern areas of the state in Durango, Mexico, from september to november of 2020, during the rainy season (June-august) months in which the species flourishes. Around one kilogram of soil and between 50-100 pods were collected in each location. Table 1 shows the geographic data of the collection sites. Seeds for each wild form were deposited in the CIIDIR herbarium, which were taxonomically identified.

The soils in Durango, Mexico, exhibit diverse characteristics influenced by various environmental factors and land use practices (Table 2). The predominant soil type in Durango, Mexico, specifically in the characterized watershed, is regosol. This soil type is associated with the dry scrubland land use in the area, which features average slopes of 7.8 %. (Hurtado *et al.*, 2013)

Soil analysis

Samples of 1000 g of soil were collected from each location at a depth of 20 cm. For the analysis, two samples from the same site were grouped together. The samples from each site were combined and stored in plastic bags until analysis. The soil parameters analyzed were: silica (Si), aluminum (Al), iron (Fe), potassium (K), calcium (Ca), strontium (Sr), magnesium (Mg), titanium (Ti), manganese (Mn), zinc (Zn), sodium (Na), phosphorus (P), sulfur (S), rubidium (Rb), barium (Ba), zirconium (Zr), arsenic (As), molybdenum (Mo), copper (Cu), and chlorine (Cl), as well as chromium (Cr).

Collected soils were air-dried, homogenized, and sieved for standard lab analysis (Figure 1). The size distribution of the soil particles will be reduced, with the pipette method (Remillard, 2022). The samples were prepared for the method, Identity test of the main bands. The analysis of the samples was obtained, according to the parameters indicated: Sample: solid, Gas used: He, Analysis time: 7 min, Type of analysis: complete analysis.

Morphometric data *in situ*

Data collection *in situ* was conducted on seven plants per population, recording three trifoliate leaves per plant, resulting in a total of 21 trifoliate leaves per population. Regarding reproductive structures (pods and seeds), a total of 100 pods and 100 seeds were recorded per population. The dimensions of the leaves, pods, and seeds were measured using a digital caliper (Surtek, 0-150 mm; model 122200). The weight of the seeds was determined using an analytical balance (Velab, model Ve204, with a maximum capacity of 220 g and a minimum of 0.1 g, with a precision of 0.0001 g).

Fourier Transform Spectroscopy

The samples were analyzed by Fourier transform infrared spectroscopy with attenuated total reflection (FTIR-ATR), using an FTIR spectrometer (Vertex 70, Bruker) in the Attenuated Total Reflectance (ATR) mode to obtain information on functional groups and chemical structures

Table 1. Origin and geographic coordinates of the sites of origin of the bean populations used in the soil analysis.

Municipality	Latitude N*	Longitude O*	Altitude (m)	Temperature Max/Min °C**	Precipitation (mm)**
El Mezquital	23° 26' 48.1''	104° 21' 49.5''	1400	42.1/-0.2	391.8
Súchil 1	23° 39' 24.7''	104° 02' 20.9''	1963	35.7/-6.4	511.0
Súchil 2	23° 39' 02.4''	104° 02' 26.7''	1964	35.7/-6.4	511.0
Nombre de Dios	24° 04' 71''	104° 14' 23''	1877	36.7/-3.5	446.6
Canatlán	24° 51' 03.4''	104° 51' 44.8''	2039	35.1/-7.3	525.6
Nuevo Ideal	24° 45' 11.9''	105° 00' 05.6''	2037	36.2/-5.0	692.5

* The data were taken with coordinates WGS84

** Data obtained from CONAGUA, average for the year 2018.

Table 2. Soil type according to the book *Vegetation and Ecoregions of Durango* (Elizondo *et al.*, 2007).

Municipality	Soil type	Characteristics	Region	References
El Mezquital	Leptosol	The soil has low content of organic matter and nutrients, which can be influenced by local factors such as parent material and vegetation.	Dry or tempered climates	Warren, 2022; Kimeklis <i>et al.</i> , 2021
Canatlán				
Súchil 1	Regosol	They have a low organic matter and nutrient content, making them less suitable for agriculture without improvements.	Hillslopes and regions with volcanic activity	Pedron <i>et al.</i> , 2024
Súchil 2				
Nombre de Dios	Kastañozem	These soils show a high mobility of chemical elements (CHEs) such as Ca, Mn and K, which are weakly bound by acid-soluble compounds.	Semiarid	Kimeklis <i>et al.</i> , 2021
Nuevo Ideal	Pheozem	These soils usually have a favorable texture that supports agricultural productivity, making them suitable for various crops.	Tempered	Hu <i>et al.</i> , 2021

Edaphological information taken from INEGI 2020.

