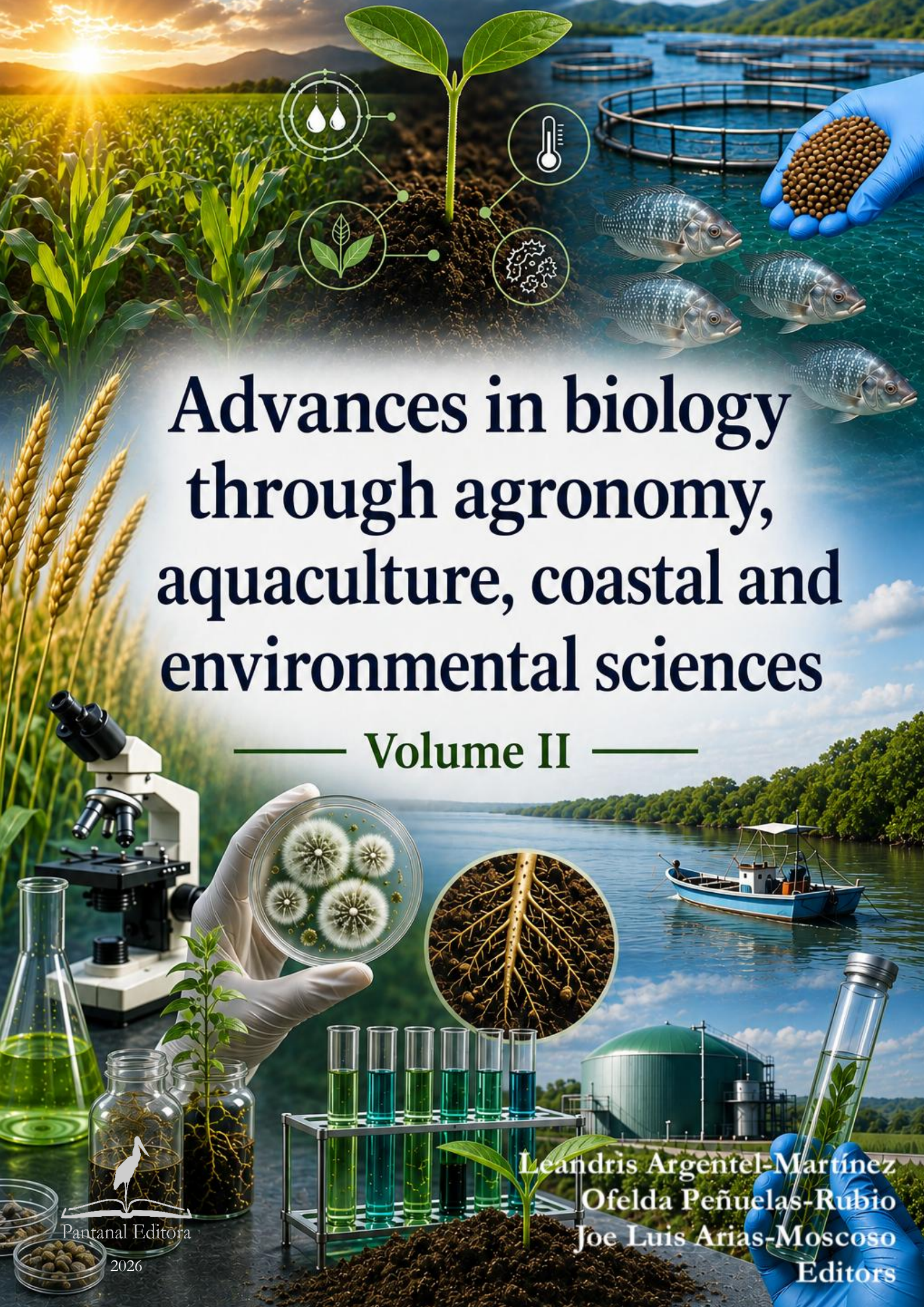


Advances in biology through agronomy, aquaculture, coastal and environmental sciences

Volume II

Leandris Argentel-Martínez
Ofelda Peñuelas-Rubio
Joe Luis Arias-Moscoso
Editors

Pantanal Editora
2026

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Joe Luis Arias-Moscoso
Editors

Advances in biology through agronomy, aquaculture, coastal and environmental sciences

Volume II

Pantanal Editora

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Prologue

Advances in Biology through Agronomy, Aquaculture, Coastal and Environmental Sciences, Volume II, is an e-book that continues the work of the first version (https://editorapantanal.com.br/ebooks/2023/advances-in-biology-through-agronomy-aquaculture-coastal-and-environmental-sciences/ebook.pdf), published in 2023 by Leandris Argentel-Martínez and Ofelda Peñuelas-Rubio.

Our e-book compiles 25 manuscripts that present scientific results and reviews based on biological aspects. All of they contribute to explaining the responses of living organisms to agricultural, aquaculture, and coastal ecosystems, as well as those facing fragility or threat of climate change. This e-book was edited by Leandris Argentel-Martínez, Ofelda Peñuelas-Rubio, and Joe Luis Arias-Moscoso, members of the Consolidated Academic Group ITVAYA-CA-3.

We appreciate the contribution of al authors for their careful attention to the editorial committee's recommendations. The review process was double-blind and conducted by internationally renowned researchers.

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The Editors

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Agronomy

Chapter 1

The reduction in chemical fertilization combined with PGPM under a controlled deficit irrigation system for maize

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ABSTRACT

This study was conducted under open field experimental conditions on a Vertisol soil located in Block 611 of the Yaqui Valley, Sonora, Mexico. The objective of the trial was to evaluate the effect of a 50% reduction in nitrogen fertilizer on the phenology and biomass yield of the Hipopótamo maize hybrid. The timing of phenophases, chlorophyll content, normalized difference vegetation index (NDVI), and biomass yield were evaluated in two treatments: T1: application of 100% of the nitrogen dose, and T2: a 50% reduction in nitrogen fertilizer with the aid of the bacteria *Enterobacter cloacae*, applied 24 hours before planting as a pre-germination treatment. The 50% reduction in nitrogen fertilizer did not cause a significant variation in phenology compared to T1. Although chlorophyll content and NDVI increased significantly in T2 during growth stages V1 and V2, no differences were observed between T1 and T2 from V3 to V12. However, during reproductive phenophases, T1 maintained higher chlorophyll and NDVI values, as well as a longer duration of phenophase occurrence. Biomass yield was not affected by the reduction in nitrogen fertilizer, reaching an average of 218 t ha^{-1} in both treatments. The experiment demonstrated that with the aid of *Enterobacter cloacae*, it is not necessary to apply 400 kg ha^{-1} of nitrogen for Hipopótamo corn cultivation in the Yaqui Valley.

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Keywords: *E. cloacae,* nitrogen fertilizer, NDVI, chlorophylls, Hipopótamo.

INTRODUCTION

In recent years, high doses of nitrogen fertilizers have been applied in maize production systems in northwestern Mexico, primarily in Sinaloa and Sonora, to ensure high maize yields (Santillano-Cázares, 2021). This practice aims to provide a greater amount of nitrogen to the plants from the initial stages of development, due to the low organic matter content (Parra-Cota et al., 2018). In most of these production systems, high yields are obtained due to these practices, but production costs are also high (Delgado & Rodríguez, (2019). At the same time, the application of high doses of fertilizer can cause soil salinization and contaminate aquifers.

In these regions, rainfall is coincidentally very low, and the availability of water for irrigation is reduced (González et al., 29026), leading producers to try to provide the crop with fertilizers to ensure faster field closure and prevent water loss through evaporation (Plata-Rocha et al., 2026). The use of pressurized and controlled deficit irrigation systems is an important alternative a better water use efficiency (Zou et al., 2021), even when combined with fertilization to reduce application rates. Meanwhile, the use of microorganisms that promote plant growth and contribute to the availability of essential minerals has proven to be a scientifically validated ecological and agronomic alternative for ensuring production and improving soil health (Xing et al., 2025, Krasilnikov et al., 2022.). Some genera, such as *Enterobacter* make a significant contribution to the physiological and agronomic performance of crops and contribute to tolerance to abiotic conditions such as drought (Azizoglu, 2025).

Based on the above, an open-field study was conducted to evaluate the effect of a 50% reduction in nitrogen fertilizer with the aid of the bacterium *Enterobacter cloacae* on the phenology, chlorophyll content, NDVI, and biomass yield of the commercial corn hybrid Hipopótamo, compared to when 100% of the nitrogen fertilizer is applied without the use of microorganisms.

MATERIALS AND METHODS

Study site

The work was carried out at an experimental site in the Yaqui Valley, Sonora, Mexico. During the winter-spring period, the soil was a vertisol that had been cultivated for more than 30 years, primarily with wheat and maize. The experimental area covered 0.12 ha (35 x 35 m^2). The crop was planted from east to west, with a spacing of 0.11 m between plants and 0.8 m between rows (Figure 1).



Figure 1. Image of the experiment.

Climate variability

Edaphoclimatic variables were evaluated in situ at the experimental area. Ambient temperatures and relative humidity were measured by means of a digital hygrometer (EMA) placed at the central part of the plots, placing the sensor at 2.4 m of height. During the experimental period, the climate exhibited temperature variations (Figure 2). The lowest minimum temperature recorded was 10.3°C in January; all other months-maintained temperatures above 12°C, so there was no risk of frost damage to the crop. Temperatures remained below 36°C, except for the planting date, which reached 38.1°C.

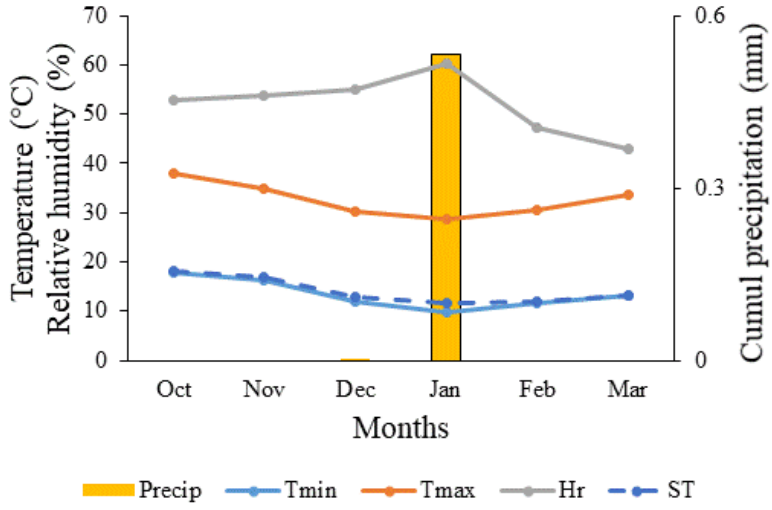


Figure 2. Climatic conditions during the crop cycle Oct 2025 - Mar 2026.

Relative humidity remained between 50% and 55% in most months, except for January, which saw only 0.55 mm of precipitation. The lowest relative humidity was observed in February and March due to reduced irrigation in the region. Soil temperature was measured by means of a FST100-2006A soil temperature sensor, placed at the central part of each plot. The minimum soil temperature consistently remained between 12°C and 18°C.

Plant material

The commercial hybrid Hipopótamo, provided by ASGROW, was used as an experimental model. This hybrid is one of the best adapted to the climatic conditions of northwestern Mexico and possesses relative tolerance to drought, salinity, and high temperatures (Argentel-Martínez et al., 2024). Its biological cycle is 154 days, with flowering occurring at 70–73 days when cultivated under four supplemental irrigations with an average water depth of 16 mm. Its average yield for northwestern Mexico is 215 t ha^{-1} of biomass, and its grain yield can exceed 16 t ha^{-1}.

Plant growth promotion microorganism used

For the experiment it was used *Enterobacter cloacae* which is a non-hemolytic endophytic rhizobacteria highly producer of siderophores, and in average production of indolacetic acid of 26.7 µL mL^{-1}. Also, this bacterium has the ability of phosphate solubilization index of 3.14 (Azizoglu et al., 2025).

Variables evaluated

Plant phenology (Phenophase occurrence time) was measured at each treatment when more than 51% of the plants presented the characteristics related to each of the phenophases described by the phenological scale used by Ibarra et al. (2020) for the northwest of Mexico.

For the chlorophyll content (SPAD units), a portable SPAD-type portable device was used. The measurements were performed on a total of 10 leaves in the central part, aiding the central nervature. These measurements were carried out during flowering phenophase in completely well-exposed leaves to sunlight between 12:00 h and 14:00 h (Li et al., 2024).

The normalized difference vegetation index (NDVI) (1 > NDVI > -1) was determined during each crop phenophase. The measurement was carried out by using a portable Greenseeker-type device (Trimble™) (Govaerts and Verhulst, 2010). All measurements were taken at 11:00 a.m., with the device positioned 0.6 m from the plant leaf area (Prakasha et al., 2020).

Biomass and grain yield evaluation

The biomass yield (BY: kg ha^{-1}) was assessed for a total of 10 plants per treatment when they were in the R3 phenophase (Figure 3). These plants were cut at the base of the stem and weighed separately to determine the average weight. This value was multiplied by the number of plants in each treatment and finally extrapolated to one hectare.



Figure 3. Image of the Hipopótamo maize Hybrid at R3 phenophase ready for harvest.

Statistical analysis

The theoretical assumptions of normality and homogeneity of variances for the evaluated variables were verified. Subsequently, a t-Student test for continuous quantitative variables was used, employing the t-test for significance levels of 1% (Gosset, 1917). These analyses were performed using the STATISTICA professional statistical package, version 15.0 for Windows.

RESULTS AND DISCULTS

Phenophase occurrence time

The phenology of the commercial hybrid Hypopótamo did not significantly differ between the vegetative phenophase and the first two reproductive phenophases. From grain filling onward, the differences were highly significant (Figure 4). Since the same irrigation regime was applied, it is assumed that the extension of the reproductive phenophases was due to the effect of the microorganism used, which has high indolacetic acid synthesis capacity, promoting greener plants and reducing the likelihood of senescence.

Phenological studies are extremely important for crop plants. In maize, in particular, a shortening of the typical phenology alters the mobilization rates of elaborate substances and decreases the efficiency of carbon nutrition, even in the reproductive stage (Irmak et al., 2024).

When plants spend more time in a reproductive phenophase, although their biological cycle is delayed, it offers more possibilities for active metabolism and the translocation of elaborate substances, resulting in higher-quality fruits (Singh et al. 2025).

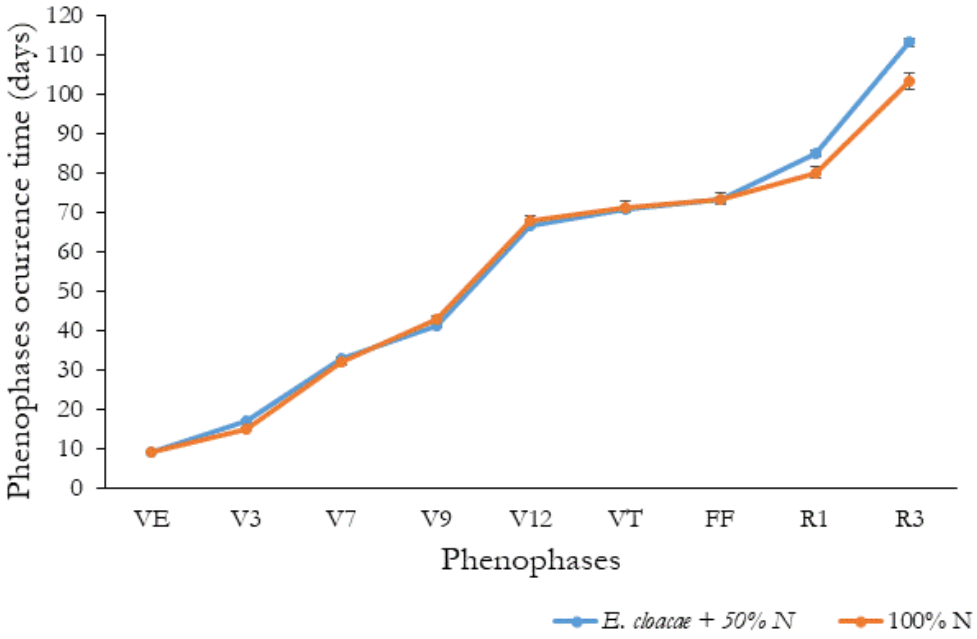


Figure 4. Phenophase occurrence time (days) of maize at the different phenophases: VE: emergence, V3; V7, V9; V12: development of the third, seventh, ninth and twelfth leaves; VT: male flowering; FF: female flowering; R1: grain formation; R3: full and milky grain.

In some regions of the world, the low biomass and grain yields of maize are due to inadequate management of nitrogen fertilizer application rates and untimely application. A high fertilizer rate applied inappropriately during phenological development alters assimilation patterns and nitrogen fertilizer use efficiency (Yadete et al., 2024).

In 2024, in a highly productive maize region of Ethiopia, the effects of low nitrogen fertilizer rates (0, 46, 92, 138, and 184 kg N ha^{-1}) and three application times (full application at planting, half at planting + half at the 5-leaf stage, and one-third at planting + one-third at the 5-leaf stage + one-third at the heading stage) were tested. The authors reported that the application rate significantly affected the phenological and agronomic variables. The study demonstrated that the highest average grain yield (9.2 t ha^{-1}) was obtained at planting, during the V5 phenophase, and at flowering when a dose of 184 kg ha^{-1} of total nitrogen was used (Yadete et al., 2024). This fertilization scheme was implemented in the treatment where only 50% of the nitrogen fertilizer was applied.

The normalized difference vegetation index (NDVI)

The NDVI in the initial phenophases of development was highly significantly different, with the indicator being highest in the treatment in which 50% of the nitrogen fertilizer was applied. This response is possibly due to the increased concentration of IAA in the plants, which promotes plant vigor (Figure 5). From the VT and FF phenophases onward, no significant differences were observed; therefore, the 50% difference in nitrogen fertilizer did not alter the NDVI in crucial phenophases such as flowering.

With respect to reproductive phenophases, the NDVI decreased significantly but more markedly in the treatment in which 100% nitrogen was applied. Perhaps the high concentration of fertilizer in the soil caused the NDVI to decrease more rapidly because of the reduction in the osmotic potential generated in the soil, consequently reducing the soil water potential and the plants' ability to absorb water.

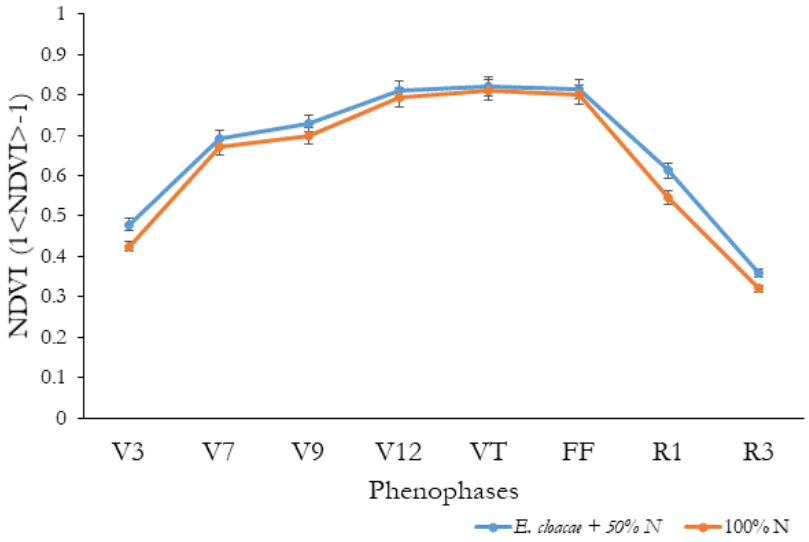


Figure 5. Normalized difference vegetation index (NDVI) in maize phenophases: VE: emergence, V3; V7, V9; V12: development of the third, seventh, ninth and twelfth leaves; VT: male flowering, FF: female flowering; R1: grain formation; R3: full and milky grain; R5: fully full and dry grain.

In both treatments, the NDVI in the VT, FF, and R1 phenophases remained above 0.8, with the highest values throughout the entire crop cycle. This value indicates very good nitrogen nutrition, which manifests as a very good physiological state (Zainuddin et al., 2025).

In a recent study conducted on maize under diverse climatic and soil conditions, testing nitrogen fertilizer doses revealed that compared with the control treatment (dose above 300 kg ha-1), 120–180 kg ha^{-1} of nitrogen yielded the best nutritional levels for maize, significantly increasing the grain yield by +5 t ha^{-1}. The statistical analyses performed demonstrated a strong correlation between the NDVI values and yield, indicating the practical utility of the NDVI measurements for predicting maize yield. These authors reaffirmed that NDVI-based monitoring is a highly effective remote sensing alternative not only for optimizing N management but also for supporting specific fertilization strategies for improving maize productivity and nutrient use efficiency (Illés et al., 2025).

Chlorophyll content

The chlorophyll content did not vary significantly during any of the vegetative growth stages or in the reproductive growth stage (VT) (Figure 6). From this stage to R3, the differences were significant. An important finding was that the chlorophyll values in both treatments exceeded 50 SPAD units during the most critical growth stages (V7 to R1). During these stages, the nitrogen demand of plants is high,

primarily because important processes such as pollen formation are highly dependent on nitrogen for plant viability. Even during the R3 growth stage, the chlorophyll content remained considerably high, indicating that the photosynthetic ability of the corn to continue grain filling increased; however, in this study, the corn was harvested for fresh consumption at the R3 growth stage.

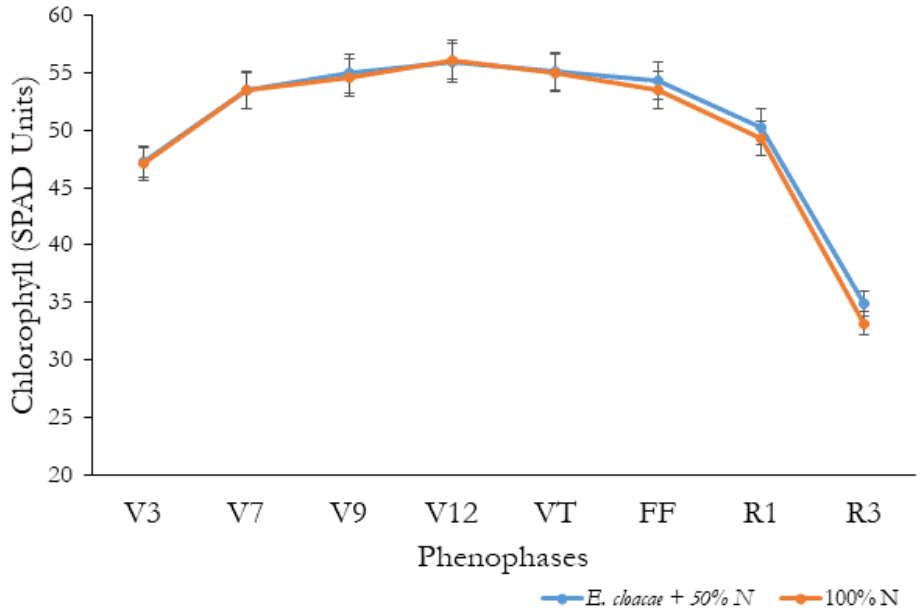


Figure 6. Chlorophyll content at the phenophases: VE: emergence, V3; V7, V9; V12: development of the third seventh, ninth and twelfth leaves; VT: male flowering, FF: female flowering; R1: grain formation; R3: full and milky grain.

The chlorophyll content is the most important variable related to nitrogen metabolism in maize (Shivashankar et al., 2025). This plant species is particularly indicative of nitrogen deficiency, as its chlorophyll concentration decreases significantly when nitrogen availability declines. This study demonstrated that 50% nitrogen fertilizer (180 kg ha^{-1}) is sufficient to achieve normal physiological and agronomic development, representing fertilizer savings and a reduction in soil pollutant load from excess nitrogen application (Ali & Bijay-Singh, 2025).

Biomass yield

When the biomass yield at the R3 phenological stage (full, tender kernels for consumption as corn on the cob) was analyzed, no significant differences were found between treatments. However, the numerical trend tended to increase in the treatment with *E. cloacae* and with only 50% nitrogen fertilizer (Figure 7). These results demonstrate that applying the regionally recommended dose does not increase the yield of the commercial hybrid used. Greater variability was observed in the data compared with the mean of the treatment with 100% nitrogen fertilizer, even though the sample size was similar. The lack of significant differences confirms the feasibility of using the growth-promoting microorganisms

employed in this research and the corresponding reduction in the nitrogen fertilizer application rate, which could be applied to other crops to avoid excess nitrogen fertilizer in the soil.

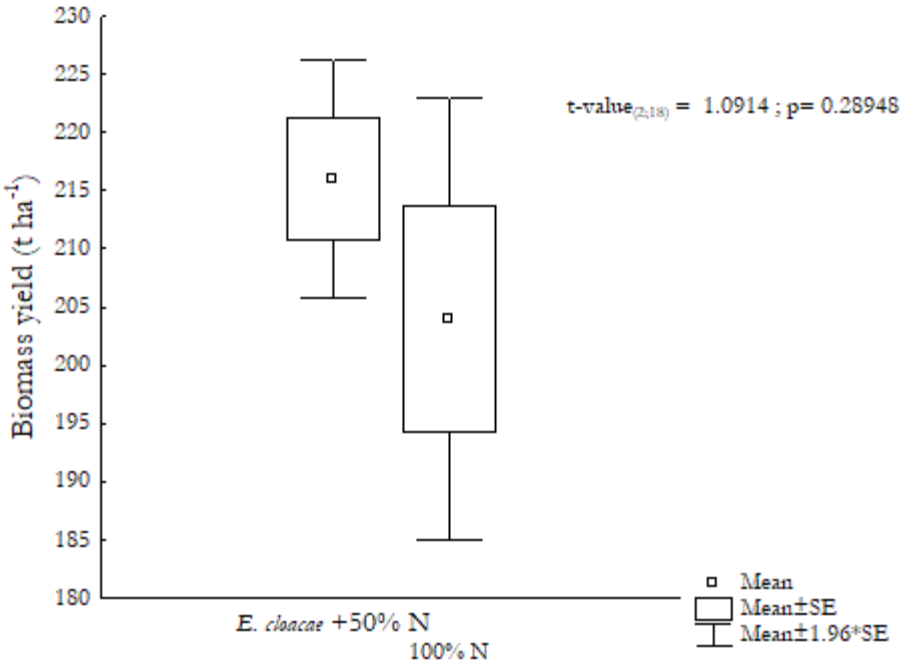


Figure 7. Biomass yield (t ha^{-1}) at R3 phenophase in maize under *E. cloacae*+50% N fertilizer and 100% N fertilizer. [t value: calculated t-student value. P: probability].

It is well known that nitrogen deficiency in the soil is among the factors that most affects the production of various crops, especially maize (Gehl et al., 2005). Reports support the idea that maize can yield close to the genetic potential of the planted material with a total nitrogen dose of 200 kg ha^{-1}, primarily with the aid of organic components or microorganisms (Yang et al., 2024). Even in soils supplemented with growth-promoting and remineralizing microorganisms, high biomass and grain yields can be obtained. Other reports have demonstrated that the increase in maize grain yield is not correlated with the increase in nitrogen dose (Patil et al., 2026).