**Figure 1.** Soil types where wild common bean populations grow (*Phaseolus vulgaris*).

present in the samples. Signal processing, peak fitting, surface fitting, statistics and signal processing were analyzed using Origin software. The spectra were recorded between 4,000 and 600 cm^{-1} , the instrument has a spectral resolution of 4 cm^{-1} . The samples were stored in paper bags and transported to the laboratory for further analysis. Table 1 shows the geographic information of the localities to which the wild common bean samples belong. The samples did not require any preparation procedure for analysis in the infrared spectrum, they were only ground in a mortar and placed on the surface of the ATR glass.

Spectral treatment

Once the FTIR spectra (raw spectra) were obtained, a standardized normal random variable (SNV) normalization was applied using the Unscrambler X version program 10.3 (CAMO Software AS). Subsequently, the

calculation of the second derivative of each spectrum was performed using the algorithm Savitzky-Golay, which applies a successive fit of adjacent subsets of data points with a low polynomial degree by least squares to the linear approximation.

Data analysis

Morphometric data were subjected to analysis of variance ($P \leq 0.05$) and the means were compared with Fisher's test, using XLSTAT 2021.2.1 (Addinsoft, 2025). To evaluate the contribution of each attribute (in each type of data) to the differentiation between wild common bean populations, matrices were constructed and subjected to principal component analysis (PCA) using Past 3.0, while to establish the similarity between the wild common, the same matrices were analyzed, they were submitted to the clustering analysis, using Past 3.0 (Hammer *et al.*, 2001)

Results and discussion

Physicochemical properties of the soil.

The results of the physicochemical analysis of the soil of each collection site are shown in Table 3. Significant variations were found between populations. The soils had low concentrations of P and Na, but high in K, Si and Al. Regarding to Mg, the soil belonging to the municipality of Canatlán had a higher relevant level than the rest of the evaluated soils.

The analyzes show that the soil where the wild form of Canatlán grows is the one with the most beneficial elements (Si, Al, Ca, Mg, K, P, S, Zn, Cu, Fe, Mn) for optimal plant growth. and the one with the fewest elements is the soil of the municipality of El Mezquital (Table 3).

The soils of Durango exhibit significant variability in organic matter content and nutrient levels, influenced by local factors such as vegetation and parental material. Research indicates that different soil types, including leptosols, luvisols, and vertisols, show distinct characteristics in organic carbon storage and nutrient availability (Kimeklis *et al.*, 2021; Kalinichenko *et al.*, 2019).

The soils with the highest amount of organic matter were those of SÚchil (Regosol) and Nombre de Dios (Kastañozem), and those with less organic matter El Mezquital and Canatlán (Leptosols). Arsenic (As) was found in the

evaluated soil of Nuevo Ideal, although in somewhat high concentrations (0.0162%) and low in the evaluated soil of SÚchil (0.0136%). Generally, the soils where the wild forms of common bean grow tend to be of low fertility (Beaver *et al.*, 2021; Romanyà & Casals, 2020).

According to Yang *et al.* (2024) guide for germplasm regeneration, the content of exchangeable bases (Ca, Mg and K) largely defines the degree of the soil fertility. Fertility soils are distinguished by their high Ca and Mg content. Of the soils evaluated, SÚchil had the highest percentage of Ca (6.58%), while the highest Mg content was found in Canatlán (1.59%). Very acidic soils generally have low Ca and Mg content, as was found in the soils evaluated: Nuevo Ideal had the lowest Ca content (1.92%), while El Mezquital had the lowest Mg content (0.359%).

In our results the soil evaluated in Nombre de Dios, presents the highest percentage in P (0.388%), compared to the rest of the soils evaluated. Phosphate is an element of great importance in plant nutrition and frequently presents limitations in soil fertility (Kaur *et al.*, 2017). Very acid soils such as red Acrisols, and soils of volcanic origin such as Andosols, have a high P fixation capacity that further decreases its availability for plants (Alakeh *et al.*, 2022).

Morphometry of reproductive structures

The comparison between the morphometric attributes of the pods in situ among the five wild common beans ana-

Table 3. Elements found in the soil expressed as a percentage (%)

Parameter	El Mezquital	SÚchil 1	SÚchil 2	Nombre de Dios	Canatlán	Nuevo Ideal
Si	54.00 ± 1.55 b	59.50 ± 0.56 a	62.5 ± 2.89 a	44 ± 0.21 c	62.00 ± 0.21 a	52.00 ± 0.77 b
Al	15.00 ± 1.27 b	12.05 ± 0.49 c	10.6 ± 0.63 c	17.95 ± 0.91 a	11.95 ± 0.92 c	16.45 ± 0.63 ab
Fe	13.80 ± 0.98 b	7.75 ± 0.74 c	7.63 ± 0.65 c	24.78 ± 0.55 a	8.50 ± 0.56 c	13.20 ± 0.14 b
K	8.63 ± 0.79 c	9.86 ± 0.61 b	10.72 ± 0.11 a	2.38 ± 0.41 d	9.57 ± 0.41 b	6.44 ± 0.29 c
Ca	3.17 ± 1.06 c	6.38 ± 0.28 a	4.32 ± 0.21 bc	5.81 ± 0.01 ab	3.65 ± 0.01 c	7.62 ± 0.41 a
Sr	0.05 ± 0.043 bc	0.04 ± 0.005 b	0.004 ± 0.004 c	0.05 ± 0.01 bc	0.07 ± 0.01 b	0.27 ± 0.03 a
Mg	1.26 ± 0.13 b	0.57 ± 0.09 cd	0.45 ± 0.03 d	0.85 ± 0.14 c	1.69 ± 0.14 a	1.62 ± 0.23 a
Ti	0.84 ± 0.14 c	0.75 ± 0.01 c	0.643 ± 0.15 a	2.18 ± 0.06 a	0.89 ± 0.06 c	1.74 ± 0.32 b
Mn	0.25 ± 0.02 a	0.25 ± 0.01 a	0.25 ± 0.11 a	0.28 ± 0.06 a	0.23 ± 0.06 a	0.23 ± 0.007 a
Zn	0.08 ± 0.003 a	0.05 ± 0.007 b	0.05 ± 0.005 b	0.07 ± 0.004 a	0.05 ± 0.004 b	0.05 ± 0.01 b
Na	1.42 ± 0.21 a	1.29 ± 0.05 ab	1.45 ± 0.13 a	0.41 ± 0.18 d	0.64 ± 0.18 cd	0.97 ± 0.14 bc
P	0.148 ± 0.03 cd	0.26 ± 0.01 a	0.002 ± 0.03 e	0.12 ± 0.01 d	0.19 ± 0.01 bc	0.20 ± 0.006 bc
S	0.15 ± 0.005 bc	0.15 ± 0.03 abc	0.13 ± 0.01 c	0.23 ± 0.06 ab	0.23 ± 0.06 a	0.11 ± 0.02 c
Rb	0.07 ± 0.00 a	0.07 ± 0.002 a	0.08 ± 0.007 a	0.03 ± 0.00 c	0.07 ± 0.00 a	0.05 ± 0.006 c
Ba	0.47 ± 0.05 c	0.32 ± 0.005 cd	0.54 ± 0.02 a	0.14 ± 0.05 b	0.25 ± 0.05 cd	1.11E-16 ± 0.00 c
Zr	0.55 ± 0.03 a	0.16 ± 0.004 b	0.16 ± 0.005 b	0.09 ± 0.006 c	0.13 ± 0.006 b	0.2 ± 0.006 b

*Units in percentages (%). Physicochemical parameters: silicon (Si), aluminum (Al), Iron (Fe), Potassium (K), Calcium (Ca), Strontium (Sr), Magnesium (Mg), Titanium (Ti), Manganese (Mn), Zinc (Zn), Sodium (Na), Phosphorus (P), Sulfur (S), Rubidium (Rb), Barium (Ba), Zirconium (Zr), Arsenic (As), Molybdenum (Mo), Copper (Cu), Chlorine (Cl), Chromium (Cr). Tukey's analysis of means ($P \leq 0.05$). Only in Mn it was not significant with a $P = 0.776$, for the other parameters the difference was significant.

lyzed revealed significant differences in the pod (length, width and thickness) and seed (length, width and thickness) characteristics (Figure 2).

The differences in the types of soil of the places of origin of the wild forms of common bean can be seen in Table 1. In the municipality of Nombre de Dios, the type of soil is Kastañozem (chestnut earth), alkaline soil found in semi-arid areas, while the one in the municipality of Canatlán is Leptosol, a very stony soil with a depth of less than 10 cm, which depends sufficient water. The soil of Canatlán has high concentrations of beneficial elements for plants, resulting in seeds with exceptional characteristics.