The amount of nitrogen applied in some maize-producing regions can reach 400 kg N ha^{-1}, which is equivalent to one ton of urea. This is a very expensive fertilizer and has very adverse effects because of the environmental pollution generated by its excessive use, which can cause environmental degradation and resource depletion. In this context, low doses of nitrogen induce the overexpression of important genes in maize that increase yield (Nazir et al., 2016).

The excessive use of nitrogen fertilizers by farmers to increase productivity can also contribute to global warming and other types of environmental pollution from greenhouse gas nitrogen (Srikanth et al., 2016). Recent studies, some reported as early as 2016 (Wen et al., 2016), have shown that maize cultivars differ in their nitrogen absorption and metabolism capacity. Currently, the development of

maize cultivars that are tolerant to low levels of nitrogen fertilizers is the subject of research and is undoubtedly among the best ways to reduce pollution (Zhang et al., 2018).

CONCLUSIONS

Compared with the high doses recommended in the Yaqui Valley (up to 400 kg ha^{-1} of nitrogen), the 50% reduction in nitrogen fertilizer with the aid of *E. cloacae* did not cause a significant reduction in the phenology of the Hypopótamo maize hybrid. The high values obtained for the physiological variables chlorophyll content and NDVI demonstrated that when 50% of the nitrogen fertilizer was applied in the Yaqui Valley for a maize crop, a good nutritional status of the plants and a high biomass yield at R3 (for sweet corn) could be achieved when plant growth-promoting microorganisms with a high capacity for indolacetic acid synthesis, such as *E. cloacae, were used*. This work contributes to sustainable production with the consequent incorporation of microorganisms into the soil and a reduction in the pollutant load from excess nitrogen.

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Chapter 2

Effects of sowing date and size on the physical and physiological quality of bread wheat seeds in southern Sonora, Mexico

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ABSTRACT

This study aimed to determine the influence of sowing date and seed size on the physical and physiological quality of bread wheat seeds. Eighteen lines provided by the International Maize and Wheat Improvement Center (CIMMYT) and two commercial checks were used. A randomized complete block design with three replications and a factorial arrangement was applied, where factor (A) was the genotype, factor (B) was the sowing date, and factor (C) was the small and large seed size. The grain yield, hectoliter weight, thousand-seed weight, emergence rate, emergence percentage, seedling length, and root length were determined. The results revealed statistically significant differences in all the study factors and their interactions for the recorded traits. Lines L-1, L-18, L-6, and L-7 presented superior agronomic characteristics and seed physical and physiological quality, achieving optimal expression of these traits and surpassing those of the commercial controls. The best sowing date was November 19th, and the use of large seeds favored agronomic expression, yield, seed physical and physiological quality, and initial seedling vigor in most of the evaluated genotypes.

Keywords: *Triticum aestivum* L., seed quality, sowing dates, seed size.

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INTRODUCTION

In Mexico, 567,000 hectares of wheat are planted in 2023. Sonora was the state with the largest planted area, at approximately 230,000 hectares, representing 41% of the national total. Its production reached 1.53 million tons, contributing 52.76% of the national total. In Sonora, durum wheat predominates, covering approximately 135,000 hectares, in contrast to bread wheat, with only 96,000 hectares (SIAP, 2023).

Genotype establishment in the field is crucial for successful plant development and the achievement of desirable yields, along with the use of high-quality seeds. This process is determined by germination. However, this test must be performed under favorable environmental conditions. Therefore, while it can be efficient for counting seeds with good germination that will produce viable plants in the field, it is important to consider the type of substrate, humidity, and temperature, as these factors can have adverse effects on seed quality (Baldini et al., 2018). For this reason, seed quality germination systems are conducted under controlled environments. These systems develop rapid, simple, and precise techniques for farmers to ensure good seed quality during sowing, growth, harvesting, and storage (Baldini et al., 2018).

Physical quality depends on size, bulk density, analytical purity, and the absence of weed seeds and seeds from other crops (Rathore et al., 2021). Furthermore, physical quality is evaluated using the variables of thousand-seed weight, seed size, bulk density, and physical purity (Fernández et al., 2015). It can also be characterized by the flotation index, hardness, and grain density (Kang et al., 2020; Beyaz, 2019).

Physiological quality, particularly seed vigor, is associated with germination uniformity, seedling development, and field adaptation. In this sense, seed vigor is defined as the property that determines behavior during germination and seedling emergence (ISTA, 2005). The main parameter of physiological quality deterioration is seed vigor, followed by reduced germination or a relatively high percentage of abnormal seedlings (Rathore et al., 2021; Finch-Savage, 2020).

In wheat, greater viability and germination occur before physiological maturity, whereas maximum vigor is expressed at physiological maturity (Wu et al., 2017). Physiological quality can be evaluated by conducting germination tests in the laboratory (Cardoso et al., 2021). Additionally, seedlings must have the capacity to emerge in the field (Derebe et al., 2022). Vigor tests have been used to determine the potential for rapid seedling emergence and development under diverse environmental conditions (Bayisa et al., 2023; Chaichi et al., 2022). Various evaluations have demonstrated that early planting dates result in optimal yields (Gadisa, 2019; Beyzi et al., 2019) and strong initial seedling vigor with good crop development in the field. Similarly, Baloch et al. (2010) demonstrated that early plantings, aligned with the agricultural cycle, tend to produce better expression of agronomic characteristics and grain physical quality.

The effects of seed size on germination and vigor have been studied in various plant species (Cardoza-Viera et al., 2024; Do Nascimento et al., 2022). Several studies have reported positive associations between seed vigor and productivity when larger seeds are used than when smaller seeds are used. Larger seeds tend to increase germination and emergence (Escobar-Álvarez et al., 2021; Domic et al., 2020). In wheat, larger seed size not only influences germination and establishment (Carranza-González et al., 2022) but also affects yield and its components (Llivicura et al., 2021); these results are attributed to the greater number of reserves in the seed. Seed size depends on the genotype and the environmental conditions in which the plants develop. According to Gadisa et al. (2019), Ceseski and Al-Khatib (2021) reported that seed size is important because it affects the percentage of total emergence, survival, growth, and seedling development; therefore, seed size is strongly related to physiological quality. However, Gadisa et al. (2019) reported that there is no significant effect of seed size on the germination percentage of bread wheat varieties. In this context, the objective of this study was to determine the influence of sowing date and seed size on the agronomic traits, physical quality, and initial seedling vigor of elite bread wheat lines in southern Sonora, Mexico.

MATERIALS AND METHODS

Study site

The research was conducted at the Technological Institute of the Yaqui Valley, located in Bácum, Sonora, between the Sierra Madre Occidental Mountain range and the Sea of Cortez, at 27°24'49" north latitude and 11°07'47" west longitude, with an average annual rainfall of 45 mm and an altitude of 13 meters above sea level. The predominant climate is BW(h), which is extremely hot, with an average annual temperature of 24°C and an average maximum of 31°C; from July to August, the maximum reaches 48°C, and in January, the minimum is 16°C, with an average annual rainfall of 450 mm. The arable soils are Vertisols, fertile, and rich in clay and are generally located in arid subhumid zones with hydration and humid exposure and cracks when dry (García, 1973).

In the fall–winter of 2020–2021, 18 previously selected lines were established from a total of 276 lines from an experiment conducted during the fall–winter cycle of 2019. Two commercial controls were used (Table 1) on two sowing dates, November 19, 2020, and January 12, 2021, with two seed sizes: large and small. A 19 × 3 mm oblong sieve was used to separate the seeds; those that remained on the sieve were considered large, and those that passed through were considered small.

The factorial design consisted of three study factors, factor A (genotypes = 20), factor B (sowing dates = 2), and factor C (seed size = 2), under a randomized complete block design with three replications, resulting in a total of 240 experimental units. The experimental unit was composed of four rows three meters long, separated by 80 cm and sown in double rows; the usable area was 9.6 m^2. The harvest was carried out on May 16 and June 20, 2021, corresponding to the first and second sowing dates, respectively.

Table 1. Commercial lines and varieties of bread wheat used in the field and sand bed evaluation in Yaqui Valley, Sonora, Mexico. 2020-2021.

Line	Genealogy	Line	Genealogy
L-1	CMSS07Y01083T-099TOPM	**L-11**	CMSS12Y00235S-099Y-099 N
L-2	CMSS10Y00374S-099 M-1WGY-0B	**L-12**	CMSS12Y00276S-099Y-0WGY
L-3	CMSS10Y00023S-099Y-4WGY	**L-13**	CMSS12Y00276S-099Y-0WGY
L-4	CMSS12Y00031S-38Y-0WGY	**L-14**	CMSS12Y00276S-099Y-16Y
L-5	CMSS12Y00064S-130 M--0WGY	**L-15**	CMSS12Y00307S-129Y-0WGY
L-6	CMSS12Y00069S-119Y-20Y	**L-16**	CMSS12Y00311S-130Y-7Y
L-7	CMSS12Y00070S-112 M-21Y	**L-17**	CMSS12Y00395S-099Y-3Y-0WGY
L-8	CMSS12Y00072S-9Y-0WGY1B	**L-18**	CMSS12Y00072S-115Y-9Y-10B
L-9	CMSS12Y00083S-099Y-5Y-6Y	**Control**	Borlaug F100
L-10	CMSS12Y00163S-152 M-099NJ-3	**Control**	Kronstad F2004

In the field, the grain yield (GR) was determined. The four rows were harvested, the seeds were cleaned, and a digital scale was used to record the data in kilograms. This was later converted to tons per hectare, adjusted for 15% moisture. Once the seed was cleaned, the weight of one thousand grains (PMG) was recorded using a digital scale, and the data were recorded in grams and hectoliter weight (PH). For the latter, a 1 L container was filled with seed until it overflowed and leveled off in a zigzag motion to remove the excess. The contents were weighed on an Ohaus volumetric weight balance (Parsippany, NJ, USA), and the readings were recorded in kg hL^{-1}. The standard germination percentage was determined at two points in time: on the fourth day (GE4) and on the seventh day (GE7), according to the ISTA (2015) guidelines.

Once the experiment was completed, the seeds obtained from each line were identified and stored in a warehouse at 22°C and 55% relative humidity until September, at which point the seed quality evaluation began.

The initial seedling vigor was subsequently determined in sand beds under nursery conditions during the period of September–November 2021. A completely randomized design with three replicates was used in this experiment. The experimental unit consisted of 100 seeds per genotype, and the following traits were measured: emergence rate (ER), percentage of emergence (PE), seedling length (SL), and root length (RL). The emergence rate was determined using the following formula (Maguire, 1962).

$$VE = \frac{\text{number of normal plants} + \ldots..}{\text{day of the first acount}} \frac{\text{number of normal plants}}{\text{day of the final acount}}$$

Emergence counts were performed at 4, 5, 6, and 7 days after sowing (DAS). The percentage of emergence (PE) was determined by the number of seedlings that emerged on the seventh day using the following equation:

$$PE = \frac{Number\ of\ emerged\ plants\ at\ the\ seven\ day}{Number\ of\ total\ seed\ sowed}\ (100)$$

Seedling length (SL) and root length (RL) were recorded 15 days after the plants were planted in a random sample of 10 plants in full competition. Coleoptile length was measured from the substrate level to the leaf apex, and root length was measured from the base of the coleoptile to the extreme tip of root growth.

Statistical analysis

The characteristics were subjected to a combined analysis using the PROC GLM procedure of the SAS statistical package Ver. 9.3 (2012). The comparison of means was performed using Tukey's multiple range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Analysis of variance revealed significant differences ($P \leq 0.01$) in the main effects of genotype (G), date (D), and seed size (SS) across all the study variables, indicating that all the genotypes differed because of their genetic makeup (Table 2). These results may be attributable to the expression of traits that are strongly influenced by the environment (Gharoobi, 2011).

Table 2. Mean squares of the analysis of variance for the agronomic and physical quality characteristics of the bread wheat seeds. Yaqui Valley, Sonora. 2020-2021.

SV	DF	TSW (g)	HW (kg hL^{-1})	GY (t há$^{-1}$)	SL (cm)	RL (cm)	ER (%)	PE (%)	G4 (days)	G7 (days)
Repetition (R)	2	10.09	2.08	0.29	0.16	0.39	0.061	0.46	3.42	0.67
Genotype (G)	19	383.31**	175.81**	3.99 *	6.25**	5.09**	0.33**	20.21**	384.51**	18.53**
date (D)	1	16.85**	0.14*	0.41 *	0.33**	0.004*	0.40**	3.26**	11.70**	0.26*
Size (S)	1	0.006 *	0.16**	1.71**	4.03**	0.024*	0.007*	4.81**	1.50**	2.40**
G × F	19	6.18**	0.31 *	0.10 *	2.53**	1.82**	0.205**	15.06**	2.02**	4.52**
G × T	19	5.52**	0.48 *	0.26 *	0.74*	0.30*	0.14*	10.06**	0.61*	4.23**
F × T	1	2.15**	0.10 *	0.02*	0.39**	0.17*	0.10*	0.41*	0.33*	2.40**
G × D × S	19	6.18**	0.45 *	0.18 *	0.66**	0.81**	0.25*	4.86**	0.78**	1.23**
Error	158	5.59	0.4	0.06	0.45	0.08	0.11	2.30	0.98	1.69
CV (%)		5.86	0.86	3.9	11.71	5.37	1.91	1.96	5.26	5.23

SV: source of variation; DF: degree of freedom; *, **, and **, indicate significance at the 0.05 and 0.01 probability levels, respectively. TSW: thousand-seed weight, HW: hectoliter weight, GY: grain yield, SL = seedling length, RL = root length, ER = emergence rate, PE = percentage of emergence, G4 = germination on the 4th day after sowing, G7 = germination on the 7th day after sowing, and CV: coefficient of variation.

The simple interactions GxF, GxT, and FxT significantly differed across all the variables studied, whereas the triple interaction GxFxT significantly differed across four of the five study variables. These results demonstrate that the response of the genotypes varied according to environmental conditions, or this could also be attributed to the maturity cycle.

On the other hand, the analysis of variance generated by the three study factors and their interactions revealed that genotypes contributed most to the total variation detected, as the variability encompassed by this factor in the expression of the variables PMS, PH, and RG exceeded that recorded for planting date (F) and seed size. The interaction (GxF) was the most statistically significant because of its impact on the expression of variability for the PMS variable; however, for (PH) and (RG), the interaction (GxT) made the greatest contribution to the total variation. The coefficients of variation for the characteristics studied in the experiment are low and therefore considered acceptable (Khan et al., 2010).

The mean comparison test (Table 3) revealed that L-18 had the highest hectoliter weight at 79.5 kg hL^{-1}, followed by Borlaug F-100 and L-1 at 78.5 and 77.2 kg hL^{-1}, respectively. These results are indicators of the quality obtained in the field in relation to the agronomic management and environmental conditions present during the experiment, which are ultimately expressed in the seed. However, the results obtained in this study are lower than the 83.1 kg hL^{-1} reported by Moreno (1993) but higher than the values reported by Baloch et al. (2010). The averages of at least eight lines were consistent with the Mexican Official Standard NMX-FF-036-1996, which ranges from 74 to 76.3 kg hL^{-1} for high-quality materials.

Table 3. Comparison of means for physical seed quality traits in bread wheat genotypes. Yaqui Valley, Sonora. 2021.

Line	HW (kg hL^{-1})	WTG (g)	GY (t ha^{-1})
L-1	77.2 c	47.9 ab	7.0 bc
L-2	75.1 f	38.9 g	5.9 fgh
L-3	69.9 k	36.2 i	5.8 gh
L-4	76.1 e	39.1 fg	6.1 f
L-5	70.9 ij	34.7 ij	5.8 hi
L-6	76.0 e	47.1 bc	6.9 c
L-7	76.6 d	45.8 cd	7.0 bc
L-8	70.5 j	43.9 de	6.6 d
L-9	68.4 l	32.2 kl	5.7 hi
L-10	76.8 cd	45.1 de	6.8 c
L-11	71.9 h	35.1 ij	5.8 h
L-12	67.9 lm	33.2 jk	5.7 hi
L-13	73.9 g	43.1 e	6.4 e
L-14	71.4 i	38.1 gh	5.8 fgh

Line	HW ($kg\ hL^{-1}$)	WTG (g)	GY ($t\ ha^{-1}$)
L-15	67.6 m	31.1 l	5.6 i
L-16	76.5 de	40.9 f	6.4 de
L-17	68.1 lm	36.5 h	5.8 gh
L-18	79.5 a	47.9 ab	7.4 a
Borlaug F100	78.5 b	49.1 a	7.1 b
Kronstad F2004	75.1 f	40.0 fg	6.0 fg
DSH (0.05)	0.93	3.47	0.36
Seed size			
large	73.4 a	40.3 a	6.39 a
Small	72.8 b	39.7 b	5.25 b
DSH (0.05)	0.16	0.5	0.8
Sowing date			
19 November 2020	75.31a	41.57 a	6.36 a
12 January 2021	74.14 b	40.04 b	5.82 b
DSH (0.05)	0.8	1.2	0.7

With respect to PMS (processed dry matter), the control variety Bourlaug F-100 had the highest value of 49.1 g, followed by lines L-18, L-1, and L-6, whose values ranged from 47.1 to 47.9 g. Lines L-5, L-9, L-12, and L-15 presented the worst performance. These results indicate that thousand-seed weight is the most important direct trait in the indirect selection of yield for genotype selection. In this regard, the yields obtained in this research can be attributed to the accumulation of chilling hours during crop development, which were strongly related to the thousand-seed weight, which accumulated 796 chilling hours, exceeding the average of 600 hours required for good production (CONAGUA, 2021).

In terms of field yield, the experimental line L-18 showed outstanding performance (7.4 t $ha^{-1)}$, surpassing the commercial controls Bourlaug F100 and Kronstad F2004 (300 and 1400 kg ha^{-1}, respectively). Furthermore, it is important to highlight that lines L-1 and L-7 obtained 7.0 t ha^{-1}, making them competitive, as they exhibited the same response as the commercial variety Bourlaug F-100. The results of this research are superior to those reported by Castañeda et al. (2009), who reported average yields of 5 t ha^{-1} in bread wheat but are similar to those reported by Buenrostro-Rodríguez et al. (2022), who reported average yields of 7.2 t ha^{-1} in the 2015/2016 cycle in varieties in the state of Guanajuato.

Large seed size yielded the best average results, and the best planting date was November 19, 2020, for all the recorded agronomic traits. In this context, Zareian et al. (2013), Akhter et al. (2017), and Huang et al. (2015) reported that large seed size in wheat favors yield expression in the field. These results were reflected in the production environments, highlighting positive effects on yield and grain physical quality, as reported by Villaseñor et al. (2012). They also mention that while production environments present critical conditions, these are not recommended for seed production, as they affect seed quality.

Table 4 presents the results of the Tukey test for comparing means for the physiological quality traits. Seedling length (SL), root length (RL), emergence rate (ER), emergence percentage (EP), standard germination on day four (EG4), and standard germination on day seven (EG7) were measured. With respect to SL, the lengths of the L-18, L-1, L-7, Borlaug F-100, and L-6 genotypes were consistently the greatest, with values exceeding 6 cm. In terms of root length, the L-7, L-1, L-18, and Borlaug F-100 genotypes again presented the greatest length, with L-10 having the longest roots. Furthermore, these genotypes consistently demonstrated the best performance for ER and EP, surpassing the commercial control Kronstad F-2004. These findings suggest that in this study, any of these genotypes offered guaranteed uniformity in terms of field establishment on the basis of their seedling vigor performance. Furthermore, 17 to 18 plants emerged per day, with 74 to 78% emergence in sand beds. These results differ from those reported by Noriega-Carmona et al. (2019), who reported higher values for both characteristics in wheat genotypes from the Bajío region of Guanajuato. It is important to note, however, that a significant number of lines—L-2, L-3, L-4, L-5, L-9, L-11, L-12, L-14, L-15, and L-17—exhibited poor performance in terms of all recorded physiological characteristics and were inferior to the commercial controls (Yousefi et al., 2020).

Table 4. Comparison of means for physiological seed quality traits among bread wheat genotypes. Yaqui Valley, Sonora. 2021.

Genotypes	G4 (%)	G7 (%)	LC (cm)	RL (cm)	VE (p dia^{-1})	PE (%)
L-1	22.75 de	94.16 bcde	7.1 ab	6.2 a	17.9 a	78.7 ab
L-2	24.41 ab	95.52 a	5.5 cde	5.4 b-f	17.7 ab	77.1 abc
L-3	24.25 bc	94.91 abc	5.3 de	5.2 f	17.7 ab	76.5 bcd
L-4	21.58 efg	94.31 bcde	5.7 cde	5.4 b-f	17.7 ab	77.5 abc
L-5	23.16 cd	95.08 ab	5.2 de	4.3 gh	17.6 ab	75.5 cd
L-6	21.41 fg	93.75 cde	6.2 cd	5.5 bc	17.8 a	78.4 ab
L-7	22.41 def	94.58 abcd	6.4 bc	6.4 a	17.9 a	78.6 ab
L-8	22.41 def	94.08 bcde	5.8 cd	5.6 b	17.7 ab	78.4 ab
L-9	21.25 fg	91.41 f	5.1 de	4.6 g	18.4 a	75.5 cd
L-10	21.52 fg	93.53 de	5.9 cd	5.7 b	17.8 a	78.5 ab
L-11	21.41 fg	93.08 e	5.2 de	5.1 ef	17.6 ab	76.9 abc
L-12	24.41 ab	95.09 ab	5.1 de	4.2 h	17.5 ab	74.4 d
L-13	21.75 efg	93.83 cde	5.6 cde	5.5 bcd	17.8 ab	77.5 abc
L-14	23.08 cd	94.91 abc	4.7 e	5.1 def	17.7 ab	75.7 cd

Genotypes	G4 (%)	G7 (%)	LC (cm)	RL (cm)	VE (p dia^{-1})	PE (%)
L-15	21.19 g	93.52 de	5.1 de	4.2 gh	17.5 ab	78.2 ab
L-16	23.34 d	94.41 abcd	5.9 cd	5.6 b	17.8 ab	78.2 ab
L-17	21.58 efg	93.58 de	5.3 de	5.1 c-f	17.6 ab	76.8 abc
L-18	23.41 bcd	94.91 abc	7.8 a	6.2 a	17.9 a	78.6 ab
Borlaug F-100	25.58 a	95.07 ab	6.2 cd	5.7 b	17.3 b	79.5 a
Kronstad F-2004	21.75 efg	94.31 bcde	5.6 cde	5.4 b-f	17.7 ab	77.2 abc
Tukey (0.05)	1.46	1.2	0.99	0.42	0.5	2.23
Tamaño semilla						
Large	18.92 a	95.28a	5.79 a	5.34 a	17.79 a	77.51 a
Small	17.67 b	94.04b	4.81 b	4.16 b	16.14 b	76.83 b
Tukey (0.05)	0.85	0.65	0.47	0.07	0.9	0.58
Sowing date						
19 November 2020	19.06 a	95.15 a	5.88 a	5.35 a	17.76 a	77.54 a
12 January 2021	18.62 b	94.19 b	5.62 b	4.71 b	16.08 b	76.25 b
Tukey (0.05)	0.25	0.75	0.18	0.36	1.25	1.05

A comparison of the means for the physiological traits revealed that the percentage of emergence on day 4 ranged from 21.08% to 25.58%, with the commercial variety Borlaug F-100 consistently showing the highest percentage, followed by lines L-2, L-3, and L-12. On the other hand, the Kronstad F-204 variety and the L-15, L-9, and L-11 lines exhibited low germination in this first count. For standard germination recorded in the second count (day 7), lines L-2, L-5, L-12, and L-3 and the variety Borlaug F-100 presented the highest germination values; however, lines L-9, L-11, and L-15 presented the worst performance.

The early sowing date of November 19, 2020 had a significant positive effect on the expression of initial seedling vigor. These findings indicate that early planting dates are favorable for wheat seed production, as confirmed by Villaseñor et al. (2012) and García-Rodríguez et al. (2018). Conversely, in late planting, all the genotypes performed poorly for all the variables studied. These results are consistent with those reported by Noriega-Carmona et al. (2019) and Martínez et al. (2010).

Large seed size favors the expression of physiological quality and initial seedling vigor, as has been reported in several studies on various crops, indicating that large seeds tend to germinate more successfully in the field and produce more vigorous seedlings (Gutiérrez-García et al., 2006; Zareian et

al., 2013), mainly because of the nutritional reserves of the seeds. In contrast, small seeds are characterized by low percentages of germination and seedling vigor (Bockstaller; Girardin, 1994); in addition, the large size of the grain in wheat favors the characteristics of milling in the agro-industrial sector (Dziki; Laskowski, 2004).

CONCLUSIONS

The evaluated genotypes responded differently to the planting date and seed size. Lines L-1, L-18, L-6, and L-7 presented high values for agronomic characteristics and physical and physiological seed quality, achieving optimal expression of these traits and outperforming the commercial controls. Finally, the early planting date of November 19th and the use of large seeds favored agronomic expression, yield, physical and physiological seed quality, and initial seedling vigor in most of the evaluated genotypes.

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Chapter 3

Morphophysiological response and yield of tomato (*Solanum lycopersicum* L.) to growth regulators and glutamic acid

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ABSTRACT

As a strategy to optimize the productive efficiency of hydroponic tomatoes under greenhouse conditions, this study aimed to analyze the effects of foliar-applied growth regulators and glutamic acid on the modulation of vegetative growth, photosynthetic activity, and yield of the indeterminate growth habit of the Malinche hybrid (*Solanum lycopersicum* L.) in a protected hydroponic system. The following treatments were evaluated: paclobutrazol (25, 50, and 75 ppm), cytokinins (0.3, 0.4, and 0.5 ppm), calcium prohexadione (125, 175, and 200 ppm), glutamic acid (1000, 3000, and 5000 ppm), and a control with no application. The experiment was established using a completely randomized design with a two-factor arrangement and three replicates. Morphological (plant height and stem diameter), physiological (net photosynthesis, stomatal conductance, and transpiration), and productive (total yield and average fruit weight) variables were quantified. Analysis of variance revealed significant effects of the treatments ($p<0.05$) on the morphophysiological variables. Tukey's test ($p<0.05$) indicated that compared with the control, the treatments with 0.4 and 0.5 ppm cytokinins and 3000 and 5000 ppm glutamic acid significantly increased net photosynthesis. In terms of stomatal conductance, the groups treated with cytokinins (0.4–0.5 ppm) and glutamic acid at 3000 ppm were significantly superior, whereas the group treated with paclobutrazol at 75 ppm presented significantly reduced photosynthetic and stomatal

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activity. The pattern of transpiration was physiologically consistent with that of stomatal modulation, with statistically significant differences between treatments. Furthermore, the SPAD units significantly varied after application. The average yield was 8.95 kg plant^{-1}, with an average fruit weight of 99.87 g, with no significant differences between treatments (Tukey, $p<0.05$), demonstrating sustained productive stability, even under the morphophysiological modulation induced by the treatments.

Key Words: Plant growth regulators, tomato, hydroponics, biostimulants.

INTRODUCTION

The tomato (*Solanum lycopersicum* L.) has become among the most important horticultural crops globally in terms of agronomy, economics, and nutrition, both for its production volume and its contribution to food security and added value in protected agriculture systems, where intensive management has allowed for the maximization of productivity and quality under controlled conditions (Heuvelink; Dorais, 2018; FAO, 2023). Given agricultural intensification and increasing climate variability, optimizing physiological efficiency and yield in protected systems represents a strategic challenge for productive sustainability, particularly in scenarios of heat and water stress that affect photosynthesis, stomatal regulation, and photoassimilate partitioning (Zhang et al., 2020; Lawson; Vialet-Chabrand, 2019). In this context, the exogenous modulation of morphophysiological processes using plant growth regulators (PGRs) and biostimulants is emerging as an agronomic tool of great scientific and technological interest (Bano et al., 2022; Rouphael; Colla, 2020; Du Jardin et al., 2015).

PGRs, including gibberellin inhibitors, auxin analogs, and cytokinin modulators, act on highly integrated endogenous hormonal networks that regulate cell elongation, plant architecture, photoassimilate partitioning, and reproductive transition—processes linked to productivity (Rademacher, 2015). Recent evidence has demonstrated that manipulating the hormonal balance can modify the source–sink relationship, increase photosynthetic efficiency, and stabilize yield under abiotic stress conditions by altering cross-hormonal signaling and stomatal regulation (Anfang; Shani, 2021). In tomatoes, these effects are reflected in modifications to plant height, stem diameter, leaf area, stomatal conductance, and chlorophyll content—variables closely associated with agronomic performance, photosynthetic efficiency, and fruit quality (Heuvelink; Dorais, 2018; Flexas et al., 2014). Glutamic acid, for its part, has been studied as a nitrogen biostimulant with signaling and metabolic functions of high physiological relevance. In addition to its central role in nitrogen metabolism and amino acid synthesis, it acts as a signaling molecule involved in the regulation of root growth, redox homeostasis, and the stress response (Liao et al., 2022; Forde; Roberts, 2014). Studies indicate that in intensive horticultural crops, exogenous applications of amino acids can increase nitrogen use efficiency, the photosynthetic rate, and productivity, particularly when these compounds interact with endogenous hormonal networks (Malécange et al., 2023; Rouphael; Colla, 2020; Ertani et al., 2013). However, the interactions between

growth regulators and glutamic acid and their combined effects on the overall morphophysiological response of tomatoes remain insufficiently characterized under hydroponic conditions. Understanding these interactions is particularly relevant in protected systems, where precise nutritional control allows for the isolation of regulatory effects on photosynthesis, stomatal conductance, transpiration, and yield components. The application of growth regulators and glutamic acid represents a physiologically sound strategy for optimizing resource use efficiency and productive stability in intensive systems (Bano et al., 2022).

Considering the above context, the objective of the present study was to analyze the effects of growth regulators and glutamic acid applied via foliage on the modulation of vegetative growth, photosynthetic activity and yield of a Malinche hybrid (*Solanum lycopersicum* L.) in a protected hydroponic system.

MATERIALS AND METHODS

Study area and location of the experimental area

This research was conducted during the 2025 spring–summer cycle at the National Technological Institute of Mexico in a greenhouse located in the experimental field of the Roque Technological Institute, Celaya, Guanajuato, Mexico, at 20°31' N and 100°49' W, at an average altitude of 1760 m.a.s.l., with a temperate semiarid climate (BS1kw) according to the Köppen classification modified by García (2004). The average temperature is 18 °C, and the average annual rainfall is 550–650 mm (Morales-Martínez et al., 2025).

Experimental Design

The treatments consisted of foliar applications of three doses (low, medium, and high) of the growth regulators; in this study, paclobutrazol (25, 50, and 75 ppm), cytokinins (0.3, 0.4, and 0.5 ppm), calcium prohexadione (125, 175, and 200 ppm), and glutamic acid (1000, 3000, and 5000 ppm) were used, along with an untreated control. The experimental design was completely randomized and arranged in a two-factor factorial scheme (regulator type × dose), with three replicates per treatment and six plants per experimental unit.

Plant material used

The plant material consisted of the Malinche tomato hybrid (*Solanum lycopersicum* L.), an indeterminate growth hybrid. It produces elongated and uniform (120–150 g) saladette-type fruits, with postharvest firmness and stable total soluble solids (4.5–5.5 °Brix). In greenhouses, its indeterminate growth habit promotes source–sink plasticity and maximizes yield with optimal nutritional and hormonal management (Heuvelink et al., 2018).

Agronomic management of the crop and fertigation

Sowing was carried out in 60-cell polystyrene trays, with an approximate volume of 200 mL per cell, using a 2:1 (v/v) mixture of commercial peat (Sunshine Mix 3®) and perlite (Multiperl®) as the substrate. This ensured adequate aeration and moisture retention during the seedling stage. One seed was placed in each cell to guarantee uniformity in emergence and initial crop establishment. Transplanting was performed 30 days after sowing (DAS), when the plants reached a suitable physiological state for establishment in the production system. The duration of the crop cycle, from transplanting to harvest, was 120 days. For transplanting, 12 L black polyethylene bags filled with red volcanic sand (tezontle) were used, with one plant per bag. The growth of a single stem was managed. From transplanting until the end of the growing cycle, the nutrient solution used was complete with (mg L^{-1}) 237 N, 57.3 P, 250.1 K, 253.3 Ca, 53.8 Mg, 180 S, 2.7 Fe, 0.5 B, 0.6 Mn, 0.1 Cu, and 0.05 Zn. The electrical conductivity was maintained between 2.0 and 2.5 dS m^{-1}, and the pH was between 6 and 6.5.

MORPHOLOGICAL VARIABLES EVALUATED

Plant Height

Plant height was determined using a graduated measuring tape (STANLEY®), and the distance from the base of the stem to the leaf apex was recorded as an indicator of the longitudinal growth of the main axis. Measurements were taken weekly in each experimental unit, with the purpose of systematically monitoring the dynamics of vegetative growth throughout the evaluation cycle (Aurdal et al., 2022).

Stem Diameter

Stem diameter was determined using an electronic caliper (HER-411®), and measurements were taken at the base of the stem to standardize the evaluation point and reduce variability associated with morphological differences along the stem axis. This parameter was recorded weekly in each experimental unit to monitor the dynamics of radial stem growth during the evaluation period (Mngoma et al., 2022).

PHYSIOLOGICAL VARIABLES

Photosynthesis (A), stomatal conductance (Gs), and transpiration (E)

The environmental parameters were standardized during the measurements, maintaining a photosynthetically active radiation (PAR) of 800 µmol m^{-2} s^{-1} and a CO_2 concentration of 400 µmol mol^{-1}, to ensure homogeneous conditions between treatments and guarantee the comparability of the net CO_2 assimilation rates (A). The determinations were made on the fifth fully expanded leaf from the growth apex, according to previously established physiological criteria (Cadahía, 2005). In each experimental unit, two plants were randomly selected, and gas exchange measurements were carried out using an LI-6400XT portable photosynthesis system (LI-COR®).

PRODUCTIVE VARIABLES

Yield

The agricultural yield of the tomato plants was evaluated by quantifying the total fruit weight per plant and is expressed in kg plant^{-1}. Its components (number of fruits per plant and average fruit weight) were also analyzed, as these constitute the yield of *Solanum lycopersicum* and allow for the interpretation of the contribution of each productive component to the final crop yield (Sekara et al., 2019; Mawasid et al., 2024).

Statistical Analysis

The assumptions of normality and homogeneity of variance were verified for the experimental data. Once the suitability of the parametric model was confirmed, analysis of variance (ANOVA) was performed to identify statistically significant effects between treatments. When overall differences were detected, the means were compared using Tukey's test with a significance level of $p<0.05$. All the statistical analyses were performed using the Statistical Analysis System (SAS, NC, USA) software package, version 9.0 (2002).

RESULTS

The data were subjected to analysis of variance, which revealed a significant treatment effect on plant height ($p<0.05$). However, no statistically significant differences attributable to this factor were detected for stem diameter according to Tukey's multiple comparison test (Table 1). In terms of plant height, the paclobutrazol treatment resulted in the lowest average value (101.4 cm), which differed significantly from those of the control (110.9 cm), cytokinins (113.2 cm), prohexadione-Ca (110.4 cm), and glutamic acid (108.9 cm), indicating that the plants formed a homogeneous group ($p<0.05$; HSD=5.5) (Table 1). The reduction induced by paclobutrazol represented an approximate decrease of 8.6% compared with that of the control, demonstrating a significant inhibitory effect on stem elongation.

With respect to stem diameter, the values ranged approximately 1.2 cm, and no statistically significant differences were observed between treatments (HSD=0.06), indicating that hormone modulation did not significantly alter radial stem growth at the evaluated stage (Table 1). With respect to the dose factor, a significant effect on both stem height and diameter was detected (Table 1). The high dose resulted in the lowest average height (102.7 cm), differing statistically from the medium (113.2 cm) and low (111.0 cm) doses, which did not differ from each other (HSD=3.6). This behavior demonstrates a dose-dependent response with restriction of longitudinal growth at high concentrations.

Table 1. Growth and yield indicators of the Malinche tomato hybrid. Source: the authors.

Treatment	PH (cm)	SD (cm)	Yield (kg planta^{-1})	NF (no. planta^{-1})	MFW (g)	RI
Control	110.9 a	1.25 a	8.95 a	44.6 a	99 a	1.3 a
PBZ	101.4 b	1.27 a	8.29 a	44.7 a	93 a	1.3 a
CK	113.2 a	1.25 a	8.78 a	46.3 a	95 a	1.4 a
Pro-Ca	110.4 a	1.27 a	8.42 a	43.3 a	97 a	1.4 a
Glu	108.9 a	1.25 a	8.00 a	45.7 a	95 a	1.4 a
HSD	5.5	0.06	1.74	5.9	9.3	0.04
Dose						
Low	111.0 a	1.29 a	7.57 a	45.9 a	90 a	1.3 a
Medium	113.2 a	1.20 b	8.53 a	45.7 a	94 a	1.3 a
High	102.7 b	1.24 ab	8.44 a	43.8 a	96 a	1.4 a
HSD	3.6	0.04	1.74	5.9	9.3	0.04

Means with different letters in a column are significantly different (Tukey, $p<0.05$). PBZ: paclobutrazol, CK: cytokinins, Pro-Ca: calcium prohexadione, Glu: glutamic acid, PH: plant height, SD: stem diameter, NF: number of fruits, MFW: mean fruit weight, RI: roundness index, HSD: honest significant difference.

In terms of stem diameter, the low dose had the greatest effect (1.29 cm), which was significantly greater than that of the medium dose (1.20 cm), while the high dose (1.24 cm) had an intermediate effect (HSD=0.04) (Table 1). Although the differences were statistically significant, their magnitude was relatively small ($p<0.1$), suggesting structural stability of the stem in response to variations in the applied concentration. The results indicate that the variation in height responded mainly to the type of regulator and the applied dose, whereas the sensitivity of stem diameter was less significant under the evaluated experimental conditions (Table 1).

The treatment factor for yield indicators did not significantly differ ($p>0.05$) for any of the evaluated variables: yield per plant, number of fruits, average fruit weight, or roundness index (Table 1). Statistical uniformity was confirmed using Tukey's multiple comparison test ($p<0.05$), in which all the means were grouped under the same category within each variable, and no significant separation was detected between treatments.

The yield ranged from 8.00 to 8.78 kg plant^{-1}, with a total range of 0.78 kg, which was below the critical comparison value (HSD=1.74). The maximum observed difference represents approximately 44.8% of the threshold required to declare statistical significance, indicating that the variability between treatments was less than the experimental error estimated by the model. The percentage variation between the maximum (8.78 kg) and minimum (8.00 kg) values was 9.75%, which was insufficient to generate inferential separation under $p<0.05$.

The number of fruits ranged from 43.3 to 46.3 fruits per plant, a value equivalent to 50.8% of the HSD (5.9). The distance between means did not exceed the multiple comparison criterion. The absence of significant differences in yield components indicates stability in reproductive partitioning, demonstrating that the allocation of photoassimilates to the fruits was not altered by the treatments under the evaluated experimental conditions.

The average fruit weight ranged from 93 to 97 g, with a range of 4 g, which is considerably lower than the critical threshold (HSD=9.3). The maximum difference corresponds to 43.0% of the value required to establish significance. These results indicate that the small fluctuations observed are within the experimental error and cannot be attributed to the effects of the treatments.

The morphometric index (roundness index) ranged from 1.3 to 1.4, with a maximum range of 0.1 units. Although the critical value was relatively small (HSD=0.04), the literal grouping in Tukey's test confirmed that the differences did not reach statistical significance under the applied model. The observed response suggests the morphological stability of the fruit in response to the application of regulators and glutamic acid.

Table 2. Photosynthesis (A) before, during and after the application of growth regulators and glutamic acid in plants of the Malinche tomato hybrid. Source: the authors.

Treatment	Before	During	After
	μmol CO_2 m^{-2} s^{-1}		
Control	36.34 a	35.85 b	36.94 a
PBZ	36.06 a	38.63 a	35.16 a
CK	39.15 a	38.42 a	36.01 a
Pro-Ca	34.94 a	36.74 a	36.41 a
Glu	37.18 a	39.08 a	35.36 a
HSD	8.81	4.03	3.89
Dose			
Low	39.38 a	37.25 ab	35.96 a
Medium	36.05 a	39.93 a	35.83 a
High	35.56 a	39.87 a	34.20 a
HSD	8.81	4.03	3.89

Means with the same letter within each variable are statistically equal, according to Tukey's test (P<0.05). PBZ: paclobutrazol, CK: cytokinins, Pro-Ca: calcium prohexadione, Glu: glutamic acid, HSD: honest significant difference.

Analysis of variance for net photosynthesis (A) revealed no significant treatment effect at any of the three evaluation times (before, during, and after application) according to Tukey's multiple comparison test ($p<0.05$) (Table 2). Prior to application, (A) values ranged from 34.94 to 39.15 μmol CO_2 m^{-2} s^{-1}, with no statistically significant differences between means (HSD=8.81), demonstrating baseline physiological homogeneity and adequate experimental randomization. During the application, although increases were observed in some treatments (glutamic acid: 39.08 μmol CO_2 m^{-2} s^{-1}; paclobutrazol: 38.63 μmol CO_2 m^{-2} s^{-1}), these variations did not exceed the threshold for significant difference (HSD=4.03), indicating that the variability between groups was comparable to the variation attributable to the treatment. The values of net photosynthesis were between 35.16 and 36.41 μmol CO_2 m^{-2} s^{-1}, which also did not significantly differ (HSD=3.89), indicating the functional stability of the photosynthetic apparatus after hormone application.

For the dose factor, ANOVA revealed a significant effect exclusively during the application period ($p<0.05$) (Table 2). The medium dose (39.93 μmol CO_2 m^{-2} s^{-1}) and the high dose (39.87 μmol CO_2 m^{-2} s^{-1}) had significantly greater effects than the control (35.85 μmol CO_2 m^{-2} s^{-1}), while the low dose (37.25 μmol CO_2 m^{-2} s^{-1}) had intermediate effects (HSD=4.03). The magnitude of the photosynthetic increase compared with that of the control was 11.4% and 11.2% for the medium and high doses, respectively, suggesting a concentration-dependent physiological stimulation effect.

No significant differences were observed before or after application, indicating that there were no residual effects on carbon assimilation. Statistically, the photosynthetic response of the Malinche hybrid showed low sensitivity to the type of regulator and a specific response associated only with the dose intensity during application, without altering the physiological balance of the photosynthetic process (Table 2).

Table 3. Stomatal conductance (Gs) before, during and after the application of growth regulators and glutamic acid in plants of the Malinche tomato hybrid. Source: the authors.

Treatment	Before	During	After
	mol H_2O m^{-2} s^{-1}		
Control	0.26 a	0.31 a	0.28 a
PBZ	0.27 a	0.31 a	0.36 a
CK	0.25 a	0.29 a	0.30 b
Pro-Ca	0.28 a	0.28 a	0.29 b
Glu	0.26 a	0.31 a	0.26 b
HSD	0.05	0.06	0.05
Dose			
Low	0.27 a	0.30 a	0.32 a
Medium	0.26 a	0.30 a	0.31 a
High	0.26 a	0.28 a	0.29 a
HSD	0.05	0.06	0.05

Means with the same letter within each variable are statistically equal, according to Tukey's test ($P<0.05$). PBZ: paclobutrazol, CK: cytokinins, Pro-Ca: calcium prohexadione, Glu: glutamic acid, HSD: honest significant difference.

Analysis of variance for stomatal conductance (Gs) indicated that the treatment factor had no significant effect on the periods before and during application ($p>0.05$), confirming the absence of differences between means according to Tukey's test (Table 3). Prior to application, the values ranged from 0.25 to 0.28 mol H_2O m^{-2} s^{-1}, with a HSD of 0.05, implying that the observed variation between treatments was below the minimum threshold required to establish a significant difference. During application, the values fluctuated between 0.28 and 0.31 mol H_2O m^{-2} s^{-1} (HSD=0.06), with statistical homogeneity maintained among the treatments. These results indicate the experimental uniformity and baseline physiological stability of the stomatal system. In contrast, after application, ANOVA revealed a significant effect of the treatment factor ($p<0.05$) (Table 3). The paclobutrazol treatment resulted in the

highest value (0.36 mol H_2O m^{-2} s^{-1}), differing statistically from those of the cytokinins (0.30), prohexadione-Ca (0.29), and glutamic acid (0.26 mol H_2O m^{-2} s^{-1}), which together formed a statistically homogeneous group (HSD=0.05). The absolute difference between the maximum and minimum values was 0.10 mol H_2O m^{-2} s^{-1}, far exceeding the critical comparison value, which demonstrates a differential effect on stomatal regulation.

For the dose factor, no significant differences were observed at any of the three time points evaluated (p>0.05) (Table 3). Postapplication means ranged from 0.29 to 0.32 mol H_2O m^{-2} s^{-1} (HSD=0.05), indicating that the observed variation was statistically similar and that no dose-dependent response was detectable below the established significance level. From a comprehensive statistical perspective, the stomatal conductance response depended on the type of regulator only in the postapplication phase, with no interaction associated with concentration. The stability observed in the application phases reflects the absence of significant differences, which is due to a delayed response of the treatment on the regulation of stomatal opening (Table 3).

Table 4. Transpiration (E) before, during and after the application of growth regulators and glutamic acid in plants of the Malinche tomato hybrid. Source: the authors.

Treatment	Before	During	After
	mmol H_2O m^{-2} s^{-1}		
Control	9.79 b	11.83 a	11.44 a
PBZ	10.98 a	11.49 a	12.25 a
CK	10.21 a	11.81 a	11.60 ab
Pro-Ca	11.48 a	11.54 a	11.36 ab
Glu	10.53 a	11.68 a	10.44 b
HSD	1.55	1.49	1.66
Dose			
Low	11.35 a	11.86 a	11.95 a
Medium	10.92 ab	11.76 a	10.84 a
High	11.15 ab	11.08 a	11.49 a
HSD	1.55	1.49	1.66

Means with the same letter within each variable are statistically equal, according to Tukey's test (P<0.05). PBZ: paclobutrazol, CK: cytokinins, Pro-Ca: calcium prohexadione, Glu: glutamic acid, HSD: honest significant difference.

Analysis of variance for the transpiration rate (E) indicated that the treatment had no effect on the periods before and during application (p>0.05) (Table 4). In the pretreatment phase, the values fluctuated between 10.21 and 11.48 mmol H_2O m^{-2} s^{-1}, with a maximum difference of 1.27 units, below the critical comparison value (HSD=1.55), confirming that the observed variability remained within the experimental error. Similarly, during application, the means ranged between 11.49 and 11.81 mmol H_2O m^{-2} s^{-1} (HSD=1.49), indicating that there was no statistically significant difference between the treatment

means. In contrast, after application, ANOVA revealed a significant effect of the treatment factor ($p<0.05$) (Table 4). Paclobutrazol achieved the highest average value (12.25 mmol H_2O m^{-2} s^{-1}), significantly exceeding that of glutamic acid (10.44 mmol H_2O m^{-2} s^{-1}). The absolute difference between the two treatments was 1.81 mmol H_2O m^{-2} s^{-1}, a value that exceeds the significance threshold (HSD=1.66), confirming a statistical separation. Cytokinins (11.60 mmol H_2O m^{-2} s^{-1}) and Pro-Ca (11.36 mmol H_2O m^{-2} s^{-1}) did not significantly differ between the treatments classified as intermediate ($p<0.05$) according to Tukey's multiple comparison test.

For the dose factor, a significant effect was observed only before application ($p<0.05$) (Table 4). The control (9.79 mmol H_2O m^{-2} s^{-1}) had the lowest value and differed from the low dose (11.35 mmol H_2O m^{-2} s^{-1}), whose difference (1.56) was slightly greater than that of the HSD (1.55), demonstrating a statistically valid separation according to Tukey's criterion. The medium dose (10.92 mmol H_2O m^{-2} s^{-1}) and the high dose (11.15 mmol H_2O m^{-2} s^{-1}) showed intermediate behavior, not differing from the other levels. No significant differences were detected during or after application ($p>0.05$), indicating the absence of a dose-dependent effect in the experiment.

Structural stability was detected at two of the three time points evaluated, with significant differentiation occurring only in the later phase, which was attributable to the type of regulator applied. The mean separations exceeded the HSD when significance was established, supporting the statistical consistency of the model and demonstrating delayed physiological modulation of the transpiration response in the Malinche hybrid (Table 4).

DISCUSSION

The morphophysiological response observed in the Malinche hybrid confirms that exogenous modulation of the hormonal balance selectively altered vegetative growth and gas exchange without compromising reproductive stability under controlled hydroponic conditions. The significant reduction in plant height induced by paclobutrazol, as evidenced by the significant difference in the results of the Tukey test ($p<0.05$; HSD=5.5), coincides with the physiological mechanism described for gibberellin inhibitors, which restrict cell elongation by suppressing GA biosynthesis and modifying the plant canopy architecture (Rademacher, 2015; Yamaguchi, 2008).

Research conducted in protected systems has reported reductions of 10 to 25% in the height of tomato plants treated with growth regulators, without significantly affecting radial stem growth or the structural stability of the crop (Ohashi et al., 2022; Heuvelink et al., 2018). In the present study, the approximately 8.6% decrease compared with the control suggests a moderate response in the Malinche hybrid, indicating that hormonal regulation allowed compaction of the crop architecture without compromising its vegetative vigor.

In terms of net photosynthesis (A), the recorded values ranged from 34.9 to 39.9 μmol CO_2 m^{-2} s^{-1}, a range comparable to that reported for greenhouse tomatoes under optimal radiation and CO_2 conditions (30–40 μmol CO_2 m^{-2} s^{-1}) (Lawson; Vialet-Chabrand, 2019; Taiz et al., 2017). The absence of significant differences before and after application (Tukey, $p>0.05$) indicates functional stability of the photosynthetic apparatus; however, the significant difference during application (HSD=4.03), with the medium and high doses exceeding the control, demonstrates a transient physiological stimulus dependent on the applied concentration. This behavior aligns with research showing that nitrogenous biostimulants and amino acids can temporarily increase the photosynthetic rate by 8–18% by activating nitrogen metabolism and improving photochemical efficiency (Bano et al., 2022; Rouphael; Colla, 2020; Xu et al., 2012). In this context, glutamic acid may act as a key metabolic intermediate integrating nitrogen assimilation with photosynthetic processes and chlorophyll synthesis (Liao et al., 2022).

Stomatal conductance (Gs) ranged from 0.25 to 0.36 mol H_2O m^{-2} s^{-1}, which are values consistent with those of ecophysiological studies of tomatoes under protected cultivation (0.2–0.4 mol H_2O m^{-2} s^{-1}) (Guo et al., 2023; Lawson; Matthews, 2020). The statistically significant difference observed only after application (HSD=0.05) was greatest for paclobutrazol, suggesting a delayed response in terms of stomatal regulation. These studies indicate that the hormonal interaction between gibberellins and abscisic acid can progressively modify stomatal opening dynamics and foliar gas exchange (Anfang; Shani, 2021; Yue et al., 2022). Similarly, transpiration was between 10.44 and 12.25 mmol H_2O m^{-2} s^{-1}, a range comparable to that reported for hydroponic tomatoes grown in greenhouses (Yue et al., 2022).

In terms of yield, the average yield reached 8.95 kg $plant^{-1}$, which is equivalent to approximately 17–20 kg m^{-2} depending on the planting density, a value within the range reported for saladette tomatoes in intensive hydroponics (15–25 kg m^{-2}) (Yue et al., 2022; Heuvelink; Dorais, 2018). The absence of significant differences between treatments (Tukey's test, $p>0.05$) indicates the stability of reproductive partitioning. From an ecophysiological perspective, this behavior can be explained by the strong regulation of the source–sink relationship in indeterminate tomatoes, where the fruits act as dominant sinks of photoassimilates (Taiz et al., 2017). These results suggest that hormonal modulation induced specific physiological adjustments without significantly altering the productive balance of the system.

CONCLUSIONS

The integration of morphophysiological variables indicated that the agronomic performance of the Malinche hybrid was primarily associated with the interaction between gas exchange processes and yield components. The most consistent positive correlations were inferred between net photosynthesis (A), stomatal conductance (Gs), and transpiration (E), which are variables that reflect the efficiency of the stomatal system and its relationship with carbon assimilation. The application of cytokinins and glutamic acid increased photosynthetic activity and Gs, indicating a greater capacity for CO_2 fixation and

transpiration, which physiologically favors the production of photoassimilates destined for reproductive organs. This behavior indicates a positive functional association between the net photosynthesis rate and the number of fruits per plant, a component that contributes to the variation in final crop yield. In contrast, paclobutrazol was inversely related to plant height but did not negatively affect yield, indicating that canopy compaction did not limit the crop's photosynthetic capacity. In hydroponic systems, where mineral nutrition and water availability are maintained at optimal levels, this productive stability reflects important source–sink regulation that buffers the morphophysiological variations induced by growth regulators. The strongest positive correlations for physiological and agronomic performance are concentrated among photosynthesis, stomatal conductance, and the number of fruits per plant, which are variables that act as integrating axes of yield under controlled hormonal and nutritional management.