Different characteristics were observed in the wild forms analyzed. The size of the leaflets coincides with what was reported by Meza-Vázquez *et al.* (2015), Wallander *et al.*, (2022), Morales-Santos *et al.*, (2017) the length ranges from 3.1 cm to 8.5 cm, and the width from 0.5 cm to 6 cm.

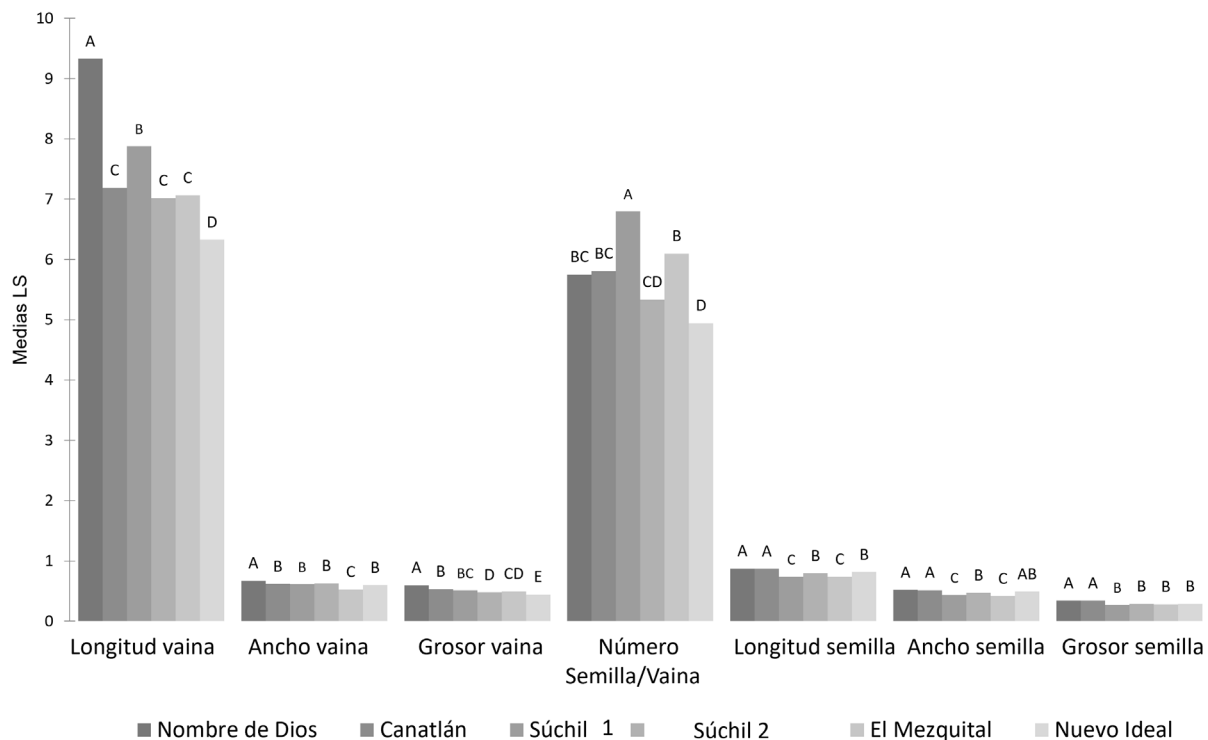
The length of the pods, presented wide variation (Figure 2) which was larger than what was Lépiz & Ramírez (2010) reported, ranging from 1.2 cm to 6.3 cm. There were also differences in the length, width, and thickness of the seeds, which agrees with the results obtained by Freytag & Debouck (2002) and Lépiz *et al.*, (2010). They defined that the morphometry of wild common bean forms is small, ranging from 0.30 to 0.79 cm in length, ranging from 0.30 to 0.54 cm in width.

The morphometry of the trefoils is shown in Figure 3. Previous studies have shown that the central leaflet is larger than the left and right leaflets, which is consistent with our results. The El Mezquital and Súcil populations had the largest clovers, and the Canatlán population, had the smallest clovers. This information is useful, since beans are generally used as livestock fodder.

Analysis of principal components and grouping.