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Chapter 4

Influence of benzoxazinoid metabolites in maize roots interacting with diverse microbes

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ABSTRACT

The rhizosphere has been considered one of the fundamental interacting zones in ecological, chemical, and biological studies. Many plants root-soil microbe interactions occur in the rhizosphere, allowing communication between neighboring plant roots. The chemical reactions occurring in the soil depend greatly on the plant species and on the organisms that coexist around their roots. One of these fundamental processes is detoxification, mainly of potentially toxic secondary metabolites, such as benzoxazinoids (BX). These compounds are released in root exudates as defense against phytopathogens, allowing the selection of beneficial bacteria and the uptake of soil nutrients. Although BX are produced at basal levels, their production tends to increase in response to biotic stimuli. Since *Fusarium verticillioides* (*Fv*) is one of the main fungal pathogens affecting maize, in this chapter, we discuss BX detoxification as a possible evasion mechanism that *Fv* can display against plant defense systems. *Fv* can survive 2-benzoxazolinone (BOA), a metabolite present in root exudates, by transforming it into less toxic compounds, such as 2-aminophenol (AP), which facilitates the successful infection of root cells. Within the rhizosphere, plant growth-promoting rhizobacteria (PGPR) play a crucial role in plant roots and soil-microorganism interactions. They contribute to plant growth, fungi control and may even colonize plant tissues as endophytes. Our group studies the complex interactions among maize roots, *Fv* (a fungal pathogen), and PGPR. The role of PGPR in BX detoxification remains unclear; however, it has been hypothesized that they can participate in plant-bacteria communication during the selection and establishment of endophytic colonization. Transcriptomic data suggest that BX metabolism may be altered in interactions among plant roots, PGPR and *Fv*. Therefore, studying the metabolic strategies

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involved in the production and detoxification of BX in root exudates could provide valuable insights into how PGPR and phytopathogenic fungi coexist or compete in the rhizosphere.

Key words: benzoxazinoids, PGPR, *Fusarium*, bacteria, root exudates, metabolites

BENZOXAZINOIDS: KEY METABOLITES FOR PLANT GROWTH

Secondary metabolites are important for plant growth and defense. The molecules produced by plant secondary metabolism have allowed the development of many pharmaceuticals for human health treatment, and compounds used in agricultural management (Ono; Murata, 2023). The chemical structures of metabolites are highly diverse across plant species, leading to a wide range of biological functions, including antimicrobial and antioxidant activities. However, many of the biological functions of plant secondary metabolites are still undescribed (Twaij; Hasan, 2022).

Secondary metabolites can be classified according to their biological function and/or chemical structure. The major classes include terpenes, phenolics, nitrogen-containing compounds, and sulfur-containing compounds (Saxena et al., 2013). Terpenes are the principal constituents of essential oils and have important functions in plant cell walls and growth. They are hydrocarbons composed of isoprene units (C_5H_8) and can be modified by cyclization reactions (Khanam et al., 2025). Phenolic compounds include many derivative molecules such as flavonoids and non-flavonoids. They are classified according to the number of aromatic rings and their connection patterns. They also play crucial roles in plant responses to environmental and biotic stresses, as well as in the organoleptic properties of plant products (Xu; Wang, 2025). Nitrogen-containing compounds can be associated with peptides, amino acids, and nucleic acids. However, the most important N-containing secondary metabolites are alkaloids which exhibit antimicrobial and antioxidant properties and have great potential for industrial production in the pharmaceutical and biotechnological sectors (Zandavar; Babazad, 2023).

N-containing compounds include the benzoxazinoids (BXs) which are indole-derived and are found primarily in maize, wheat, and rye. BXs contain a 1,4-benzoxazin-3-one skeleton, also known as benzoxazinones, while benzoxazolinones are their degradation products. BXs are classified according to the nitrogen substituents: N-H, N-OH, and N-OCH_3 (de Bruijn et al., 2016; Li et al., 2025a) and are related primarily to plant defense against insects and phytopathogenic fungi. The BXs biosynthetic pathway has been extensively studied in various plant species, particularly in maize. This pathway begins primarily in the chloroplast (Figure 1), where the BX1 lyase enzyme catalyzes the conversion of indole-3-glycerol phosphate into indole, thereby initiating BX synthesis (Frey et al., 2000). Indole can enter the endoplasmic reticulum, where it is transformed into the 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) aglycone product through the sequential action of four monooxygenase enzymes (BX2-BX5). Subsequently, this product is glycosylated by UDP-glucosyltransferases (BX8/BX9), yielding the compound DIBOA-Glc (von Rad et al., 2001). DIBOA-Glc can follow two distinct metabolic routes:

the first involves transport to the vacuole of this compound, whereas the second route uses DIBOA-Glc as a substrate for cytoplasmic enzymes BX6, BX7, BX10/11/12, resulting in the formation of the final product HDMBOA-Glc. However, the production of this compound appears to be primarily induced by herbivory or pathogen attack (Glauser et al., 2011), as HDMBOA-Glc is more toxic than 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA)-Glc, even in the producing plant (Meihls et al., 2013). Due to their toxicity these compounds accumulate in the vacuole. The induction and release of the different glycosylated BXs depend on the severity of the infection. DIBOA-Glc and DIMBOA-Glc are considered constitutive, or basal defense molecules, whereas HDMBOA-Glc and HDM_2BOA-Glc represent high-level defense molecules (Oikawa et al., 2001; Handrick et al., 2016) (Figure 1). However, deglycosylation is required to generate the active forms of BX compounds stored in the vacuole and may occur either before or after their release into the rhizosphere in roots exudates.

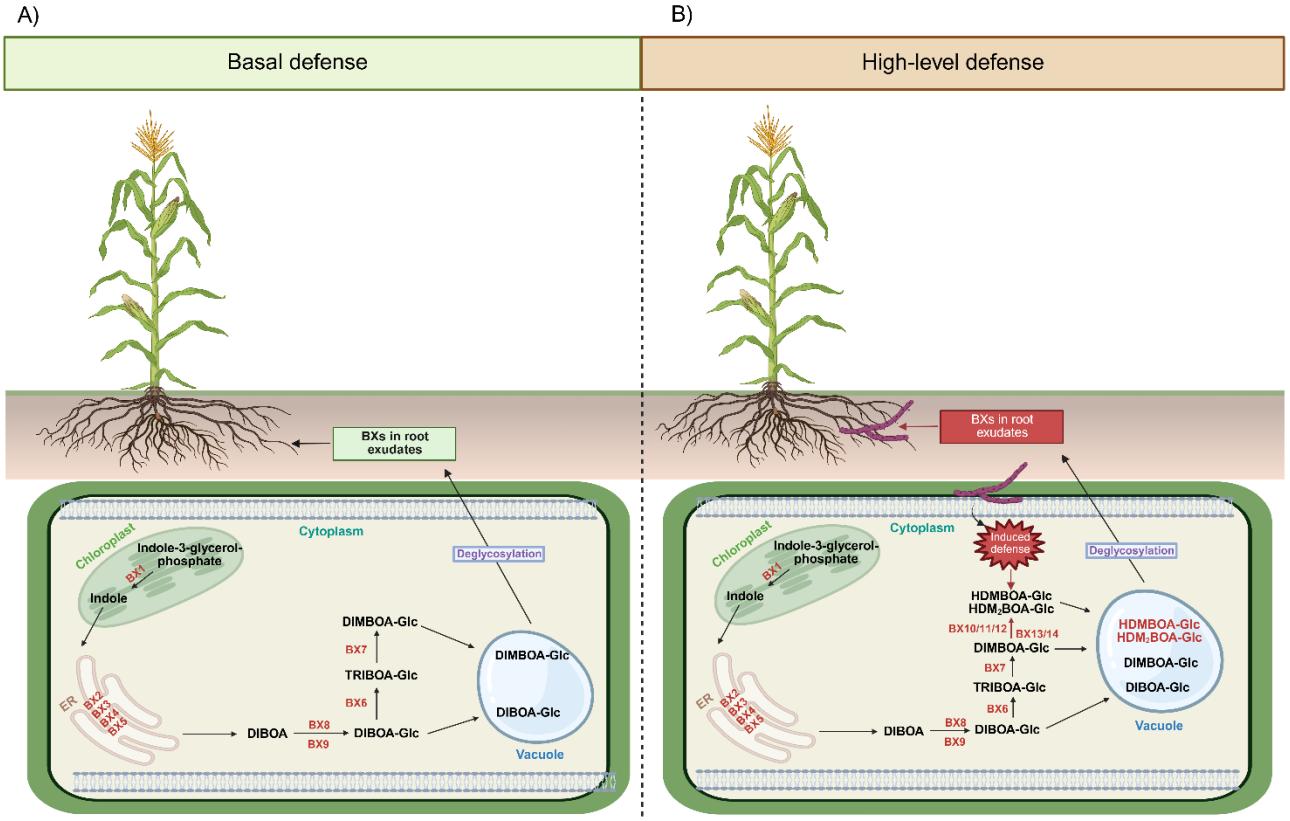


Figure 1. BXs biosynthesis model in A) basal and B) high-level defense against fungi. In response to pathogenic fungal attack, plants can induce the production of BX compounds, which exhibit increased antifungal activity. BioRender was used for the illustration.

Extensive studies have investigated the effects of BXs on phytopathogenic fungi, including reductions of *in vitro* mycelial growth of *Gaeumannomyces graminis* var. *graminis* and *Fusarium culmorum* when MBOA is used as a substrate (Martyniuk et al., 2006). Likewise, a reduction in the pathogenicity of *F. oxysporum* f. sp. *fabae* has been observed using DIMBOA and MBOA treatment (Ma et al., 2024).

Transcriptomic analyses have shown that BXs-related genes are induced in resistant wheat cultivars (04 Zhong 36) infected with *F. pseudograminearum*, and wheat metabolomic profiles mainly detected the accumulation of BOA and MBOA (Duan et al., 2022).

BXs are widely distributed in the Poaceae family species. However, the presence and amounts of each compound vary across plant species. For example, BXs are found in wheat grains but absent in mature maize seeds (Hanhineva et al., 2011). The presence of BXs in different tissues is also related to the production of their methylated products, such as DIBOA and DIMBOA (Rice et al., 2005). Cambier et al. (2000) detected high concentrations of DIMBOA-Glc and DIM_2BOA-Glc during maize seed germination, but the main BX product in roots was HDMBOA-Glc (N-O-methylated DIMBOA-Glc). Xu et al. (2021) demonstrated that benzoxazinoid biosynthetic pathway genes were differentially expressed between WT wheat and the quick development mutant (*qd*), which affects stem elongation. The results indicate that the mutant exhibits alterations in the BX biosynthesis pathway, with *bx1*, *bx3, bx4, bx5, bx8,* and *bx9* being differentially expressed between *qd* and WT, suggesting that this pathway plays an important role in stem regulation, which associates BX with the elongation phenotype. Park et al. (2004) found that BXs are released around the emerging lateral and crown roots of maize, suggesting that BXs are produced during maize growth to protect the plant against fungi. Furthermore, benzoxazolinones can inhibit auxin-induced growth in roots and coleoptiles by auxin-receptor binding affinity (Hasegawa et al*.,* 1992), suggesting that they can control plant growth indirectly. BXs also function as chelator compounds of iron, aluminum, and molybdenum; and it has been demonstrated that BX, especially DIMBOA-Glc and DIBOA-Glc, form complexes with Fe(III) (Farkas et al., 1998). In addition, Poschenrieder et al. (2005) investigated the potential involvement of BX compounds in aluminum tolerance in maize, finding that BX may contribute to aluminum resistance by forming complexes with Al^{3+}, reducing its phytotoxicity in root tissues. Therefore, BXs cannot be considered solely as metabolites involved in plant defense, but they can also play a role in maize nutrition, growth, and development.

ALLELOPATHIC PROCESSES INVOLVED IN BENZOXAZINOID PRODUCTION

BXs are primarily considered as defense metabolites (Niemeyer, 2009). As we have pointed out before, the main benzoxazinoid products, DIBOA and DIMBOA, are synthesized by enzymatic glycosylation, and can reach the extracellular environment or be stored in the vacuole (Frey et al., 2009). Upon plant root tissue damage or fungal attack, the aglycone forms are released as root exudates, where they act as potent allelochemicals. Allelopathy is a well-studied mechanism involving chemical interactions between plants or between plants and fungi, and can be mediated by secondary metabolites, which may negatively affect neighboring organisms (Meiners et al., 2012). Likewise, soil bacteria play a major role in controlling certain invasive species. Some bacteria can degrade allelochemicals that

accumulate in the soil, primarily released by invasive plants of the genus *Argeratina*. These bacteria can adapt and mitigate the effects of these allelochemicals (Li et al., 2015).

The potential of BXs to control weed growth in maize (Jabran, 2017) and wheat (Hussain et al., 2022) has been investigated. Similarly, this allelopathic mechanism has also been analyzed in various plant-microbe interactions. For example, arbuscular mycorrhizal fungi (AMF) colonization in maize has been shown to induce the production of diverse metabolites in root exudates, thereby reducing infection by *F. graminearum*, *F. oxysporum*, and *Rhizoctonia solani* (Ma et al., 2022). Belz and Hurle et al. (2005) demonstrated variations in the proportion of DIBOA and DIMBOA production in root exudates of different Triticeae species, which can inhibit the growth of the white mustard (*Sinapis alba* L.). The release and modification of BXs can mediate communication between plants and microorganisms by regulating plant growth and interactions with neighboring plants through altering the dynamics of the soil microbial community (Li et al., 2025b). Soil microbiota plays an important role in modifying BXs to produce various derivatives, such as phenoxazinones, acetamides, and malonic acids (Schulz et al., 2013).

Communication between plants and soil microorganisms is highly complex; however, root exudates are among the main mediators acting as signal molecules. These exudates strongly influence the rhizosphere microbiota, which, in turn, affects the release and composition of specific root exudates (Dowarah et al., 2025). These compounds enable the plant to recruit different bacterial species to its benefit; for example, leguminous plants can release exudates such as isoflavonoids, which, in turn, promote symbiotic relationships with nitrogen-fixing bacteria (Santoyo, 2022). Another important group of bacteria that directly interacts with plants is plant growth-promoting rhizobacteria (PGPR), which are recruited by plant root exudates. This communication is mediated by the exudates and by bacterial derived responses, such as movement towards the rhizoplane mediated by bacterial flagella and/or pili, as well as the production of extracellular proteins and lipopolysaccharides (Chen; Liu, 2024). PGPR-plant interactions are dynamic and possibly strain-specific, as plants modify the compound release patterns in their root exudates. Ankati et al. (2019) demonstrated that two PGPR strains, *Bacillus sonorensis* RS4 and *Pseudomonas aeruginosa* RP2, induced the production of diverse root exudate compounds in groundnut plants when inoculated either individually or in consortium. In the latter case, metabolites such as serine, pentanoic acid, glucopyranoside, tartaric acid, and 2-pyrrolidinone were detected by gas chromatography (GC) coupled to mass spectrometry (MS) [GC-MS], or MS analyses compared with the uninoculated control, indicating that PGPR have a significant influence on the release of metabolites in root exudates.

PRODUCTION OF BENZOXAZINOIDS IS NOT SUFFICIENT TO CONTROL *FUSARIUM VERTICILLIOIDES* INFECTION IN MAIZE

Fungal infections are one of the main problems affecting agriculturally important crops, including maize, rice, and wheat. It is estimated that around 3.5 million tons of maize grain are lost due to biotic

factors (Ávila et al., 2023). Among infectious agents of maize, *Fusarium* species are important. The life cycle of *Fusarium* in maize has been well studied (Ajmal et al., 2022; Li et al., 2025c). *Fusarium* spp. can survive in soil for up to 10 years as highly resistant conidia. It is considered a cosmopolitan organism, found in various regions of the world, primarily in soil, plant debris, seeds, and inside living plants. Furthermore, it is not exclusive to maize, as these fungi can infect various agriculturally important crops, including wheat, tomato, and potato (Alisaac et al., 2023; Srinivas et al., 2019; Xue et al., 2023).

However, the main problem with *Fusarium* is not its presence in the soil or its easy entry into the plant, but rather its potential to act as an endophytic fungus in maize cells, since it can colonize the root epidermis and vascular vessels (Leyva-Madrigal et al., 2014). Therefore, one of the main control methods is the use of chemical fungicides; however, these compounds can accumulate in soils, affecting microbial diversity and enzymatic activity (Wang et al., 2025). *Fusarium verticillioides* (*Fv*) has maintained a long-standing interaction with maize for thousands of years, co-evolving to become able to evade several plant defense mechanisms, including salicylic acid and jasmonate signaling (Wang et al., 2016), PAMP (Pathogen-Associated Molecular Patterns) recognition (Li et al., 2025d), modification of chitinases (Naumann et al., 2011), and benzoxazinoid detoxification (Gao et al., 2022) (Figure 2).

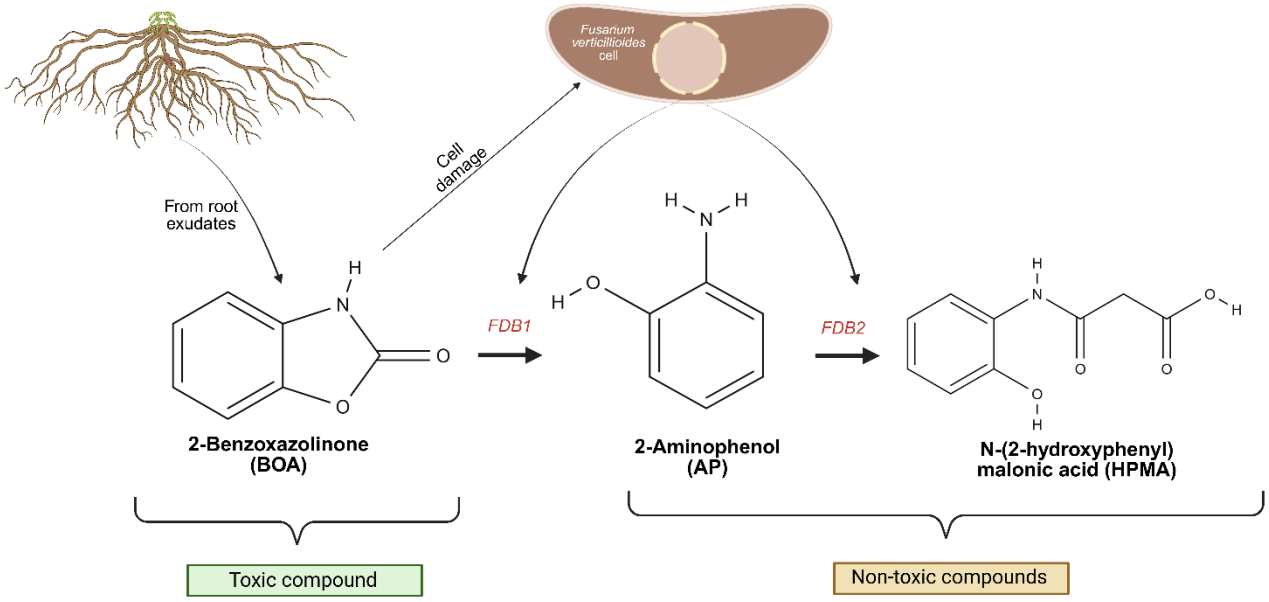


Figure 2. Detoxification pathway of maize BOA in *Fusarium verticillioides*. The first step involves the *FDB1* locus, which produces 2-aminophenol, a non-toxic compound. Furthermore, the *FDB2* locus participates in the transformation of 2-AP into malonic acids. BioRender was used for the illustration.

Fusarium verticillioides has demonstrated high tolerance to MBOA and BOA, transforming these compounds into non-toxic metabolites (Glenn et al., 2001). However, detoxification capacity varies among *Fusarium* species; *F. verticillioides*, *F. subglutinans*, *F. cerealis*, and *F. graminearum* are the most tolerant. The genes involved in BOA detoxification in *Fv* are in two major loci, FDB1 and FDB2 (Glenn et al.,

2002). These loci are not physically linked, but most of their genes are essential for detoxification. Among them, the *MBL1* gene (β-lactamase, *FVEG_08291*) from the *FDB1* locus and the *NAT1* gene (arylamine N-acetyltransferase, *FVEG_12636*) from the *FDB2* locus (Glenn; Bacon, 2009) are particularly important during BOA exposure (Glenn et al., 2016). Primarily, *FDB1* catalyzes the first step of BOA detoxification by producing 2-AP, followed by the involvement of *FDB2*, which leads to the production of malonamic acids (HPMA) [see Figure 2].

BENEFICIAL BACTERIA CAN INDUCE THE PRODUCTION OF MORE TOXIC BX COMPOUNDS TO CONTROL PHYTOPATHOGENIC FUNGI

From an ecological perspective, root exudation plays a key role in shaping interactions among rhizosphere organisms. Due to the high abundance of microorganisms and fungi in soil, plants can selectively recruit different microbial species to their benefit through root exudates. However, the interactions between rhizosphere bacteria and root-secreted benzoxazinoids remain poorly understood. BXs significantly influence soil microbial and fungal community composition (Kudjordjie et al., 2019), and their presence in the rhizosphere has enabled some microbial species to become tolerant to these compounds. For example, *Azospirillum brasilense* strain A-V5 can induce several genes in response to MBOA *in vitro*. These genes are primarily involved in transport, signal transduction, transcriptional regulation, and cellular respiration. Additionally, MBOA enhances biofilm formation, resulting in increased biomass associated with the *Arabidopsis thaliana* roots (Baatsen et al., 2025). Furthermore, rhizosphere bacteria adapt to the soil environment surrounding plant roots by metabolizing BXs. For instance, the *bxdA* gene of *Microbacterium*, which encodes an N-acyl homoserine lactonase, catalyzes the conversion of MBOA to AMPO *in vitro* (Thoenen et al., 2024). Notably, enzymes of this family are generally characterized by degrading N-acyl homoserine lactones, key quorum-sensing signaling molecules in bacteria (Morohoshi et al., 2009). Likewise, Neal et al. (2012) demonstrated that *Pseudomonas putida* is tolerant of DIMBOA and possesses the metabolic capacity to detoxify BXs. In addition, transcriptomic analyses revealed the induction of genes associated with benzoate catabolism and chemotaxis. The bacterial BX detoxification pathway is not yet fully elucidated; however, based on analyses of N-acyl homoserine lactonase enzymes, this gene family may be involved in bacterial adaptations to the rhizosphere environment. Such adaptations could occur through potential enzymatic coordination with multicopper laccases, as these enzymes have been reported to oxidize o-aminophenols (Sousa et al., 2021). During the maize-*Fv* interaction, the *BX6* and *BX8* genes are induced (Tran et al., 2021), along with other relevant BX-related genes, suggesting that this pathway plays an important role in controlling *Fv* infection.

Plant growth-promoting rhizobacteria (PGPR) have an important effect on plant growth and the control of *Fv* in maize. Species of the genera *Bacillus* (Cavaglieri et al., 2005), *Pseudomonas* (Nayaka et al.,

2009), and *Enterobacter* (Omotayo et al., 2023) have been reported to inhibit mycelial growth and reduce *Fusarium* root rot. *Bacillus cereus sensu lato B*25 is a rhizosphere bacterium that has been reported to act as a PGPR and to exhibit antagonistic activity against *Fv* (Lizárraga-Sánchez et al. 2015). Furthermore, during direct interactions between *B*25 and *Fv*, *B*25 induces several genes associated with antagonism, including chitinases, antibiotics, and siderophores (Báez-Astorga et al., 2022).

Transcriptomic analyses of root samples inoculated with either the bacterium *Bacillus cereus sensu lato B*25 or the fungus *Fusarium verticillioides*, or both at 7 days post inoculation, demonstrated that several BX biosynthetic genes are induced, mainly *bx2* (*Zm00001eb165620*), *bx5* (*Zm00001eb165580*), *bx8* (*Zm00001eb165590*), and *bx13* (*Zm00001eb116010*) (Báez-Astorga et al., 2025) (Table 1).

Table 1. Differentially Expressed Genes (DEGs) involved in the BX biosynthesis were identified in transcriptomic analyses of maize root samples in interaction with the PGPR *B*25, the fungal pathogen *Fv*, and both simultaneously.

ID	Name	FC (Fold change)		
		Maize-***B*25**	Maize-***Fv***	Maize-***B*25-*Fv***
Zm00001eb165620	BX2	2.55	2.85	4
Zm00001eb165580	BX5	2.53	2.04	2.45
Zm00001eb165590	BX8	14.12	10.78	4.79
Zm00001eb116010	BX13	2.38	2.48	2.68

NE: not expressed gene

It is noteworthy that the *Zm00001eb165620* gene (BX2) was highly induced in the tripartite interaction, suggesting that maize responds more rapidly to *Fv* infection. In contrast, the remaining BX-related genes were also induced across the other treatments, suggesting that the BX biosynthesis pathway is activated during the early stages of root development. Consistent with previous reports (Tran et al., 2021), a strong induction of *bx8* was observed in maize-*Fv* treatment. Notably, a similarly high level of *bx8* expression was detected in the maize-*B*25 interaction. This response may be attributed to the plant growth-promoting rhizobacteria activity of the *B*25 strain, which could prepare the plant by activating defense-related mechanisms in anticipation of potential pathogen attack. These findings suggest that BXs are involved in tripartite plant-microbial interactions. The study of BXs in these interactions may help elucidate novel mechanisms of molecular crosstalk between plants and their microbial partners when they interact simultaneously.

CONCLUSIONS

Interactions within the plant rhizosphere are highly complex. Hundreds or thousands of microorganisms may co-exist and compete for survival and reproduction in this environment. Root

exudates are an essential resource for many, particularly for bacteria that can colonize root tissues. Plant-bacteria communication has been extensively studied over the past decade; however, important questions remain regarding more complex interactions involving plants, beneficial bacteria, and pathogenic fungi.

Benzoxazinoids are secondary metabolites that play a crucial role in the early stages of plant-microbe communication. These compounds may contribute to the suppression of fungal infections, the recruitment and selection of beneficial bacteria, and possibly to plant growth and development. Nevertheless, the mechanisms by which PGPR perceive and respond to BX compounds remain poorly understood. Given their roles in plant defense against pathogenic fungi and in the induction of bacterial antagonistic genes, future research should explore whether bacterial detoxification of BX functions as a mechanism of plant-microbe communication and a strategy for fungal biocontrol.

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Chapter 5

Comparison of RGB spectrum vegetation indices to estimate agronomic variables in wheat and oats

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ABSTRACT

Vegetation indices (VIs) are key tools for gathering crop information; however, different vegetation indices are applicable depending on the crop and the data to be collected. The objective of this study is to compare 11 RGB vegetation indices and evaluate their effectiveness in estimating various agronomic characteristics. Orthophotos of five oat varieties and five wheat varieties obtained through programmed drone flights during the grain-filling stage were analyzed. The 11 RGB vegetation indices were obtained from the orthophoto data and compared with the following agronomic data: days to heading (DH), days to physiological maturity (DPM), and grain yield (GY). The Pearson and Spearman correlation coefficients strongly positively correlated between the DPM and the vegetation indices MPRI, vDVI and GCC and negatively correlated with the PRI and RCC. These indices were most strongly correlated with the evaluated agronomic variables, which could indicate a high level of reliability for future estimates of these agronomic characteristics.

INTRODUCTION

Within the framework of precision agriculture (PA), vegetation indices (VIs) are among the most versatile tools for crop monitoring. VIs are numerical values that are calculated from the reflectance of different bands of the electromagnetic spectrum (mainly infrared and visible light), and their main function is to measure various variables during crop development to monitor their status throughout the

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entire production cycle (Solis-Pino et al., 2021). VIs can be qualitative or quantitative and have been developed to increase the efficiency of monitoring the development of production cycles, helping to estimate a wide variety of factors related to yield and biomass production. In this way, they can be used to determine the influence of these factors in a given area and period on agricultural production, considering the different phenological stages of the crop (Rubio et al., 2020). They are especially useful for monitoring high-demand crops such as cereals (Sierra et al., 2024).