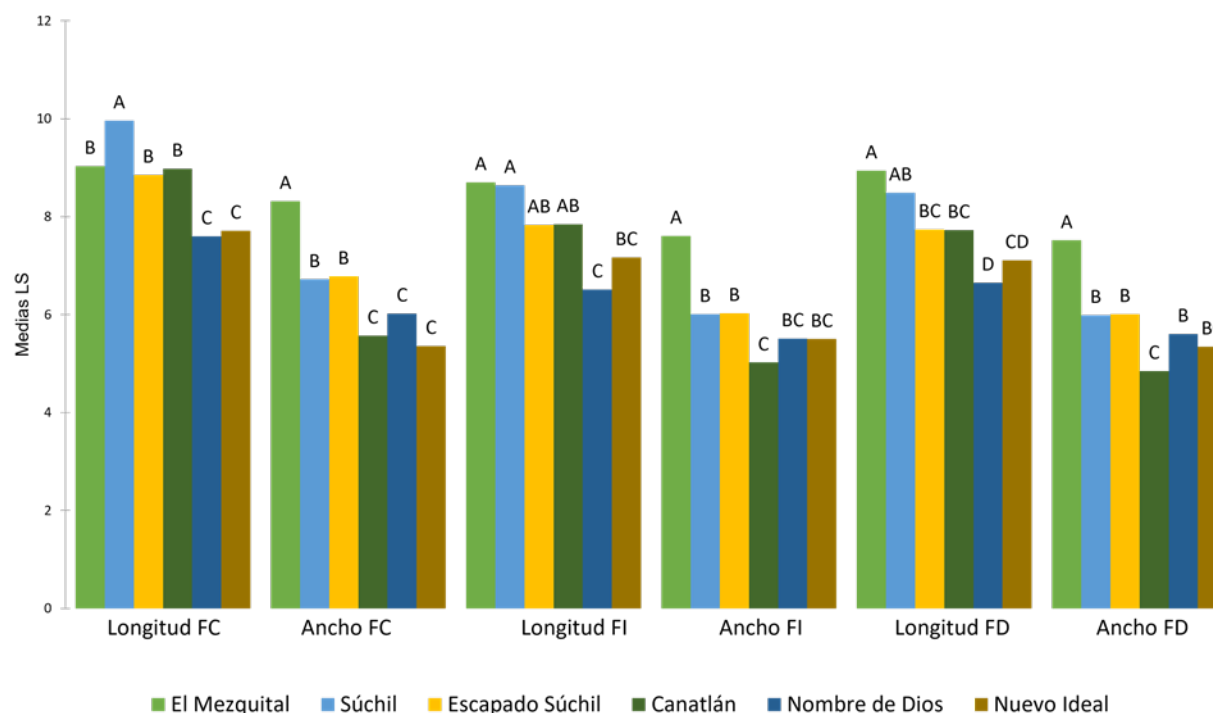
Global chemical variation among the analyzed populations was evaluated using a PCA (Figure 4A). The PCA was then plotted in two dimensions, based on the morphometric characteristics of the pod and seed. The principal components PC1 and PC2 explained 86.80 % of the total variance in the data, (61.32% and 25.48%, respectively).

PC1 grouped the wild forms into two subgroups and PC2 into another group with two subgroups. It can be observed that, within the group, PC1 clearly separated Súcil 1, and Súcil 2 and El Mezquital, in group 2 from PC2 Nombre de Dios, Canatlán and Nuevo Ideal. PC1 was correlated with the morphometric characteristics of the trefoils and the number of seeds, and had a higher discriminative power (4.82). PC2 was correlated with the morphometric characteristics of the pod (length, width and thickness) and seed (length, width and thickness), and had a lower discriminatory power (3.43), while the results of a clustering analysis, based on the same data are shown



Different letters indicate significant differences between populations, Tukey's analysis of means ($P \leq 0.05$).

Figure 2. Morphometric data of pods and seeds of six wild forms of common bean growing *in situ* in Durango.



Different letters indicate significant differences between populations, Tukey's analysis of means ($P \leq 0.05$).

Figure 3. Morphometric data of trefoils, Central Leaf (FC), Left Leaf (FI) and Right Leaf (FD) of six wild forms of common bean growing *in situ* from Durango.

in Figure 4B. These results confirmed the discrimination between the populations of wild forms, showing at the same time a greater similarity between the populations of Súčil 1 and Súčil 2, and Canatlán and Nuevo Ideal, different from the wild forms of El Mezquital and Nombre de Dios.

Correlation between physicochemical and environmental variables.

A Multiple Correspondence Analysis (MCA) test was performed to determine the relationship among these variables.

The CCA showed that the CCA1 and CCA2 axes explained 94.70% of the total variance (Figure 5). CCA1 axis was mainly associated with soil physicochemical parameters. The significantly correlated variables were ($P \leq 0.05$) were Mn ($r = 0.73$), Al ($r = 0.96$), Si ($r = 0.44$) and Fe ($r = 0.11$).