Plant phenology can be defined as the periodic cycles of seasonal biological activities and how they are related to environmental and edaphoclimatic conditions, such as solar radiation, humidity, wind, temperature, and soil conditions. Different types of crops respond differently to these conditions (Girimonte; García, 2020). Different types of vegetation indices (VIs) are used depending on the data required from the crops. The normalized difference vegetation index (NDVI) is the best known of those derived from optical remote sensing and is formulated using the following equation: NDVI = (NIR − R)/(NIR + R), where R is the reflectance in the red band and NIR is the infrared reflectance (Marín et al., 2020). It is the most widely used method because it retains the ability to minimize topographic effects and produces a linear measurement scale. Their measurements are stable, allowing meaningful comparisons of changes in crop growth and activity (Rubio et al., 2020). An alternative to indices that use the infrared region are those obtained with visible light sensors, such as red, green, and blue (RGB) indices.

In particular, the use of aerial images captured by unmanned aerial vehicles (UAVs) has facilitated the application of RGB spectral indices for the discrimination and evaluation of crop status (Gallego et al., 2024). This also provides precise, high-spatial-resolution information, allowing for the monitoring of vegetation conditions and their updated form changes (Buendía-Rodríguez, 2024). Conventional visible light sensors typically have only three bands: red, green, and blue. This limited number of bands means that the information obtainable from RGB indices is limited and that they are primarily used for basic image capture. However, owing to their lower costs, ease of production, and relatively simple data processing, the use of RGB image data is often an alternative to more expensive multispectral and hyperspectral images (Hu et al., 2024).

Vegetation characteristics can be distinguished using vegetation indices (VIs), which provide a dataset derived from cross-band operations on remote sensing images, from which various RGB vegetation indices can be obtained (Chuvieco, 2010; Reddy and Reddy, 2013; Villamil et al., 2020). However, to optimize the information obtained from vegetation indices, it is necessary to use an appropriate index according to the type of crop and the variables to be studied. Research has shown that compared with individual indices, the collective performance of different vegetation indices is more stable and reliable when monitoring pests and certain crop stresses (Hu et al., 2024).

MATERIALS AND METHODS

Experiment Location

The experiments were conducted at the Bajío Experimental Field (CEBAJ) of INIFAP, which is located at km 6.5 on the Celaya–San Miguel de Allende highway in Celaya, Guanajuato, with geographic coordinates 20° 32' N; 100° 48' W, at an altitude of 1765 m above sea level, during the 2021–2022 fall-winter (F-W) production cycle.

Experimental Design

The experiment was established during the 2021–2022 fall–winter growing season. The trials were established using the same experimental design for both seasons in a 6×6 triple-lattice pattern with three replications per irrigation schedule. Sowing was performed at a density of 120 kg ha^{-1} of seed. The experimental and usable plot consisted of two 2.75 m long rows sown in double rows, separated by 75 cm, for a total area of 4.125 m^2.

Genetic Material

Five wheat genotypes (Table 1) and five commercial varieties of oats (Table 2) were used, which were provided by the INIFAP Bajío experimental field.

Table 1. Wheat genotypes used and their variety numbers. Source: The authors.

No. Variety	Variety Name	Prodution cicle
1	CORTAZAR S94	F-W 2021-2022
2	ALONDRA F2014	F-W 2021-2022
3	FAISAN S2016	F-W 2021-2022
4	LUMINARIA F2012	F-W 2021-2022
5	CISNE F2016	F-W 2021-2022

Table 2. Oat genotypes used and their variety numbers. Source: The authors.

No. Variety	Variety Name	Prodution cicle
1	ÁGATA	F-W 2021-2022
2	CHIHUAHUA	F-W 2021-2022
3	JADE	F-W 2021-2022
4	KARMA	F-W 2021-2022
5	TURQUESA	F-W 2021-2022

Generation of RGB orthophotos

Photographic sampling was carried out using a DJI Phantom Pro 3® UAV as follows: Geographic sampling consisted of georeferencing the area to be evaluated through programmed flights using the DJI Pilot® mobile application, which is compatible with the DJI Phantom Pro 3® UAV.

Weekly photographic sampling. The parameters used for the programmed flights with the UAV were 25 m above ground level at a constant speed of 5 m/s, with automatic capture of photographs every two seconds.

Orthophoto creation. The orthophotos were created using individual images obtained from weekly flights with Pix4D® software. Weekly photographic sampling was performed on the scheduled weekly flights with the UAV to take photographs of the four irrigation calendars from 26 days after planting until harvest.

Orthoimage Production. During the weekly photographic sampling, an image database was created for processing and obtaining the orthoimages (Figure 1). The images obtained were analyzed individually using ImageJ software to obtain the 12 vegetation indices.



Figure 1. Orthoimages of the four irrigation calendars. Source: The authors.

Evaluation of Agronomic Characteristics

Days to Heading (DH): This variable was quantified in days, starting from the initial irrigation at planting until 50% of the plants within each experimental plot had fully exposed their ears. This parameter is essential for understanding crop development, as it reflects the transition from vegetative to reproductive stages and allows comparisons of phenological behavior among genotypes.

Days to Physiological Maturity (DPM): This was determined as the number of days elapsed from planting irrigation until 50% of the ears and peduncles exhibited a characteristic golden coloration, indicating the end of grain filling and the attainment of maximum dry matter accumulation. This variable

is particularly relevant for assessing crop cycle duration and potential adaptation to environmental conditions.

Grain yield (GY): Grain yield data were collected in grams per experimental unit and subsequently converted to kg ha^{-1} to standardize the results and facilitate comparisons. This variable represents the final productivity of each genotype and integrates the effects of all the agronomic and environmental factors throughout the crop cycle.

Vegetation indices: The vegetation indices (VIs), presented in Table 3, were calculated using specific formulas referenced for each index. These indices are derived from reflectance values in the RGB spectrum and are widely used to estimate plant vigor, biomass, and overall crop health. Their inclusion allows for a nondestructive and efficient evaluation of crop performance over time.

Statistical Analysis: The images captured by the UAV were processed using ImageJ software, which incorporates the RGB Measure plugin to extract basic descriptive statistics for each image. RGB-based vegetation indices were subsequently calculated using the mean reflectance values of all pixels within each image, following the formulas provided in Table 3. This approach enables a comprehensive quantitative analysis of spatial variability and crop conditions, strengthening the interpretation of agronomic and spectral data.

Table 3. Formulas used for generating the RGB spectrum vegetation indices. Source: The authors.

Name	Abbreviation	Formula
Green Leaf Index	GLI	(2G-R-B)/(2G+R+B)
Normalized Green Blue Difference Index	NGBDI	(G-B)/(G+B)
Normalized Green Red Difference Index	NGRDI	(G-R)/(G+R)
Photochemical Reflectance Index	PRI	R/G
Triangular Greenness Index	TGI	G-0.39*R-0.61*B
Modified Photochemical Reflectance Index	MPRI	(G-R)/(G+R)
Visible Band Difference Vegetation Index	VDVI	(2G-R-B)/(2G+R+B)
Green Percentage Index	GCC	G/(R+G+B)
Blue and Red Vegetation Index	BRVI	(B-R)/(B+R)
Blue Chromaticity Coordinate Index	BCCI	B/(R+G+B)
Red Chromaticity Coordinate Index	RCCI	R/(R+G+B)

The results obtained with the 11 vegetation indices were correlated with the agronomic data used (DH, DPM, and GY) using Pearson and Spearman correlations. Owing to the sample size, the Spearman correlation index was used to compare the results of the Pearson correlation analysis, and heatmaps were generated using RStudio 4.2.2 software (R Core Team, 2022).

RESULTS AND DISCUSSION

To obtain the 11 vegetation indices from the RGB spectrum, four orthomosaics—one from each image—were generated and analyzed for both crops. The indices were subsequently obtained from these orthomosaics using ImageJ software.

A graphical representation of the 11 vegetation indices of the RGB spectrum evaluated for both wheat and oat crops are shown in Figure 2.

The Pearson correlations between the vegetation indices and agronomic variables during oat cultivation are shown in Figure 3a. No correlation was observed between the yield and vegetation indices. However, the days to maturity variable was strongly positively correlated with the vegetation indices GLI, NGRDI, MPRI, vDVI, and GCC and strongly negatively correlated with the PRI. The BRVI was the only index that showed no correlation with other indices or agronomic variables. The lack of correlation between vegetation indices and grain yield differs from what has been reported by other authors, such as Buenavista-Jaralillo et al. (2025), where a positive correlation between yield and the GLI was observed.

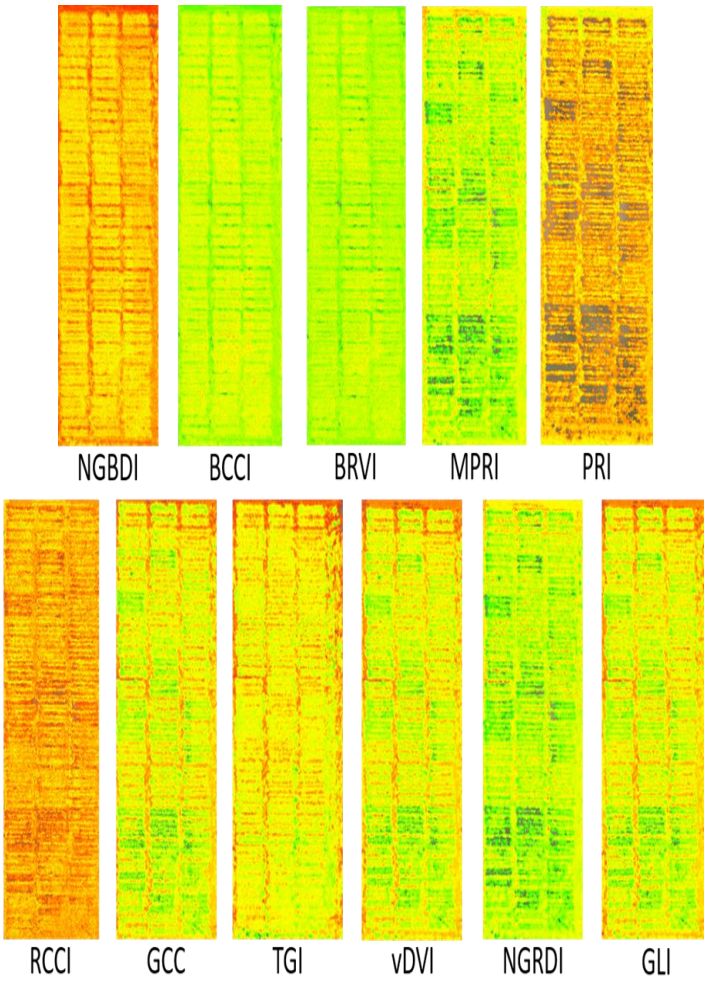


Figure 2. Graphical representation of the 12 evaluated indices of the RGB spectrum. Source: The authors.

According to the results of the Pearson correlation analysis for the vegetation indices and agronomic variables related to wheat cultivation, the agronomic variable of days to maturity is positively correlated with the vegetation indices NGRDI (0.93) and MPRI (0.93) and negatively correlated with the PRI (-0.93) and RCCI (-0.95), whereas it is not correlated with the GLI, vDVI, or GCC indices, unlike what was observed for oat cultivation (Figure 3b). Furthermore, no correlation was detected between the BRVI and the agronomic variables of days to heading and grain yield or between the other vegetation indices and the agronomic variable of days to maturity.

With respect to oat cultivation, in the Spearman correlation analysis, no correlation was observed between the agronomic variables and the vegetation indices. Similarly, the correlation between the BRVI and the rest of the indices and variables remained zero, whereas the remaining indices were strongly positively correlated and negatively correlated only with the PRI and BCCI, as shown in Figure 3c.

The results of the Spearman correlation analysis between vegetation indices and agronomic variables for wheat cultivation are shown in Figure 3d. Consistent with previous results, the BRVI was the only index that was not correlated with the other vegetation indices or agronomic variables; thus, it was ruled out for grain yield estimation. Similarly, the only variable showing strong positive correlations was days to maturity, with the NGRDI (1) and MPRI (1) indices and a negative correlation with the RCCI (-1).

In terms of oat cultivation, genotype three resulted in the highest yield; however, genotype CISNE F2016 was the most consistent in terms of higher expression of the evaluated agronomic variables, as well as the highest vegetation indices. Conversely, genotype two stood out negatively by not showing high expression for any of the evaluated variables, including the vegetation indices, as shown in the heatmap of Figure 4a.

In terms of oat cultivation, the TURQUESA genotype presented the highest values for the vegetation indices NGRDI, MPRI, GLI, vDVI, and GCC (Figure 4a).

The heatmap in Figure 4b shows that in the wheat crop, genotype CORTAZAR S94 was the most consistent in terms of the highest vegetation indices and had the greatest expression of the agronomic variables of days to heading and days to maturity; however, genotype LUMINARIA F2012 had the highest grain yield, in addition to having the highest RCCI and PRI.

Additionally, these findings highlight the importance of selecting appropriate vegetation indices depending on the crop type and the agronomic variable of interest. The contrasting behavior observed between wheat and oat suggests that physiological and phenological differences between species influence the sensitivity of spectral indices. These findings reinforce the need for crop-specific calibrations when remote sensing tools are used in precision agriculture. Moreover, the lack of correlation in certain indices, such as the BRVI, indicates that not all spectral information is equally useful for yield prediction, emphasizing the importance of careful variable selection. Overall, these results provide a

better understanding of how vegetation indices can support decision-making in terms of crop monitoring and management practices.

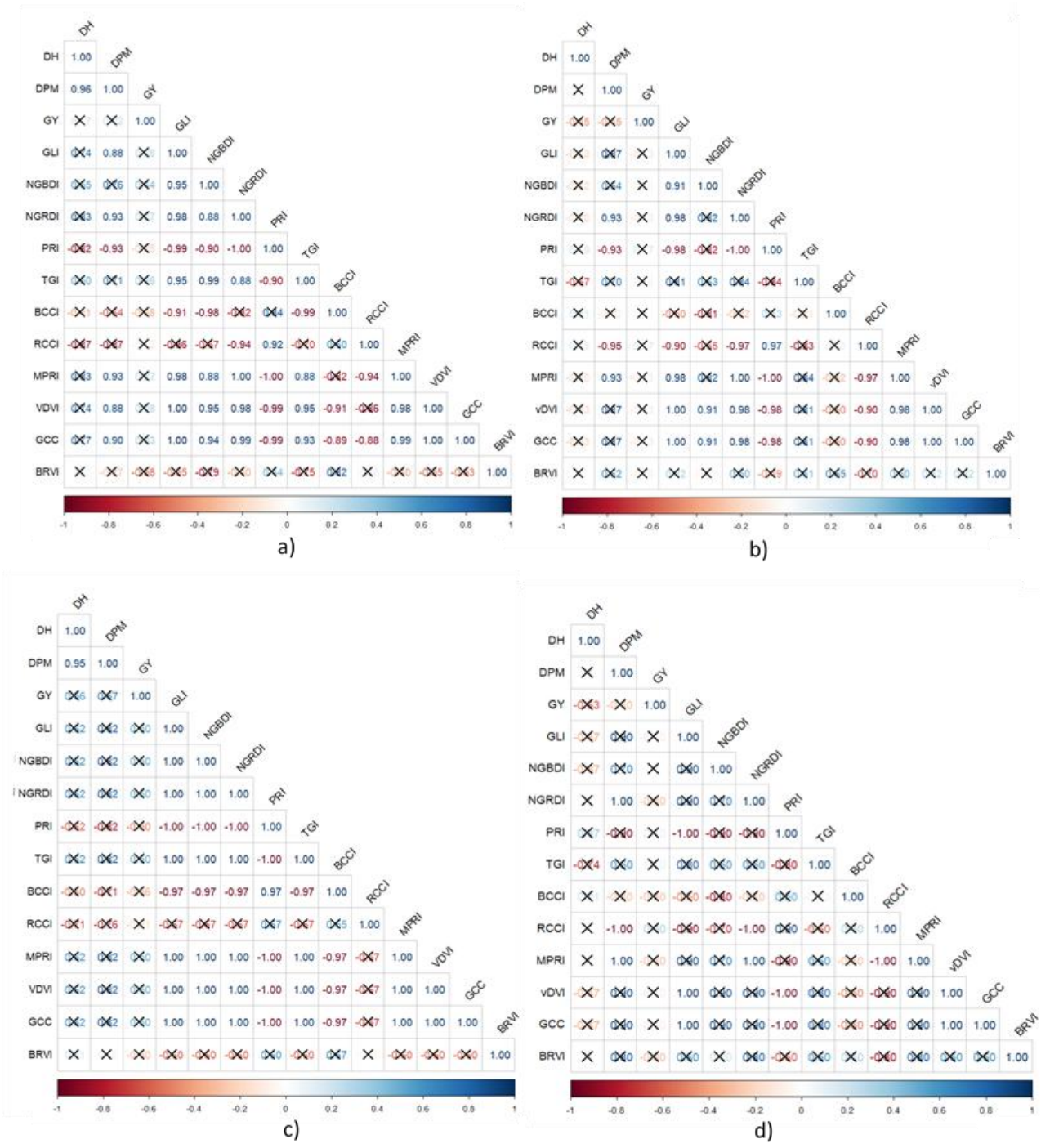


Figure 3. **a)** Pearson correlation graph of vegetation indices and agronomic variables during oat cultivation. **b)** Pearson correlation graph of vegetation indices and agronomic variables in response to wheat cultivation. **c)** Spearman correlation graph of vegetation indices and agronomic variables during oat cultivation. **d)** Spearman correlation graph of vegetation indices and agronomic variables in response to wheat cultivation. Source: The authors.

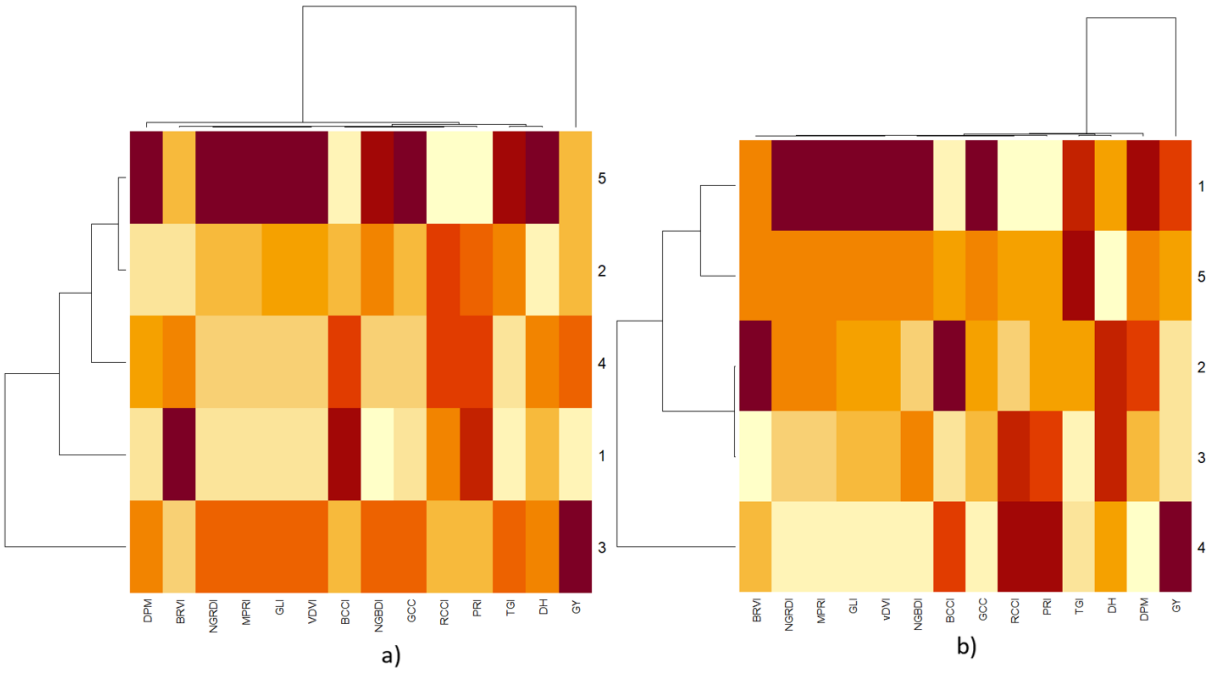


Figure 4. a) Heatmap of the expression of the variables evaluated in the oat crop. b) Heatmap of the expression of the variables evaluated in the oat crop. Source: The authors.

CONCLUSIONS

The results of the Pearson and Spearman correlation statistical analysis revealed a strong positive correlation between the agronomic variable of days to maturity and the vegetation indices NGRDI and MPRI and a negative correlation with the PRI in both oat and wheat crops. These findings suggest that these indices are suitable for predicting this variable. The BRVI was not correlated with the agronomic variables or vegetation indices for either crop, ruling out it as an option for predicting the evaluated agronomic variables.

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Chapter 6

Alleviation of saline stress in maize and peanut plants induced by *Bacillus aryabhattai* strain CMAA 1363

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ABSTRACT

Salinity caused by the excess dissolved salts in the soil solution or irrigation water is among the abiotic stresses that most limit the development and yield of agricultural crops. This study was conducted with the objective of evaluating the effect of inoculation of the *Bacillus aryabhattai* strain CMAA 1363 on the induction of tolerance in maize (*Zea mays* L.) and peanut (*Arachis hypogaea* L.) plants grown under high-salinity conditions. Two bioassays were conducted to investigate the effects of *Bacillus aryabhattai* strain CMAA 1363 on the growth and vigor of maize (Bioassay 1) and peanut (Bioassay 2) plants grown under different salinity levels. The two bioassays were arranged in a completely randomized experimental design in a 2 × 4 factorial scheme with four replicates of 50 seeds. The studies consisted of two microbial treatments (noninoculated plants (control) and plants inoculated with *B. aryabhattai* strain CMAA 1363) and four levels of irrigation water salinity [0.0 MPa (control), –0.1 MPa (mild saline stress), –0.4 MPa (moderate saline stress) and –0.8 MPa (severe saline stress)]. The germination capacity (GC), germination speed index (GSI), mean germination time (MGT), mean germination rate (MGR) and early growth of the maize and peanut plants were measured. Inoculation with the *Bacillus aryabhattai* strain CMAA 1363 effectively mitigated the negative effects of salt stress on maize and peanut plants. These findings indicate that inoculation with *B. aryabhattai* in maize and peanut plants may be a sustainable alternative to enhance

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the development of maize and peanut crops under saline soil conditions or with the use of saline water for irrigation.
Keywords: Salinity; agricultural crops; *Zea mays* L.; *Arachis hypogea* L.

INTRODUÇÃO

Salinity caused by the excess dissolved salts in the soil solution or irrigation water is among the abiotic stresses that most limit the development and yield of agricultural crops (Zuffo et al., 2020; Atta et al., 2023; Khasanov et al., 2023). Currently, approximately 40% of the world's food is produced in irrigated areas, with Brazil being the sixth largest producer of food in irrigated areas in the world (i.e., 8.5 million hectares irrigated) (FAO, 2023). However, approximately 20–25% of the world's irrigated areas suffer from soil salinization (Hassan et al., 2021; Khasanov et al., 2023). Soil salinization has intensified in recent years because of the inappropriate and excessive use of mineral fertilizers, inadequate irrigation and drainage management, and irrigation with saline water (Stavi et al., 2021). In Brazil, soil salinity is more common in the arid and semiarid regions of the northeast and in the Pantanal region (Pessoa et al., 2022).

The effects of soil or irrigation water salinity on plants can occur directly and indirectly. Salinity stress directly affects plant growth through osmotic, ionic, and oxidative effects (Acosta-Motos et al., 2017; Taiz et al., 2017). The osmotic effect reduces the water potential of the soil solution, delaying the germination rate and plant growth because of limited water absorption by seeds and plants (Acosta-Motos et al., 2017; Zuffo et al., 2020). Ionic and oxidative effects result from the accumulation of specific phytotoxic ions that increase the production of reactive oxygen species (ROS), ionic imbalance of the cytosol, damage to the functions of cell membranes and walls, and interference in the physiological processes of plants (indirect effects) (Acosta-Motos et al., 2017; Zelm et al., 2020; Gong, 2021).

The response of plants to saline stress includes a series of biochemical, physiological, and morphological changes (Acosta-Motos et al., 2017; Kumar et al., 2020; Gao et al., 2022), which constitute the strategies and/or mechanisms of plant tolerance to salinity. The main tolerance strategies for mitigating saline stress are associated with increased water use efficiency, the production of plant hormones, osmotic adjustment, the production of antioxidant enzymes, and the activation of cellular signaling and messengers that improve plant metabolic reactions under saline conditions (Numan et al., 2018). These strategies aim to maintain plant growth and development under saline conditions. However, the plant response depends on the severity of the stress and the plant species or genotype (Taiz et al., 2017). Maize (*Zea mays* L.) and peanut (*Arachis hypogea* L.) plants are considered moderately salinity-tolerant crops, with soil salinity thresholds of 1.7 and 3.2 dS m^{-1}, respectively (Gheyi et al., 2016). However, relatively high salinity levels can drastically affect plant germination and growth.

Among the strategies used to mitigate the adverse effects induced by salinity, the application of biofertilizers based on plant growth-promoting rhizobacteria (PGPR) has been used to improve the germination uniformity and plant growth rate under saline stress conditions (Numan et al., 2018; Kumar et al., 2020; Mishra et al., 2021; Gao et al., 2022). Some PGPR possess specialized mechanisms that play key roles in salinity tolerance and plant growth stimulation. These bacteria improve hormonal and nutritional metabolism, osmotic regulation, signal transduction, and the synthesis of volatile organic compounds (acetoin and 2,3-butanediol), which can stimulate plant growth, especially in the root system, and improve the recovery capacity of seedlings when they are exposed to saline stress (Numan et al., 2018; Sarkar et al., 2018; Ayaz et al., 2022).

Among the PGPR with the potential to mitigate the adverse effects of salinity, species of the genus *Bacillus* have been extensively studied for the inoculation of some agricultural plants (Liu et al., 2017; Ferreira et al., 2018; Ansari et al., 2019; Ibarra-Villarreal et al., 2021; Ayaz et al., 2022). Upadhyay & Singh (2015) reported that the application of *Bacillus aquimaris* increased nutrient uptake by wheat plants, resulting in greater tolerance to saline conditions. Ansari et al. (2019) reported that inoculation with *B. pumilus* stimulated plant growth and alleviated saline stress in wheat plants. Similarly, Ferreira et al. (2018) reported that inoculation with *B. subtilis* improved plant growth and attenuated the biochemical damage induced by saline stress in maize plants. Other studies have also proven the effectiveness of using *B. pumilus* in rice plants (Khan et al., 2016), *B. paralicheniformis* in wheat plants (Ibarra-Villarreal et al., 2021), and *B. amyloliquefaciens* in *Arabidopsis thaliana* plants (Liu et al., 2017) and rice (Nautiyal et al., 2013) in improving plant tolerance to salinity. However, the effects of *Bacillus aryabatthai* inoculation on the induction of plant tolerance to salinity are still unclear.