In the grouping analysis, three main groups were observed, according to the correlation of the morphometric variables, separating from the rest of the populations that of Nombre de Dios, having very variable values in the elements found in the soil, high in Si (63.2%), K (11.7%) Na (1.27%), and low in Al (12.1%), Fe (7.11), Sr (0.0256%), Mg (0.359%), and Mn (0.172%), among others, the related morphometric variables are larger seeds and

Pods, compared to the rest of the wild forms analyzed. The subgroups formed group those from the same locality, Súčil 1 and 2.

Fourier Transform Spectroscopy

The FTIR spectra for the evaluated soil showed different peaks. Peaks were found in all analyzed soils at 1004 cm^{-1} , 785 cm^{-1} , 694 cm^{-1} , 521 cm^{-1} , 459 cm^{-1} , and 426 cm^{-1} (Figure 6). In the Nombre de Dios and Nuevo Ideal populations, nine peaks were identified, one more than in the rest of the analyzed soils. The spectra were grouped into three categories based on the number and distribution of peaks: Group 1, consisting of Súčil 1, Súčil 2, and Canatlán; Group 2, include Nombre de Dios and Nuevo Ideal; and Group 3, is represented by El Mezquital, whose spectrum exhibits distinct peaks.

Functional groups, found in the samples after extraction are shown in Table 4. The different peaks observed in the soil samples correspond to different molecular vibrations, each of which is characteristic of specific bonds in the molecule. The results indicated that many functional groups were associated.

The band observed at $1632\text{--}1621 \text{ cm}^{-1}$ can serve as an indicator of water-resistant soil, which relates directly to its wettability (Margenot et al., 2017). The aromatic

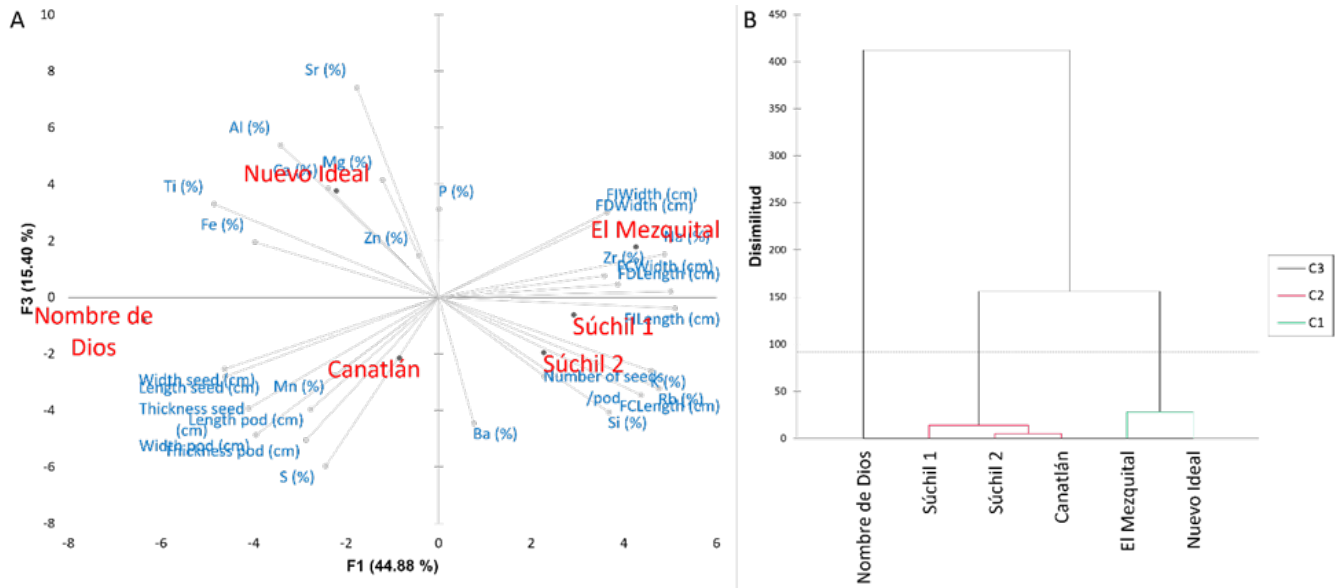


Figure 4. A) Results of a principal component analysis and B) Dendrogram resulting from the cluster analysis of soil data, as well as trefoils, pod and seed morphometry, for six wild populations of common bean (*Phaseolus vulgaris*) from Durango, Mexico.