This study was conducted with the objective of evaluating the effect of inoculation of the *Bacillus aryabhattai* strain CMAA 1363 on the induction of tolerance in maize (*Zea mays* L.) and peanut (*Arachis hypogaea* L.) plants grown under high-salinity conditions.

MATERIAL E MÉTODOS

Plant material and treatments

Two bioassays were conducted at the Laboratory of Plant Ecophysiology and Sustainable Agriculture (L-PESA), State University of Mato Grosso do Sul, Cassilândia, Mato Grosso do Sul, Brazil, to investigate the effects of *Bacillus aryabhattai* strain CMAA 1363 on the growth and vigor of maize (Bioassay 1) and peanut (Bioassay 2) plants grown under different salinity levels. Seeds from commercial maize and peanut genotypes were purchased from agricultural retailers in the municipality of Chapadão do Sul, Mato Grosso do Sul, Brazil. These genotypes were chosen because they are widely recommended for cultivation in the Brazilian Cerrado region. The main characteristics of the maize and peanut seeds are shown in Table 1.

Table 1. Water content, thousand-seed mass and germination rate of the maize and peanut seeds used in this study

Crop	Cultivar	Water content (%)	Thousand seed mass (g)	Germination (%)
Maize	DKB 360 PRO3	11.58	242	100
Peanut	IAC 503	6.30	778	94

Microorganisms

The *B. aryabhattai* strain CMAA 1363 used in this study originated from the commercial inoculant Auras®, a product licensed by Embrapa and marketed by NOOA Ciência e Tecnologia Agrícola and registered in the Ministry of Agriculture, Livestock and Food Supply—MAPA under Nº 0007579000003. This bacterial strain was initially isolated from the mandacaru root in the Brazilian caatinga biome and belongs to the microorganism collection of Embrapa Meio Ambiente (Kavamura et al., 2017). For inoculum development, the commercial strain CMAA 1363 was grown in Petri dishes (9.0 × 1.5 cm) with solid Luria Bertani (LB) culture medium (10 g L^{-1} peptone, 5 g L^{-1} yeast extract, 5 g L^{-1} NaCl, 15 g L^{-1} agar; pH 7.0) for 72 h at 28 ± 2 °C, 60% relative humidity and a 12 h photoperiod. After the incubation period, the concentration of the strain CMAA 1363 was evaluated using the serial dilution method and standardized to 1.0 × 10^9 colony forming units per milliliter (CFU mL^{-1}).

Experimental Design and Treatments

The two bioassays were arranged in a completely randomized experimental design in a 2 × 4 factorial scheme with four replicates of 50 seeds. The studies consisted of two microbial treatments (noninoculated plants (control) and plants inoculated with *B. aryabhattai* strain CMAA 1363) and four levels of irrigation water salinity [0.0 MPa (control), –0.1 MPa (mild saline stress), –0.4 MPa (moderate saline stress) and –0.8 MPa (severe saline stress)].

The strains of *B. aryabhattai* CMAA 1363 were applied to the sowing furrow at a dosage of 2.0 L ha^{-1} of the suspension containing the microbial inoculum (100 µL of microbial solution per meter of sowing furrow) at a concentration of 1.0 × 10^9 CFU mL^{-1}. The solution containing the microbial inoculum was applied to the seeding furrow using a micropipette and adjusted to apply 6.0 mL of solution per meter of furrow (equivalent to 120 L ha^{-1} of spray volume).

The plants were irrigated daily with iso-osmotic solutions of 0.0, –0.10, –0.40 and –0.80 MPa prepared with sodium chloride (NaCl). The amount of NaCl added to obtain saline solutions with distinct levels of osmotic pressure was calculated by the van't Hoff equation (Hillel, 1971): $\Psi s = -RTCi$, where ΨS is the osmotic pressure (MPa), R is the universal constant of noble gas (0.008314 MPa mol^{-1} K^{-1}), T is the absolute temperature (273.15 + °C), C is the molar concentration of the solute (mol L^{-1}) and i is

the van't Hoff factor, that is, the number of ions released when the solute is dissolved in water [i.e., for NaCl, this value is 2.0 (Na^+ and Cl^-).

Plant Growth Conditions

The seeds were sown in plastic containers (44 × 30 × 7.5 cm) filled with sterilized quartz sand at a depth of 2.0 cm. The sand was sieved through a mesh with a diameter of 0.05 to 0.8 mm and then moistened with different solutions at 70% of the substrate's water retention capacity (BRASIL, 2009), which was equivalent to 150 mL kg^{-1} aqueous solution. The plastic containers were kept under laboratory conditions using artificial light supplemented with red (620–630 nm) and blue (455–475 nm) wavelengths at ratios of 85% (red) and 15% (blue) from light-emitting diodes (LEDs) at a 180 ± 50 μmol m^{-2} s^{-1} light intensity, a 12/12 h photoperiod (light/darkness), and a temperature of 25.4 °C (± 2.1 °C) for 18 days. The substrate moisture content was monitored daily at 3:00 p.m. using the gravimetric method by weighing the plastic containers. The moisture content of the substrate was maintained at 70% of the water retention capacity of the sand through daily irrigation.

Measurements of Germination and Seedling Growth

The germination capacity (GC), germination speed index (GSI), mean germination time (MGT), mean germination rate (MGR) and early growth of the maize and peanut plants were measured. Equations 1–5 and the parameters therein were employed to express the seed germination and plant vigor index.

$$GC\ (\%) = S_{NG}/S_{N0} \times 100 \quad \text{[Eq. 1]}$$

where GC is the germination capacity, S_{NG} is the number of germinated seeds, and S_{N0} is the number of viable experimental seeds (50 seeds).

$$GSI = \Sigma(n_i/t_i) \quad \text{[Eq. 2]}$$

where GSI is the germination speed index (seed day^{-1}), n_i is the number of germinated seeds on a given day, and t_i is the time in days from the sowing day (Maguire, 1962).

$$MGT = (\Sigma n_i t_i)/\Sigma n_i \quad \text{[Eq. 3]}$$

where MGT is the mean germination time (day), n_i is the number of germinated seeds on a given day, and t_i is the time in days from the sowing day (Laboriau, 1983).

$$LVI = [TL(m) \times GC\ (\%) \quad \text{[Eq. 4]}$$

where LVI is the plant length vigor index, TL is the total plant length and GC is the seed germination capacity (Abdul-Baki and Anderson, 1973).

$$WVI = TDM(g) \times GC\ (\%) \quad \text{[Eq. 5]}$$

where WVI is the plant weight vigor index, RDM is the total dry matter and GC is the seed germination capacity (Abdul-Baki and Anderson, 1973).

After 18 days of exposure to salinity stress, ten seedlings per replicate were randomly chosen to measure the length and dry matter of the shoot and roots. The shoot length (SL), root length (RL), and total plant length (TL) were measured using a meter scale. The shoot dry matter (SDM), root dry matter (RDM), and total plant dry matter (TDM) were recorded on an analytical balance (±0.0001 g) after oven drying at 85 °C for 48 h.

Statistical analysis

The data were previously tested for homoscedasticity of variances (Levene test; $p > 0.05$) and normality of residues (Kolmogorov–Smirnov test; $p > 0.05$) and then subjected to analysis of variance (ANOVA). The means of the treatments were compared by the Tukey test at the 0.05 level of confidence. The analyses were performed using Rbio® software version 140 for Windows (Rbio Software, UFV, Viçosa, MG, BRA).

RESULTADOS E DISCUSSÃO

In bioassay 1, analysis of variance revealed significant effects ($p < 0.05$) of *Bacillus aryabhattai* inoculation and salinity level on most germination, initial growth, and vigor traits of the maize plants, except for germination capacity. The interaction effect between *B. aryabhattai* inoculation and salinity level was significant ($p < 0.05$) for most of the evaluated characteristics, except for germination capacity (Table 2).

Table 2. Summary of the analysis of variance for the effects of microbial inoculation with *Bacillus aryabhattai* and salinity levels on the germination traits, initial growth, and vigor of maize and peanut plants.

Causes of variation	Probability > F										
	GC	GSI	MGT	SL	RL	TL	SDM	RDM	TDM	LVI	WVI
						Bioassay 1 (Maize)					
Microbial inoculation (I)	0.25	0.08	0.04	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.01
Salinity level (S)	0.12	0.01	0.02	0.01	0.01	0.01	0.01	0.03	0.01	0.01	0.02
I × S	0.32	0.04	0.01	0.04	0.01	0.01	0.01	0.02	0.01	0.02	0.01
CV (%)	4.78	5.34	4.78	8.95	7.56	6.92	8.39	7.64	9.21	6.71	7.47
						Bioassay 2 (Peanuts)					
Microbial inoculation (I)	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Salinity level (S)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
I × S	0.02	0.01	0.01	0.01	0.01	0.01	0.03	0.01	0.04	0.03	0.04
CV (%)	7.53	9.03	8.57	4.35	5.59	4.02	9.14	8.17	6.48	8.85	9.86

GC: germination capacity; GSI: germination speed index; MGT: mean germination time; SL: shoot length; RL: root length; TL: total plant length; SDM: shoot dry matter; RDM: root dry matter; TDM: total plant dry matter; LVI: plant length vigor index; WVI: plant weight vigor index.

In bioassay 2, the results of the analysis of variance revealed that the effects of isolation and interactions between *B. aryabhattai* inoculation and salinity level were significant ($p < 0.05$) for all the traits of germination, initial growth, and vigor of the peanut plants (Table 2).

The coefficient of variation (CV) values obtained in the two experimental trials were less than 10% for all the dependent variables measured in the maize and peanut seedlings, a value considered low for the agricultural experiments (Table 2). According to Gomes (2000), the CV is a measure of dispersion used to estimate the precision of the experiment, and when the values are less than 10%, it indicates that the results of the experimental trial have excellent precision.

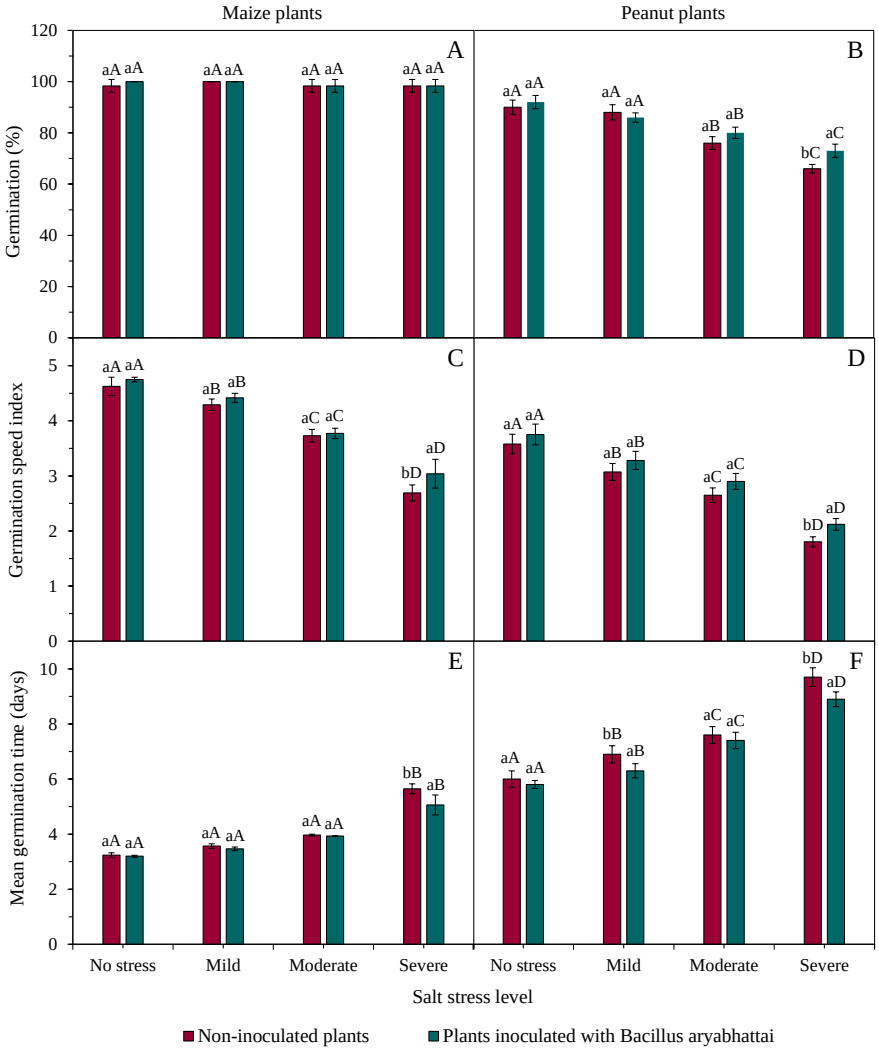


Figure 1. Effects of salinity on the germination capacity (A and B), germination speed index (C and D), and mean germination time (E and F) of maize (A, C, E) and peanut (B, D, F) plants not inoculated or inoculated with *Bacillus aryabhattai* strain CMAA 1363. Bars followed by distinct letters, lowercase for *B. aryabhattai* inoculation or uppercase for salinity levels, show significant differences according to Tukey's test at 5% probability. Data refer to mean values (n = 40) ± standard error of the mean.

Inoculation with *B. aryabhattai* and irrigation water did not affect the germination capacity of maize seeds (Figure 1A). However, when peanut seeds were subjected to moderate and severe salinity stress,

the seed germination capacity was significantly lower than that in the no-stress and mild-stress treatments. Nevertheless, under severe salinity stress conditions, the germination capacity of peanut seeds increased with increasing *B. aryabhattai* inoculation (Figure 1B). These results showed that *B. aryabhattai* inoculation was effective at improving peanut seed germination capacity under severe salinity conditions, confirming the results reported by Lessa et al. (2022).

The increased salinity resulted in a reduction in the germination speed index of maize (Figure 1C) and peanut (Figure 1D) seeds. Oliveira et al. (2022) reported a significant reduction in the germination speed index and germination capacity of tomato seeds with increasing salinity levels in irrigation water. Under saline conditions, the soil water potential decreases, resulting in a lower capacity for water absorption by the seeds, affecting the imbibition process, germination speed, and seed germination capacity (Taiz et al., 2017). However, under severe salinity stress, compared with noninoculated plants, plants inoculated with *B. aryabhattai* presented a greater germination speed index. Similar results were reported by Abdallah et al. (2016) and Jasim et al. (2015), who reported that inoculation with *Bacillus* sp. was able to stimulate both germination and initial plant growth. This increase in seed germination speed may be associated with the ability of bacteria of the genus *Bacillus* to produce plant hormones (Kang et al., 2014), which stimulate the seed germination process.

Inoculation with *B. aryabhattai* and irrigation water did not affect the mean germination time of maize seeds up to moderate salinity levels (Figure 1E). However, compared with that under other salinity levels, the mean germination time under severe salinity stress was significantly longer. Nevertheless, inoculation with *B. aryabhattai* mitigated the delay in the germination process of maize seeds. Increased salinity levels resulted in increased mean emergence time of the peanut plants (Figure 1F). However, under mild and severe salinity stress, inoculation with *B. aryabhattai* reduced the average emergence time of peanut plants. These findings indicate that inoculation with *B. aryabhattai* has a beneficial effect against saline stress, mitigating the adverse effects of salinity and promoting the germination process.

Mild saline stress resulted in greater shoot length in maize plants (Figure 2A), with no differences between inoculated and noninoculated plants. However, *B. aryabhattai* inoculation had a positive effect on the shoot length of maize plants exposed to moderate stress. With respect to peanut plants, *B. aryabhattai* inoculation resulted in greater shoot length under mild saline stress conditions (Figure 2B). Similarly, Kavamura et al. (2013) reported that compared with noninoculated plants, plants inoculated with *Bacillus* sp. were 27% taller.

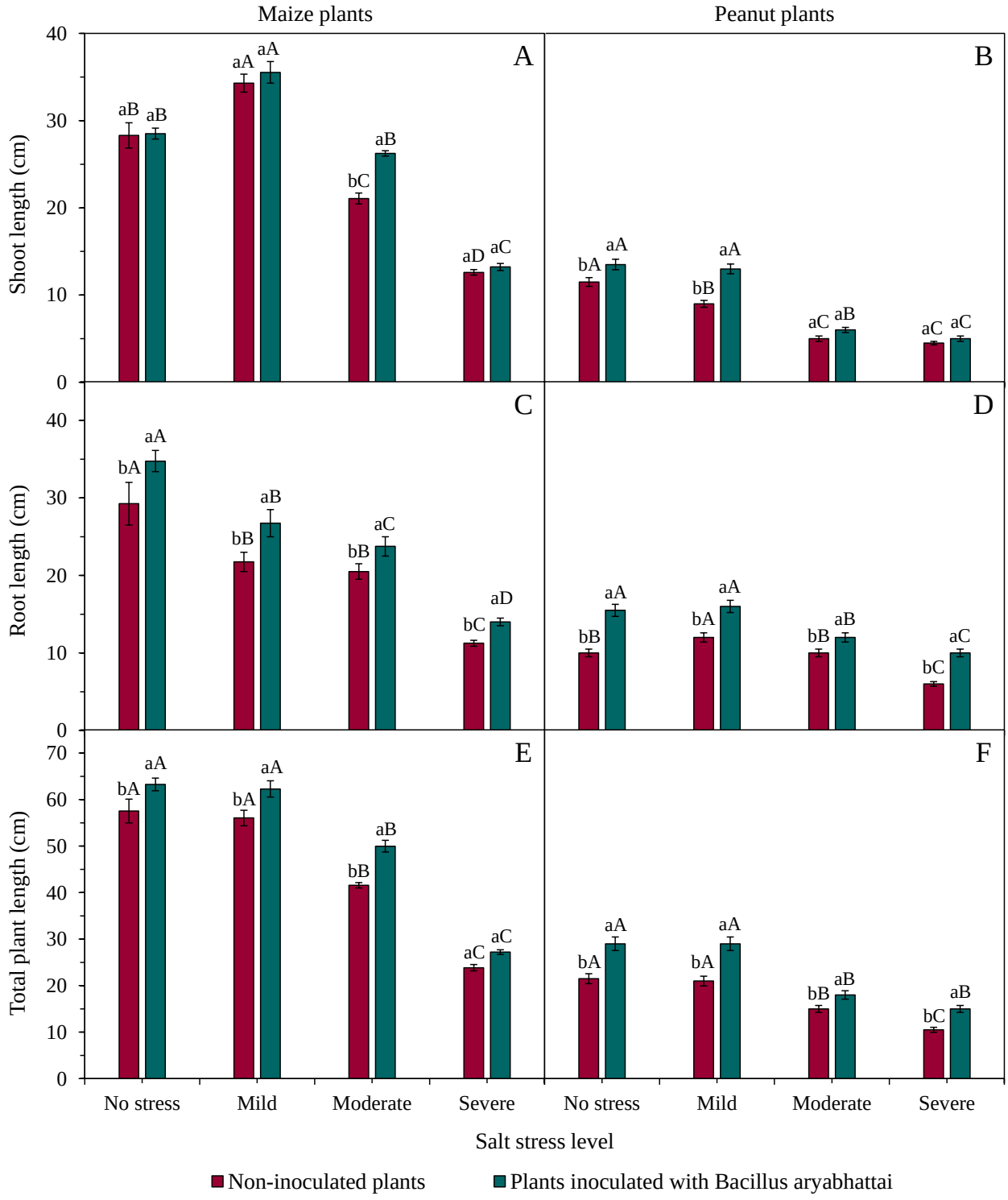


Figure 2. Effects of different levels of irrigation water on shoot length (A and B), root length (C and D) and total plant length (E and F) of maize plants (A, C, and E) and peanut plants (B, D, and F) uninoculated and inoculated with *Bacillus aryabhattai* CMAA 1363. Bars followed by distinct lowercase letters for the inoculation of *B. aryabhattai* or uppercase letters for the salinity levels are significantly different (Tukey test; $p \leq 0.05$). Data refer to mean values ($n = 40$) ± standard error.

The use of plant growth-promoting bacteria has contributed to combating the negative effects of abiotic factors (Alor et al., 2017). Tolerance to saline stress in various crops can be increased by bacteria

of the genus *Bacillus* (Paul; Harshad, 2024). Researchers report that the use of bacteria of the genus Bacillus acts on multiple mechanisms in plants, such as biological nitrogen fixation (Teixeira-Filho; Galindo., 2019; Galindo et al., 2020), increasing iron availability (Sivasakth et al., 2014), and solubilizing and transporting phosphorus and zinc (Jalal et al., 2022; Rosa et al., 2022). In addition, they improve plant growth and development through the production of plant hormones such as gibberellins, cytokinins, auxins, and salicylic acid (Zaheer et al., 2019). Tiwari et al. (2017) reported that compared with noninoculated rice plants, plants inoculated with *Bacillus amyloliquefaciens presented* increased total soluble sugar levels. In this study, inoculation with *B. aryabhattai* also improved the growth of maize (Figure 3) and peanut (Figure 4) plants, indicating the high potential for using this bacterium in the development of modern and sustainable agriculture.

The greatest root length was observed in maize and peanut plants inoculated with *B. aryabhattai* at all salinity levels (Figures 3 and 4). However, increasing the salinity level of the irrigation water limited root growth (Figures 2C and 2D). Lee et al. (2017) reported that the *B. aryabhattai* strain SR02 improved the growth of soybean and rice plants. These researchers reported that the SR02 strain can synthesize plant hormones (gibberellin and abscisic acid), which are responsible for the significant increase in the number of soybean roots. Yuan et al. (2015) reported that the NJN-6 strain of *B. amyloliquefaciens* was able to synthesize organic acids in banana roots, which improved root growth in these plants.

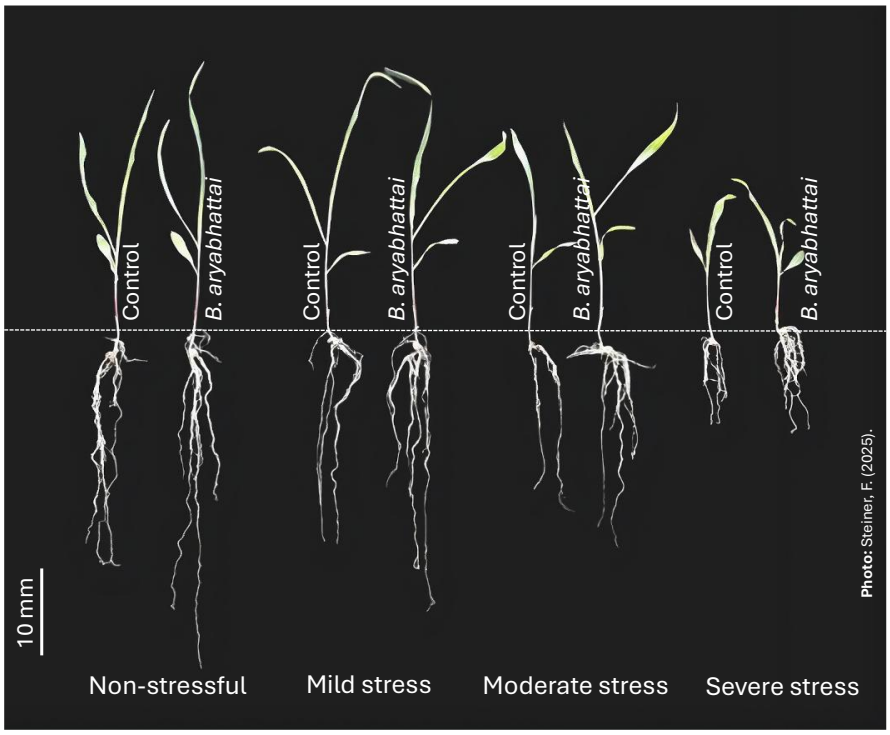


Figure 3. Maize plants (*Zea mays* L.) were irrigated for 18 days with different levels of irrigation water salinity and inoculated with *Bacillus aryabhattai* strain CMAA 1363.

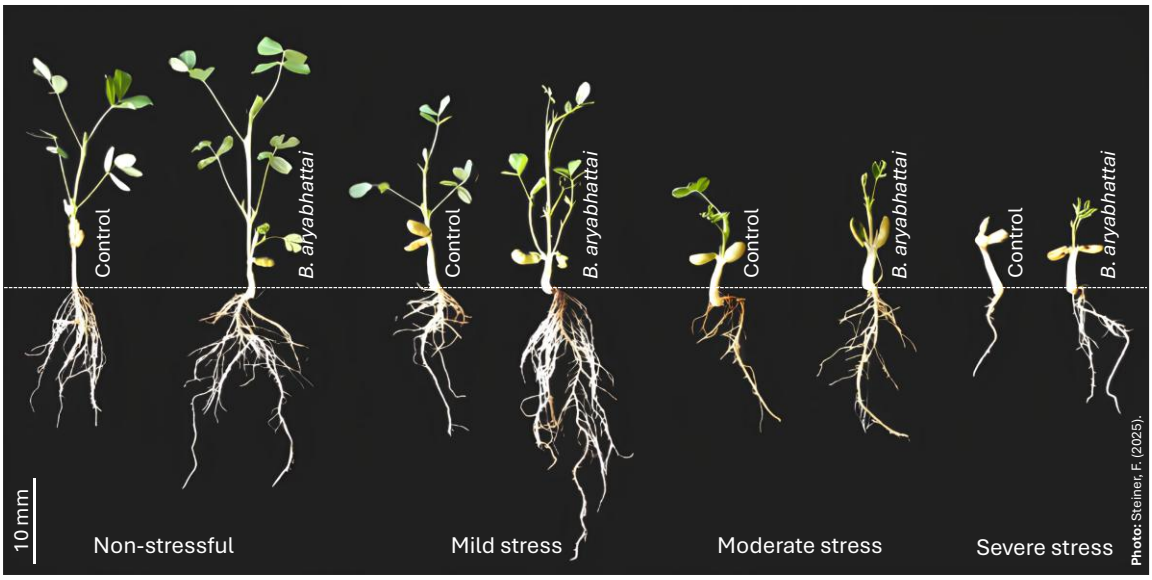


Figure 4. Peanut plants (*Arachis hypogaea* L.) were irrigated for 18 days with different levels of irrigation water salinity and inoculated with *Bacillus aryabhattai* strain CMAA 1363.

Similar results were reported by Maio et al. (2021), who reported that *B. aryabhattai* was able to stimulate root growth and promote greater soil water use efficiency. Metoui et al. (2020) reported that inoculation with *B. mojavensis* and *B. pumilus* promoted root growth and water and nutrient uptake in barley plants under saline stress. Islam et al. (2022) reported that saline stress, when induced by irrigation, limits water uptake by plants because of osmotic and physiological effects, in addition to causing biochemical changes that reduce water use efficiency.

The greatest accumulation of dry matter in maize roots occurred under control conditions and under moderate salinity stress. Regardless of the salinity level, compared with noninoculated plants, plants inoculated with *B. aryabhattai* had greater root dry matter (Figure 5C). With respect to the peanut plants, there was a reduction in root dry matter accumulation with increasing irrigation water salinity (Figure 7D). In turn, inoculation with *B. aryabhattai* did not result in a significant effect on peanut root accumulation under moderate and severe stress conditions.

Inoculation with *B. aryabhattai* had a significant effect on the total dry matter production of maize plants under severe saline stress conditions. However, there were no significant differences up to the moderate salinity level (Figure 5E). Conversely, the total dry matter production of peanut plants significantly decreased with increasing salinity levels in the irrigation water. However, inoculation with *B. aryabhattai* resulted in higher total dry matter production at all salinity levels (Figure 5F). These results demonstrate that bacteria of the genus *B. aryabhattai* can mitigate the effects of saline stress, in addition to promoting plant growth.

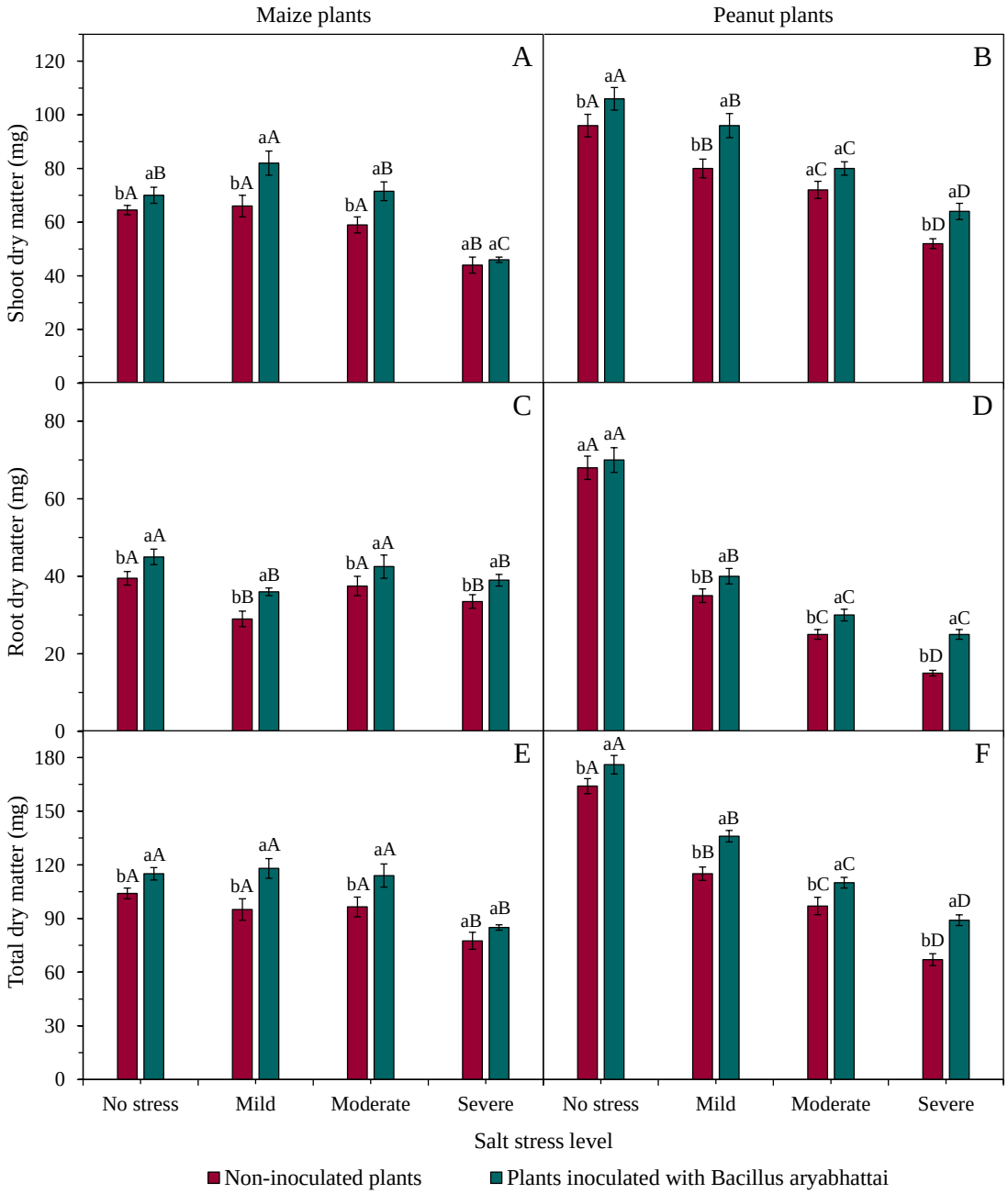


Figure 5. Effects of different levels of irrigation water on shoot dry matter (A and B), root dry matter (C and D) and total dry matter (E and F) in maize plants (A, C, and E) and peanut plants (B, D, and F) uninoculated and inoculated with *Bacillus aryabhattai* CMAA 1363. Bars followed by distinct lowercase letters for the inoculation of *B. aryabhattai* or uppercase letters for the salinity levels are significantly different (Tukey test; $p \leq 0.05$). Data refer to mean values ($n = 40$) ± standard error.

Xu et al. (2022) reported that *B. aryabhattai* isolated from wild plant species promoted the growth of Arabidopsis, *N. tabacum*, and other plant species. The isolate increased the size and dry matter of the plants evaluated, indicating that *B. aryabhattai* can stimulate the growth of a wide range of plant species. In addition to promoting plant growth through the activity of plant hormones and metabolites (Radhakrishnan; Lee, 2016), *Bacillus* species may assist in the induction of endogenous proteins, amino acids, and minerals that can promote plant growth (Radhakrishnan; Lee, 2016; Kang et al., 2014).

Mild saline stress levels did not significantly affect the plant length vigor index of maize or peanuts; however, from the moderate salinity level onward, there was a significant reduction in the plant length vigor index (Figures 6A and 6B). Nevertheless, at all the salinity stress levels tested, inoculation with *B. arybhattai* was effective, with a greater length vigor index for both the maize and the peanut plants (Figures 6A and 6B).

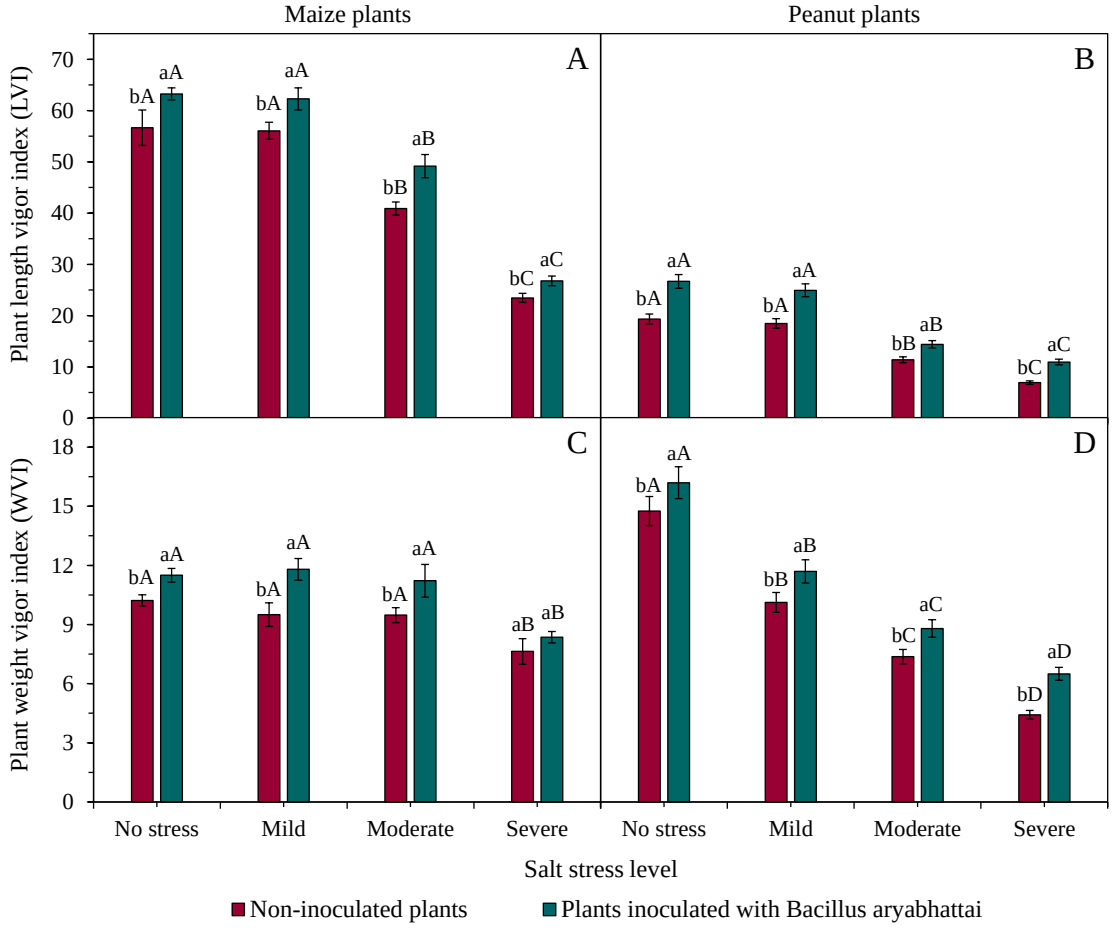


Figure 6. Effects of different levels of irrigation water on the plant length vigor index (A and B) and plant weight vigor index (C and D) of maize (A, C) and peanut plants (B and D) uninoculated and inoculated with *Bacillus aryabhattai* CMAA 1363. Bars followed by distinct lowercase letters for the inoculation of *B. aryabhattai* or uppercase letters for the salinity levels are significantly different (Tukey test; $p \leq 0.05$). Data refer to mean values ($n = 40$) ± standard error.

By studying the effects of *B. aryabhattai* inoculation under saline stress, reported that salinity affects water and nutrient absorption, resulting in a reduction in the stem diameter of maize plants. However, these effects were attenuated by the use of *B. aryabhattai*. Inoculation of *B. aryabhattai* in the early stages of plants can mitigate the adverse effects of abiotic stresses through biochemical changes in the plant, facilitating water and nutrient absorption (Maio et al., 2021; Qiu et al., 2021).

Sousa et al. (2021) reported that this decrease can be attributed to irrigation with saline water, since the salts present in irrigation water can inhibit the translocation and synthesis of the hormones

essential for plant growth, resulting in reduced dry matter production. In this study, at all the stress levels tested, the length vigor index increased in the *B. aryabhattai*-inoculated plants. Furthermore, at the other levels, the inoculated plants presented a greater weight vigor index.

Among the salinity levels, severe stress resulted in a reduction in the weight vigor index (WVI) of the maize plants, which did not significantly differ between the plants inoculated with B. aryabhattai and those not inoculated with *B. aryabhattai* (Figure 6C). On the other hand, the WVI of peanut plants decreased with increasing salinity. However, compared with noninoculated plants, plants inoculated with *B. aryabhattai* presented a greater WVI at all levels of salinity stress (Figure 6D).

Morais (2018) reported that inoculation with *B. aryabhattai* mitigated the negative effects of abiotic stress on soybean plants by activating the antioxidant defense system. Liu et al. (2017) reported that inoculation of *Arabidopsis thaliana* plants with the *Bacillus amyloliquefaciens* strain FZB42 could stimulate plant growth under both saline and nonsaline stress conditions.

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CONCLUSIONS

Inoculation with the *Bacillus aryabhattai* strain CMAA 1363 effectively mitigated the negative effects of salt stress on maize and peanut plants. These findings indicate that inoculation with *B. aryabhattai* in maize and peanut plants may be a sustainable alternative to enhance the development of maize and peanut crops under saline soil conditions or with the use of saline water for irrigation.

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Chapter 7

Bacterial consortia as a sustainable strategy for disease management and productivity improvement in maize

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ABSTRACT

Maize is among the most important agricultural crops worldwide because of its nutritional value, versatility, and role in food security; however, its cultivation faces various challenges from biotic and abiotic stressors. In recent decades, the use of agrochemicals has increased crop yields; however, environmental consequences such as biodiversity loss, pollution, and soil degradation highlight the need to integrate more sustainable strategies. In this context, plant growth-promoting microorganisms, particularly rhizobacteria (PGPR), offer alternatives; however, the use of individual strains has certain limitations under field conditions. By integrating microorganisms that can synergistically combine functions, microbial consortia have emerged as a promising biotechnological strategy, thereby improving their stability and effectiveness in real agricultural systems. This chapter explores the role of microbial consortia as a sustainable strategy for disease management and productivity improvement in maize cultivation. Additionally, the limitations of the conventional agricultural model and the role of beneficial microorganisms in terms of crop health and yield are examined. Similarly, the use of consortia as an integrated strategy and their implications for the sustainability of agricultural systems are discussed. Finally, future perspectives are proposed to optimize their design and application using emerging tools.

Keywords: microbial consortia, PGPR, maize, sustainable agriculture, plant–microbe interactions.

INTRODUCTION

In recent decades, farmers have increased agricultural production to meet global food demand. Agrochemicals, genetic improvements in crop varieties, and mechanization support this increase (Lam et al*.,* 2022; Chen, 2025). Nevertheless, this agricultural model creates sustainability challenges. It causes

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biodiversity loss and soil degradation and leads to the emergence of resistant pathogens (Miller et al., 2022; Jing et al., 2025). These limitations highlight the growing need for more sustainable strategies in current agricultural models.

Maize (*Zea mays* L.) is among the most important crop species because of its role in food security, versatility, and a wide range of uses (Shiferaw et al., 2011; Erenstein et al., 2022). However, its production is subject to various stress factors that can affect and limit yield (Nsibo et al., 2024; Hudson et al., 2024; Costantini et al., 2025). Conventional management strategies have traditionally relied heavily on agrochemicals to maximize productivity (Popp et al., 2013; Baum et al., 2025). Recently, interest in using beneficial microorganisms to improve crop health and increase yields has increased. Among these organisms, plant growth-promoting rhizobacteria (PGPR) have received attention because they are known to promote plant growth, improve nutrient uptake, and enhance plant defense against pathogens (de Andrade et al., 2023; Hasan et al., 2024). However, plants in agricultural systems interact with complex microbial communities rather than only individual strains. Therefore, addressing this complexity requires a new approach to plant–microorganism interactions. In this context, the use of microbial consortia has emerged as a promising alternative to single-strain inoculants. This approach enables the integration of diverse biological functions that can act together, increasing the likelihood of successful field application.

This chapter explores the role of microbial consortia as a sustainable strategy for disease management and productivity improvement in maize cultivation. From this perspective, the limitations of the conventional agricultural model and the role of beneficial microorganisms in crop health and yield are first examined. The chapter subsequently discusses the use of consortia as an integrated strategy and evaluates their implications for sustainability. Finally, future perspectives are proposed to optimize consortia design and application using emerging tools.

MODERN AGRICULTURE AND THE CHALLENGE OF SUSTAINABILITY

Currently, modern agriculture must increase production to meet global food demand and ensure environmental sustainability. For decades, intensification has relied on advanced technology and excessive use of agrochemicals, which have increased yields (Tilman et al., 2002; Foley et al., 2011). Despite these yield gains, intensive agriculture has also caused negative effects, including soil degradation, reduced microbial biodiversity, resistance of phytopathogens, the emergence of resistant weeds, and water pollution (VanGessel, 2001; Bijay-Singh & Craswell, 2021; Zhou et al., 2025).

The widespread use of agrochemicals such as pesticides has led to adverse health effects. These include immunosuppression, reproductive abnormalities, cancer, and, in extreme cases (exposure to high doses), death (Gupta, 2004). Given these significant risks, this evidence reinforces the need to rethink agricultural management practices.

In this context, it is essential to redirect cultivation strategies toward agricultural models that achieve optimal production without depleting resources in the long term (Beddington, 2010). Rather than fully replacing conventional practices, agriculture should instead move toward systems focused on resource conservation and ecological strategies. In line with this transition, incorporating beneficial microorganisms into crops is becoming a strategic alternative to improve plant health, increase productivity, and promote sustainability.

LIMITATIONS OF AGROCHEMICAL USE FOR CROP PRODUCTION AND DISEASE CONTROL

Historically, agrochemicals have been the primary alternative for achieving the required production levels and controlling disease. Fertilizers, pesticides, and fungicides have helped prevent and reduce losses from phytosanitary problems. They can also quickly correct nutrient deficiencies in crops (Popp et al*.,* 2013; Virk et al*.,* 2024; Sharafi et al*.,* 2025). However, despite these advantages, this approach reveals important limitations to the system's sustainability.

In disease management, repeated fungicide application exerts selective pressure on microbial populations. Sensitive populations are eliminated, and resistant populations survive (Bradley et al*.,* 2016). Over time, this leads to an increase in resistant populations. Consequently, a production system that is increasingly dependent on the application of external chemical inputs has emerged.

The intensive use of chemical fertilizers negatively alters the diversity of microbial communities in agricultural soils. This can significantly reduce plant–microorganism interactions and the benefits they provide, such as nutrition and pathogen suppression (Dasgupta et al*.,* 2026; Zhang et al*.,* 2022). Long-term studies have shown that chemical fertilization reduces fungal diversity and alters the structure of bacterial communities. It also significantly alters chemical properties, which can affect nutrient dynamics and ecological processes important for crop health (Bai et al*.,* 2020).

The conventional system focuses on agrochemical use and tends to address problems in isolation. It does not account for the complexity of biological networks, which are essential for maintaining crop health. This approach limits the ability to generate comprehensive solutions for plant nutrition, growth, and phytosanitary aspects.

BENEFICIAL MICROORGANISMS AS A STRATEGIC ALTERNATIVE

As alternatives to the limitations of agronomic models based on agrochemicals, interest in the use of beneficial microorganisms as tools to improve productivity and plant health has increased. Building on previous arguments for ecological strategies, among these, plant growth-promoting rhizobacteria

(PGPR) stand out. These bacteria colonize the rhizosphere and, in some cases, the interior of plant vascular tissues.

PGPR can influence the development of its host through both direct and indirect mechanisms. Direct mechanisms induce plant growth through biological nitrogen fixation, phytohormone production, phosphate solubilization, and siderophore production. PGPRs can indirectly function as biocontrol agents by enhancing the defense response to phytopathogen attack (Vessey, 2003; Shahwar et al., 2023; Sun et al*.,* 2024).

Experimental studies have shown that PGPR inoculation can optimize nutrient uptake, increase plant biomass and yield, and increase tolerance to both biotic and abiotic stresses in various crops, including maize. These effects are observed under both controlled and field conditions. However, the effects can vary depending on the edaphoclimatic context and interactions with the native microbiota (Pereira et al*.,* 2020; Nabati et al*.,* 2025; Al-Barakaha & Alghamdi, 2025).

Although the potential of PGPR applied individually to crops has been demonstrated, evidence shows that plant–microorganism interactions occur within complex, dynamic microbial communities. Specifically, in the field, the rhizosphere is an ecosystem in which diverse microorganisms interact with each other and host plants, forming ecological networks that can limit or amplify the effects observed under controlled conditions (Saleem et al*.,* 2021; Molina-Romero et al*.,* 2021). Therefore, the use of individual strains with specific functions may not replicate the stability and functional diversity found in natural microbial systems. Recognizing these limitations has led to the development of bioinoculants based on microbial consortia that integrate complementary functions to increase crop benefits. From this perspective, functional consortia serve as strategic biotechnological tools.

MAIZE HEALTH AND YIELD IN THE CURRENT AGRICULTURAL CONTEXT

Maize is among the most important crops worldwide because of its economic and cultural relevance, as well as its importance to global food security (Tanumihardjo et al*.,* 2020). Furthermore, it is a highly versatile crop across agricultural systems in both developed and developing countries. Its uses are diverse and include food for human consumption, bioethanol production, animal feed, and industrial products, among others (Shiferaw et al*.,* 2011; Ranum et al*.,* 2014).

From a production standpoint, maize is among the three most widely produced cereals in the world, along with wheat and rice. Global production now exceeds 1.2 billion tons annually. For example, in 2024, global production reached approximately 1.218 billion tons (FAO, 2024). Maize grows in diverse soils and climates, but yields depend on intensive agronomic management. This reliance makes maize susceptible to both biotic and abiotic stressors.

Maize is closely related to the soil microbiota, especially in the rhizosphere. These microbial communities influence plant efficiency in acquiring essential nutrients such as phosphorus and nitrogen. In turn, this influences plant growth and defense against pathogens. As a result, healthy agricultural soils and stable microbiota are essential for sustaining long-term productivity (Luo et al., 2025; Boloch, 2025).

MAIZE GROWTH AND YIELD AND THE USE OF BACTERIAL CONSORTIA

Maize growth and yield depend on interactions among plant genotype, environment, and agronomic management (Fischer et al., 2014). Many biotic and abiotic stress factors can limit plant growth and decrease crop productivity (Mittler, 2006; Gull, et al., 2019). Abiotic factors include salinity, drought, and poor nutrient bioavailability (Hu et al., 2022; Deribe, H., 2024). Among the biotic stress factors, plant pathogens pose a major threat because of their ability to cause disease, compromising health and productivity (Savary et al. 2019; Matos et al., 2024).

Maize can be affected by a wide range of pathogens. The main disease-causing agents are fungal, bacterial, and viral microorganisms. These can infect maize, limit its growth and productivity, and cause major economic losses. In some cases, pathogens cause mycotoxins to accumulate, posing health risks to humans and animals (Albarracín et al., 2012; Redinbaugh & Zambrano, 2014; Guerra et al., 2022; Bryła et al., 2022).

On the other hand, agronomic practices directly impact soil health and structure, as well as microbial communities (Meena et al., 2020). Excessive agrochemical use results in negative effects, such as loss of microbial diversity, nutritional imbalances, and reduced long-term fertility (Steiner et al., 2024; Yasir et al., 2025). A shift to sustainable agriculture cannot rely only on replacing chemical inputs. This change requires the integration of different tools and knowledge from agronomy, ecology, and microbiology. Designing and implementing integrated strategies is essential for promoting more sustainable agricultural systems.

The combination of plant stress factors highlights the need for a comprehensive approach to crop production. This approach must account for interactions among the environment, cultivated plants, and soil microbiota—microorganisms that live in the soil and influence plant growth. Given this context, the use of beneficial microorganisms, such as plant growth-promoting rhizobacteria (PGPR), which increase plant growth, is a promising way to improve crop productivity and health (Vessey, 2003; Olanrewaju et al., 2017). However, the use of individual strains may be limiting in the field because of competition with the native microbiota (Yang et al., 2024). Microbial consortia provide an alternative to these limitations. The integration of diverse biological functions within a single community enables improved plant nutrition, growth promotion, and stronger defense against pathogens (Sarma et al., 2015; Santoyo et al., 2021; Negi et al., 2024).

In the rhizosphere, microorganisms exist in a complex system. They interact with each other and with their plant host (Iqbal, 2026). Soil microbiota play key roles in pathogen suppression, nutrient availability, and plant growth promotion (Trivedi et al., 2020). On this basis, bacterial consortia aim to mimic the functions of natural microbial communities.

Consortia offer benefits not seen with single-strain PGPR inoculation. They combine microorganisms with complementary functions, resulting in their coexistence and collaboration (Niu et al., 2017; Trivedi et al., 2020). Consortia also maintain functionality under variable conditions (Shade, 2012). Their strength lies in the interactions among their members, enabling processes such as functional cooperation, metabolic complementarity, and niche partitioning (Hu et al., 2016; Carreras, 2025). These microbial interactions increase individual functions and stability (De Roy et al., 2014; Niu et al., 2017). The main differences between solitary and consortium inoculation are shown in Figure 1.

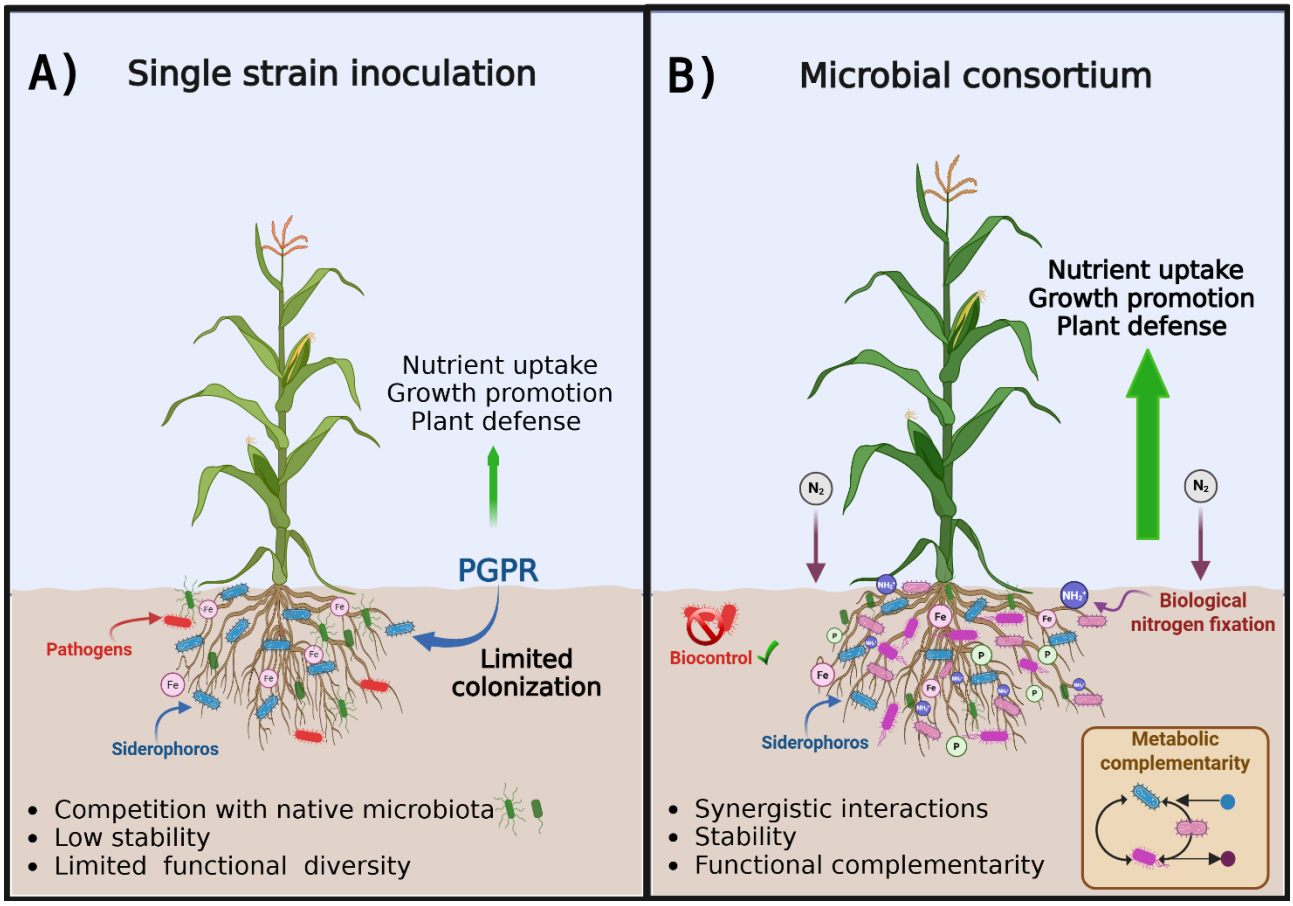


Figure 1. Schematic comparison between A) a single PGPR strain inoculation and B) inoculation with a bacterial consortium.