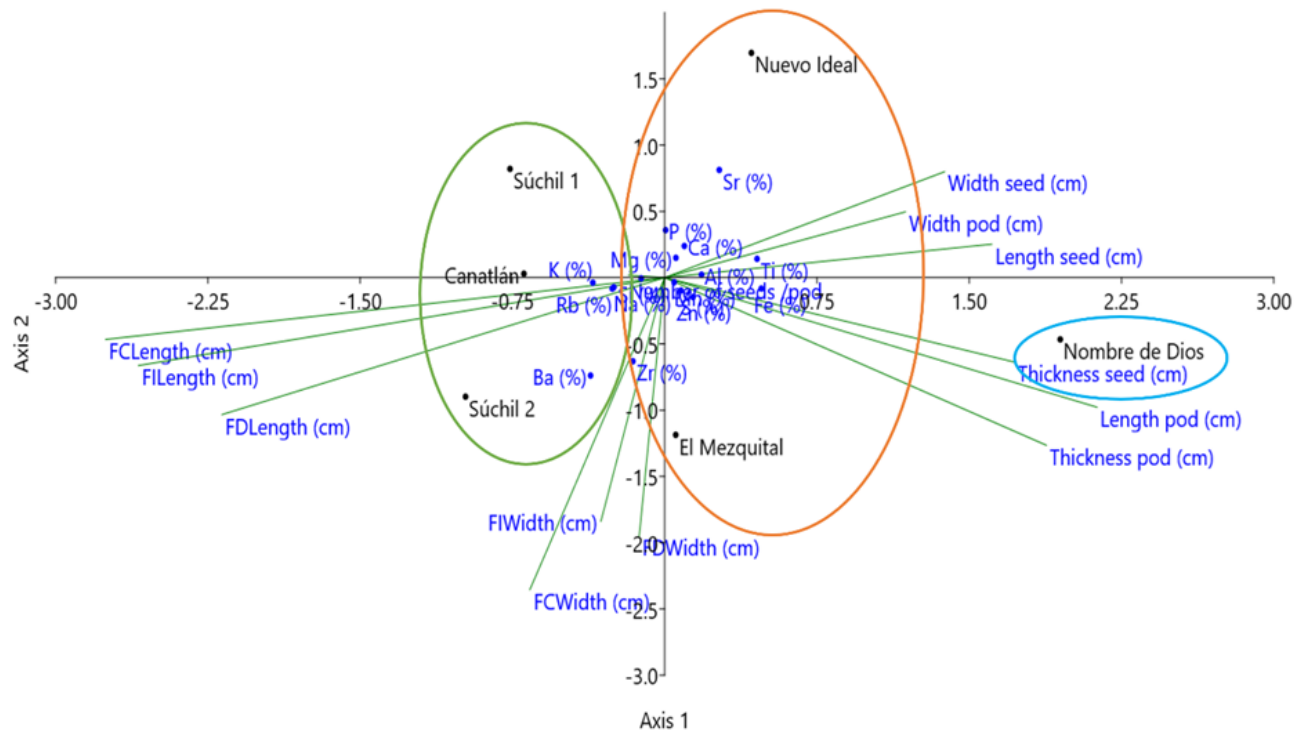


Figure 5. Results of a Canonical Correspondence Analysis (CCA) of morphometric data and soil physicochemical from six wild populations of common bean (*Phaseolus vulgaris*) from Durango, Mexico.

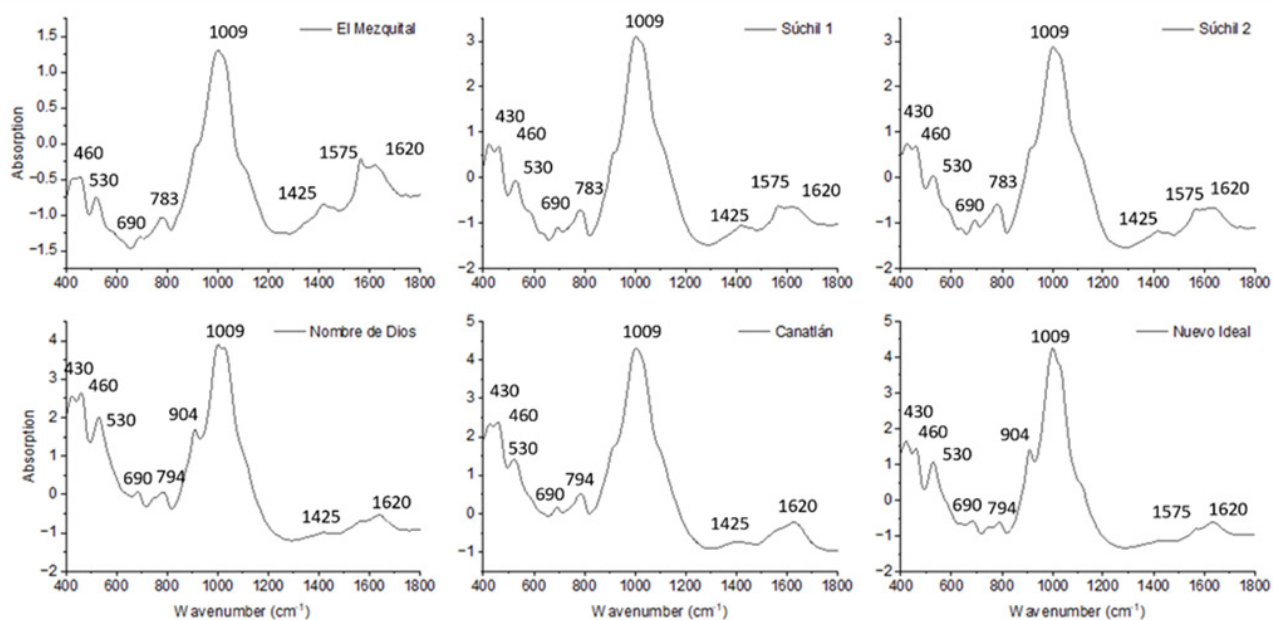


Figure 6. FTIR spectra of wild bean seeds represented in the biological fingerprint region ($1800\text{-}400\text{ cm}^{-1}$), from different locations in Durango.

Table 4. Vibrational bands corresponding to the analysis of the wavelength from 1800 to 400 in analyzed soil.

	Wavenumber (cm^{-1})	Vibration band	Molecule	Vibration mode	Reference
1	1620	(C=C)	Amide I (Protein)	stretching	Kumari & Ramakrishnan, 2023; Dovbeshko <i>et al.</i> , 2023
2	1575	N-H	Amida II (Proteína) Protein amide II 3 absorption- predominatelyb- sheet of amide II	stretching	Chatterley <i>et al.</i> , 2022
3	1425	C-H ($-\text{CH}_3$)	aliphatic compounds, particularly in methyl	bending vibrations	Bakshi <i>et al.</i> , 2014
4	1029	indicative of C-O and C-OH	carboxylic acids	stretching	Duan <i>et al.</i> , 2024
5	1009	C-OH	bonds in oligosaccharides such as mannose & galactose	stretching	Schindler <i>et al.</i> , 2017
6	904	C-H d (monosustituído)	Alqueno	bending	Dovbeshko <i>et al.</i> , 2023
7	794	C-H d (meta)	Aromatic C-H out-of-plane bend;increasing wavenumber with increasingdegree of substitution	bending	Sharma & Kumar, 2023
8	690	Vs Fe-O	Fe-O-(H)	bending	Margenot <i>et al.</i> , 2017
9	530	C=C, C-Br t	Torsion and ring torsion of phenyl (1), Haluros de alquilo	strech	Siddique, 2024
10	460	BrCN	It primarily focuses on the rovibrational analysis of BrCN isotopomers and their spectral data	strech	Siddique, 2024; Fayt <i>et al.</i> , 2002
11	430	C-OH ₃ δs (O-P-O)	Tosion methoxy group, PO4 3- in phosphates	bending	Siddique, 2024

C=C stretching ratio at 1632–1621 cm^{-1} has been used as an index of humification (Serafimova & Dedelyanova, 2023). Phyllosilicates are the most abundant class of soil minerals. They consist of aluminum coordinated with hydroxyl-bound oxygen, coupled with silicon films in tetrahedral coordination with oxygen. (Margenot *et al.*, 2017). This is because 2:1-layer silicates exhibit a single broad absorption peak at 1029–1009 cm^{-1} .

Conclusion

The population that exhibited the largest trifoliolate leaves, seeds, and pods was El Mezquital, whereas the population with smaller pods and seeds corresponded to Nuevo Ideal. In the latter site, soil analysis revealed one of the highest deficiencies in essential nutrients for plant growth.

The morphometric characteristics of pods and seeds of wild *Phaseolus vulgaris* were compared in relation to the type of soil in which they develop. Populations collected from soils with higher nutrient content produced larger pods and seeds, in contrast to those from soils with greater deficiencies in beneficial elements, which showed lower morphometric values.

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