In the field, competition for resources and space can be a limitation (Hibbing et al., 2010). Integrating consortia creates complementarity between microorganisms, which favors adaptation and stability (Pascual et al., 2020; Zhang et al., 2026). Consortia are more likely to establish and perform better than single-strain inoculants are. Their use as biocontrol agents is notable because they combine multiple

mechanisms of action to control phytopathogens more effectively (Negi et al., 2024). For example, De la Vega-Camarillo et al. (2023) reported a biocontrol effect using a microbial consortium composed of *Achromobacter xylosoxidans*, *Burkholderia sp.*, *Klebsiella variicola*, *Pantoea ananatis*, *Kosakonia pseudosacchari*, *Phytobacter diazotrophicus*, and *Pseudomonas protegens*, resulting in a reduction in maize rust incidence (8 to 12.5%) in a field experiment.

Various greenhouse and field studies have shown that the application of microbial consortia can significantly improve maize growth and yield (Table 1). For example, an experiment by Olanrewaju & Babalola (2019) evaluated native maize rhizosphere strains. The strains were inoculated individually or in pairs or triplets. The consortia of two or three strains significantly increased plant biomass and grain weight, with the three-strain consortia being the most efficient. Similarly, a study in semiarid environments revealed that bacterial consortia improved the number and weight of maize kernels. Organic matter and phosphorus availability also increased. However, these results varied by application rate and plant genotype, as four maize varieties showed different responses, highlighting the complexity of plant–microorganism interactions under real-world conditions (Kurt, 2025).

Compared with individual strains, a consortium composed of *Azospirillum brasilense*, *Pseudomonas putida*, *Acinetobacter sp.*, and *Sphingomonas sp.* increases rhizosphere colonization in maize. Inoculation with the consortium, combined with 50% chemical fertilization, maintained yields similar to those with 100% fertilization, demonstrating its potential to reduce reliance on external inputs (Molina-Romero et al., 2021).

This evidence suggests that microbial consortia can promote pathogen defense, growth, and productivity. They also help agriculture adapt to environmental variability. Understanding the basis of these interactions and their implications for functionality requires an integrated approach.

Table 1. *In vitro*, greenhouse and field experiments in maize using diverse microbial consortia**.**

Reference	Microbial consortium	Effects	Experimental conditions
Olanrewaju & Babalola, 2019	Native PGPR including *Bacillus, Pseudomonas,* and *Streptomyces*	↑ plant height ↑ root and stem length ↑ number of leaves ↑ weight of 100 seeds consortia better than individual inoculation	Field trials
Pacheco et al., 2021	*Rhizoglomus irregulare* *Pseudomonas putida*	↑ P absorption ↑ P use efficiency	Field trials

Reference	Microbial consortium	Effects	Experimental conditions
		↑ Productivity associated with P	
Saleem et al., 2021	*Bacillus* sp. *Enterobacter* sp. *Pseudomonas fluorescens*	higher efficiency of photosynthesis (14%) and an increase in antioxidant systems (20%)	Greenhouse (pots) under water stress, consortium vs. individual inoculants
Molina-Romero et al., 2021	*Azospirillum brasilense* *Pseudomonas putida* *Acinetobacter* sp. *Sphingomonas* sp.	↑ height ↑ root length and dry weight ↑ grain yield from 3.91 to 5.35 t/ha (consortium + 50% urea vs. control)	Field trials with different nitrogen fertilization regimes (0%, 50%, 100%)
Peng et al., 2021	*Pseudomonas* sp. *Peribacillus* sp. *Streptomyces* sp.	↑ plant biomass ↑ antioxidant enzymatic activity ↑ chlorophyll content ↑ abundance of beneficial rhizobacteria ↑ salinity tolerance	Greenhouse: tests in saline soils (pots)
Ahmad et al., 2022	*Bacillus subtilis* *Paenibacillus polymyxa* *Bacillus aryabhattai*	↑ grain yield +17% ↑ 1000-grain weight +12% ↑ nutrients in grain	Greenhouse (pots) and field trials (two cycles)
De la Vega-Camarillo et al., 2023	*Achromobacter xylosoxidans Burkholderia* sp. *Klebsiella variicola* *Pantoea ananatis* *Kosakonia pseudosacchari* *Phytobacter diazotrophicus* *Pseudomonas protegens*	↑ Root length (4.2 folds) ↑ Air length (3.0 folds) ↑ Yield of 11 to 16 t/ha ↓ Rust incidence from 12.5% to 8%	Greenhouse (pots) and field trials
Manjunath et al., 2023	*Pseudomonas putida* *Paenibacillus favisporus*	↑ yield from 3.02 to 3.86 t/ha (↑ 27.8% or ↑ 0.84 t/ha) ↑ biomass (stubble) from 6.93 to 9.11 t/ha (↑ 31.4%)	Field trial (three years, rainfed/subhumid conditions) with seed + soil application

Reference	Microbial consortium	Effects	Experimental conditions
Zelaya-Molina et al., 2025	*Aureobasidium* sp. *Clavispora* sp. *Pantoea* sp.	↑ Germination rate ↑ Root architecture development ↑ Seedling biomass	Early-stage *in vitro* assay
Kurt, 2025	*Azotobacter* spp. *Bacillus* sp. *Paenibacillus* sp. *Pantoea* sp. *Pseudomonas* sp.	↑ grain yield ↑ soil organic matter ↑ P availability ↑ cation exchange capacity ↑ [Zn and Cu] in plant and soil	Field trials in a semiarid region
Patil et al., 2026	*Bacillus haynesii* *Shouchella clausii* *Aspergillus versicolor* *Aspergillus proliferans*	↑ yield 8.53 t/ha ↑ aerial dry biomass ↑ root length ↑ number of leaves ↑ grains per ear	Field trials in semiarid conditions

FUTURE PERSPECTIVES

The development and use of bacterial consortia are a promising strategy in agricultural biotechnology. They are especially useful for addressing issues caused by intensive agrochemical use. However, implementing consortia in cropping systems poses several challenges. Addressing these issues requires an interdisciplinary approach that combines knowledge from microbiology, agronomy, ecology, and biotechnology.

Additionally, the effectiveness of consortia is influenced by plant genotype, environmental conditions, and native microbiota. Evidence suggests that these variables can influence the effectiveness of consortia in agronomic systems (Wagner et al., 2016; Trivedi et al., 2020; Yang et al., 2024), underscoring the need to develop consortia with strategies tailored to each system rather than more general approaches.

Recent research emphasizes the importance of designing consortia that incorporate functional interactions and complementary strategies, tailored to specific objectives, to ensure their effectiveness and stability under real agricultural conditions (Singh et al., 2023).

Combining microorganisms with complementary functions can result in synergistic effects. These processes improve pathogen defense, increase plant growth, and yield (Olanrewaju & Babalola, 2019; Molina-Romero et al., 2021; Pacheco et al., 2021; Wang et al., 2025). However, designing stable,

functional consortia remains a challenge. Key knowledge gaps exist about their interactions and performance (persistence, stability, and functionality) in the field.

In this context, the integration of omics tools (such as genomics, proteomics, transcriptomics, and metabolomics—methods for comprehensively studying genes, proteins, RNA, and metabolites) has allowed for a deeper understanding of plant–microorganism interactions. These tools enable the investigation and elucidation of biological systems and their interactions. Taken together, these approaches enable an unprecedented understanding of the mechanisms underlying plant responses to microbial colonization, facilitating the identification of key functions, gene expression, metabolic pathways, and microbial communication mechanisms that promote plant growth and defense against pathogens (Mmotla et al*.,* 2025). The integration of these tools, along with agroecological approaches, such as the reduction of chemical inputs and the optimization of soil–plant–microorganism system interactions, will allow progress toward more productive and environmentally responsible agricultural systems.

CONCLUSIONS

Microbial consortia are presented as a strategic alternative by integrating microorganisms with complementary functions, allowing greater stability and efficiency under variable agricultural conditions. Their potential to improve maize productivity positions them as a key tool for agricultural sustainability. The design of microbial consortia requires an interdisciplinary approach; in this sense, the integration of omics tools will allow for a more detailed understanding of plant–environment–microbiome interactions. The integration of these strategies will help optimize the performance of microbial consortia under real agricultural conditions, contributing to sustainable agricultural management.

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Chapter 8

Agronomic management for oat seed production in the Bajío region, Mexico

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ABSTRACT

The Bajío agricultural region in Mexico represents a viable option for the production of certified oat seed. During the fall–winter (f–w) growing season, temperature and relative humidity conditions are favorable for the crop, with a low risk of economically significant diseases such as rusts and leaf spot. However, the effects of environmental factors, particularly planting date, on genotype performance—in terms of agronomic variables, yield, and seed physical quality—are unknown. For this reason, experiments were conducted in Celaya, Guanajuato, over three f-w growing seasons, with three planting dates (December 1, 15, and 31) and five genotypes (Chihuahua, Menonita, Karma, Obsidiana, and Turquesa), under irrigated conditions, using a randomized complete block design, where the main plots were the planting dates and the subplots were the varieties, with three replications in each case. The variables evaluated were days to flowering, plant height, panicle length, seed yield, hectoliter weight, and thousand-seed weight. The SREG model was used to analyze yield stability. The model explained 89.26% of the combined effects of genotype, environment, and their interaction. Compared with Menonita, Karma, and Chihuahua, the Obsidiana variety presented the greatest agronomic stability and yield, positioning it as the most desirable; moreover, Chihuahua was classified as unstable, and Obsidiana excelled in yield across the greatest number of evaluated environments.

Keywords: *Avena sativa* L., SREG, environments, genotypes.

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INTRODUCTION

In Mexico, oats are grown primarily for livestock feed. Cultivation is concentrated in the states of Chihuahua, Durango, Mexico, and Zacatecas. In 2024, approximately 562,000 hectares were registered for forage production, with 75% of the area planted during the spring–summer growing season under rain-fed conditions (SIAP, 2026). The nutritional value of grain is high, making it a good option for human consumption because of its contents of amino acids, fiber, β-glucans, protein, and micronutrients (Alemayehu et al., 2023; Li et al., 2024). However, only 45,000 hectares are allocated to grain production under rain-fed conditions (SIAP, 2026); in many cases, farmers set aside part of the commercial crop and use it as a seed, lacking the necessary quality components.

The Mexican Bajío region comprises the states of Querétaro, Jalisco, Michoacán, and Guanajuato. During the fall–winter (f-w) growing season, the region offers favorable temperature and relative humidity conditions for crop development, making it a viable alternative for the production of certified oat seed. The risk of economically significant diseases such as rusts and leaf spots is low (Chávez-Solís et al., 2021; Villaseñor-Mir et al., 2021).

Other factors that influence the production of high-quality seeds include agronomic management. In a study of oat seed germination and seedling development using silicon- and phosphorus-based fertilization, the highest average germination rates and vigor were obtained with the highest K_2SiO_3 fertilization rates of 300 and 450 mg dm^{-3}, respectively; in addition, the phosphorus dose influenced seedling development when it was applied at the highest silicon level (Zampar et al., 2011). When organic fertilizer sources were compared with chemical fertilizers, no differences were found in terms of seed yield components, thousand-seed weight, germination percentage, or vigor (Rodríguez-Herrera et al., 2020). The seeding rate also influences yield and quality components. Bobadilla et al. (2013) evaluated the effects of three seeding rates (60, 90, and 120 kg ha^{-1}) and reported positive trends in yield and specific weight as the seed rate increased; in contrast, the thousand-seed weight was affected.

Furthermore, the planting date significantly influences the expression of yield components, phenological development, certain quality attributes, and the incidence of damage caused by biotic and abiotic factors during seed production; therefore, it must be carefully selected to maximize seed production and quality. Furthermore, there is little information on the influence of the environment on the aforementioned variables. Consequently, the objective of this study was to evaluate the performance of five oat genotypes under different environmental conditions (planting dates and growth cycles) in terms of yield components, agronomic characteristics, and seed physical quality.

MATERIALS AND METHODS

Assessment Environments

The experiment was set up on land belonging to the INIFAP Bajío Experimental Farm (CEBAJ), located in Celaya, Guanajuato (20° 35' north latitude and 100° 49' west longitude), at an elevation of 1,756 m. The climate is semiwarm (BS1Hw), with temperatures ranging from 18 to 22°C, an annual average temperature of 21.6°C, and an average annual rainfall of 670.3 mm (García, 1985). Planting was carried out on three dates—December 1, 15, and 31—for three fall–winter growing seasons (2020–2021, 2021–2022, and 2022–2023; hereinafter referred to as cycle 1, cycle 2, and cycle 3, respectively).

Agricultural management

The seeding rate was 90 kg of seed per hectare, and a double-row system with a row spacing of 0.20 m and an interrow spacing of 0.80 m was used. Irrigation was applied at 0, 45, 70, and 90 days after planting, which coincided with the stages of germination, tillering, booting, and grain filling. The fertilizer formula used ranged from 75–40–00; all nitrogen (N_2) and phosphorus ($P2O_5$) were applied at planting. For broadleaf weed control, the herbicide 2,4-D Amina was applied at a rate of 1.5 L ha^{-1}. When aphids were identified on the foliage, 50% Oxidemeton-methyl or 1000 Omethoate E was applied at a dosage of 0.25 L ha^{-1} (García et al., 2016).

Genotypes

The varieties used were Chihuahua (control), Turquesa, Obsidiana, Karma, and Menonita, released by INIFAP's Wheat and Oats Genetic Improvement Program; their agronomic characteristics were evaluated to identify the variety best suited to various environments.

Experimental design

The experiment was set up using a randomized complete block design, where the main factor was the planting date and the secondary factor was the variety, with three replicates for each. Each experimental unit was 16 m^2 in size, consisting of 4 furrows 5 m long, which were planted in double rows. The variables evaluated were days to flowering, plant height, panicle length, and seed yield at 13% moisture content, which were calculated using the following formula: P2 = [(100% - CH1)/87] P1, where P1 = field weight; P2 = weight adjusted to 13% moisture content; and CH1 = grain moisture content at harvest; for hectoliter weight, the Boerner-type determinator was used as proposed by Moreno (1996), and it was expressed in kg hL^{-1}. The thousand-seed count was performed manually; the seeds were subsequently weighed on an electronic scale (ISTA, 2022).

Statistical analysis

Two groups were formed to analyze the data obtained. Group I included the fall-winter (f-w) cycles of 2021–2022 and 2022–2023 (cycles 2 and 3, respectively) and the varieties Obsidiana, Karma, Chihuahua, and Menonita. Group II included the f-w cycles 2020–2021 and 2022–2023 (cycles 1 and 3), with the varieties Turquesa and Chihuahua. A combined analysis was performed for plots divided by space and time (Steel, Torrie, 1985). The least significant difference (LSD with $p \leq 0.05$) test was used to compare the means. To identify statistically significant differences in the genotype × environment interaction, the analysis was conducted using the methodology known as SREG (Yan et al., 2000) with GEA-R software (Alvarado et al., 2020); in addition, the yield stability of each variety was determined by assessing how predictable the genotypes' yields are through their evaluation across various environments. The data were analyzed using SAS/STAT® 9.3 software.

RESULTS AND DISCUSSION

Group I

Analysis of Variance

The combined analysis (Table 1) revealed that the growing season was the most significant source of variation for thousand-seed weight, accounting for 85.4% of the total variance in this variable; plant height accounted for 67.7%, and flowering accounted for 62%. Therefore, compared with the variance contributed by the varieties, it can be inferred that the impact of the cycles was 13, 3.2, and 2.1 times greater, respectively, than the variation contributed by the varieties in the expression of the variability of these traits.

Table 1. Mean squares from the randomized complete block design with a split-plot arrangement (divided by time and space) in the combined analysis of four oat varieties over two growing seasons in Celaya, Guanajuato.

Sources of Variation	GL	Yield	Flowering	Plant Height	Panicle Length	Weight of a Thousand Seeds	Hectoliter Weight
Blocks	3	0.82	0.53	0.009	0.002	18.46	1.07
Cycles (C)	1	5.61 **	2480.67 **	1.15 **	0.02 **	576.00 **	39.18
Planting Dates (PD)	2	1.84	110.17 **	0.15 **	0.002	15.32	75.32 **
C*PD	2	20.51 **	157.29 **	0.03 *	0.002	0.39	2.55
Variedades (V)	3	5.21 **	1231.19 **	0.36 **	0.03 **	44.41 **	135.81 **
C*V	3	0.03	1.75	0.009	0.001	8.75	11.78
PD*V	6	0.54	9.71 **	0.004	$6x10^{-4}$	11.47	2.35
C* PD *V	6	1.92 **	9.71 **	0.006	0.002	18.41	9.22 **
C. V. (C, %)		5.5	1.4	6.7	12.5	8.9	5.8
C. V. (PD, %)		15.8	1.9	6.5	17.2	11.6	2.9
C. V. (V, %)		12.6	1.6	5.6	14.0	7.1	3.4

GL: degrees of freedom; *, ** indicate significance at the 0.05 and 0.01 probability levels, respectively; CV: coefficient of variation

The varieties were the primary determinants of the expression of these traits—hectoliter weight and panicle length—accounting for 49.2% and 46.1% of the total variance in each of these variables, respectively, which was 1.8 and 1.1 times greater than the contribution of the growth stages.

With respect to the importance of interactions, the interaction between growing season and planting date was a key factor in yield expression, accounting for 57.5% of the total variance observed; this effect exceeded that between growing season and variety by 3.7 and 3.9 times, respectively. Therefore, the environmental effect—as mediated by growing season and location—was the most significant source of variation for this variable.

Furthermore, the combined analysis revealed statistically significant differences, with $p \leq 0.01$, among the varieties for all the study variables; among the evaluation cycles or years for all the variables except hectoliter weight; and among the planting dates for flowering, plant height, and hectoliter weight. In terms of the cycles × planting dates interaction, significant differences were recorded for yield and flowering ($p \leq 0.01$), in addition to differences in plant height at $p \leq 0.05$. In terms of the planting date × variety interaction, significant differences were detected for the flowering variable ($p \leq 0.01$). For the three-way interaction, statistically significant differences ($p \leq 0.01$) were detected in the yield, flowering, and hectoliter weight variables. With respect to the cycle × variety interaction, no statistically significant differences were detected in the variables studied. The coefficients of variation for cycles, planting dates, and varieties were acceptable, validating the uniformity in crop management, data collection, and treatment application, as well as the reduced significance that biotic and abiotic stress factors may have had. This allowed for an acceptable experimental error.

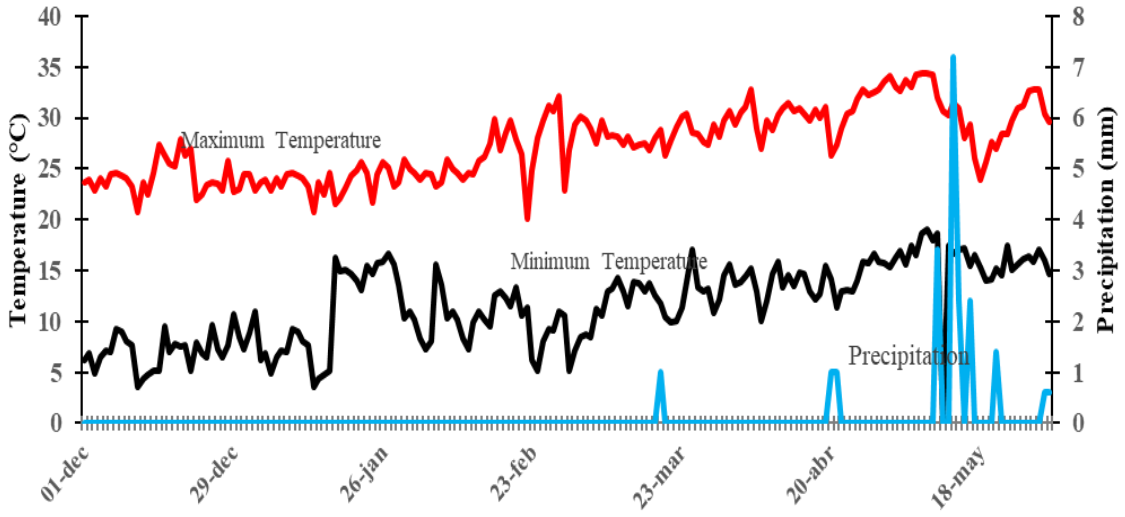


Figure 1. Minimum and maximum temperatures and precipitation in the town of Celaya, Guanajuato, during the second evaluation cycle.

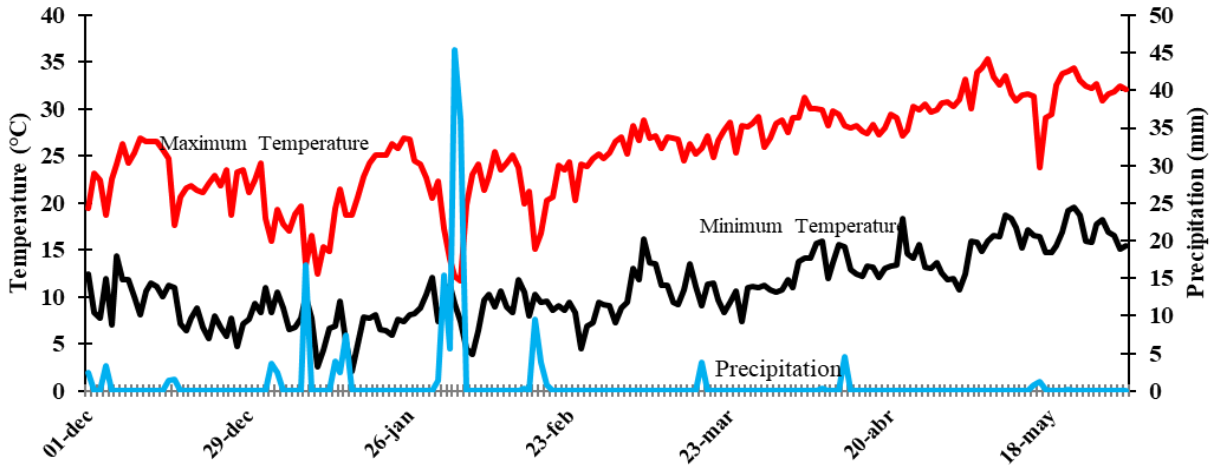


Figure 2. Minimum and maximum temperatures and precipitation in the town of Celaya, Guanajuato, during the third evaluation cycle.

Tests of means

The mean tests for the evaluation cycles (Table 2) revealed that cycle 3 yielded the highest yield, flowering, plant height, panicle length, and thousand-seed weight ($p \leq 0.05$). In other words, environmental conditions were more favorable for the evaluated varieties than for cycle 2, which can be attributed to the fact that only 21 mm of precipitation accumulated in the latter cycle, whereas 175.2 mm accumulated in cycle 3, as shown in Figures 1 and 2, respectively. High temperatures during the anthesis and grain-filling stages in small-grain cereals negatively affect yield by accelerating the rate of senescence (Pinto et al., 2016; Al-Ajlouni et al., 2016; Kim et al., 2024; Lu et al; 2025).

Table 2. Tests of means for fall–winter planting cycles with four oat varieties evaluated in Celaya, Guanajuato.

Cycles	Yield (t ha^{-1})	Flowering (days)	Plant Height (cm)	Panicle Length (m)	Weight of a Thousand Seeds (g)	Hectoliter Weight (kg hL^{-1})
Cicle 3	4.73 a	92.2 a	1.34 a	0.22 a	36.27 a	42.79 a
Cicle 2	4.25 b	82.0 b	1.13 b	0.19 b	31.37 b	44.07 a
MSD ($p \leq 0.05$)	0.162	0.802	0.054	0.016	1.968	1.644

MSD: Minimally significant difference

Analysis of the planting dates (Table 3) revealed that the highest hectoliter weight was obtained on the first planting date ($p \leq 0.05$), which could be associated with the greater number of days to flowering also observed on this same planting date ($p \leq 0.05$) and the greater plant height ($p \leq 0.05$); that is, to the extent that there was a longer growing season, these variables were most pronounced, regardless of the year or evaluation cycle. This is corroborated by the response of the same variables on December 31, which is when the lowest values were obtained for these variables. This finding is consistent with the

results for beans reported by González et al. (2008), who reported that genotypes with the highest daily yield accumulation or the highest rate of grain weight accumulation achieved the highest yield per unit area. The duration of each phenological stage is associated with absorbed radiation, with a more pronounced effect during flowering through maturity (Royo et al., 2021).

Table 3. Mean planting date test for four oat varieties, evaluated over two fall–winter growing seasons in Celaya, Guanajuato.

Planting dates	Yield (t ha^{-1})	Flowering (days)	Plant Height (cm)	Panicle Length (m)	Weight of a Thousand Seeds (g)	Hectoliter Weight (kg hL^{-1})
01 December	4.22 a	88.7 a	124 a	0.20 a	34.15 a	45.13 a
15 December	4.64 a	87.6 b	129 a	0.22 a	33.03 a	42.99 b
31 December	4.62 a	85.1 c	116 b	0.20 a	34.28 a	42.16 c
MSD ($p \leq 0.05$)	0.44	1.05	4.9	0.02	2.40	0.78

MSD: Minimally significant difference

Among the varieties evaluated, Obsidiana had the highest seed yield ($p \leq 0.05$) and the highest physical quality ($p \leq 0.05$) and outperformed the other genotypes, followed closely by Karma, which was earlier to flower (days to flowering, $p \leq 0.05$) and had a higher hectoliter weight ($p \leq 0.05$) than did Chihuahua and had the same plant height, panicle length, and stem diameter as Obsidiana were ($p \leq 0.05$). Although Menonita had the lowest seed yield ($p \leq 0.05$), it was the earliest to flower ($p \leq 0.05$) and had the highest seed hectoliter weight ($p \leq 0.05$) and the smallest panicle size ($p \leq 0.05$).

Table 4. Mean values for four oat varieties evaluated over two fall–winter growing seasons in Celaya, Guanajuato.

Varieties	Yield (t ha^{-1})	Flowering (days)	Plant Height (cm)	Panicle Length (m)	Weight of a Thousand Seeds (g)	Hectoliter Weight (kg hL^{-1})
Obsidiana	5.09 a	83.9 b	117 b	0.21 b	35.84 a	44.90 a
Karma	4.52 b	83.9 c	115 b	0.20 b	33.07 b	43.38 b
Chihuahua	4.40 b	97.5 a	141 a	0.25 a	33.40 b	40.38 c
Menonita	3.96 c	81.3 d	118 b	0.17 c	32.97 b	45.35 a
MSD ($p \leq 0.05$)	0.34	0.86	4.1	0.02	1.43	0.88

MSD: Minimally significant difference

Principal component analysis

In the principal component analysis, as shown in Table 5, the variables that contributed most to the variance in principal component 1 across varieties were days to flowering, panicle length, and hectoliter weight, whereas for principal component 2, they were thousand-seed weight, yield, and plant height. The first two principal components explain 95.2% of the total variance observed across all the

variables in the experiment; therefore, the component model is suitable for explaining the response pattern(s) of the evaluated oat varieties and their variables.

Table 5. Eigenvectors and proportion of variance explained by the two principal components (PC) in the oat study across two fall-winter growing seasons and three planting dates.

Variables	Characteristic vectors	
	PC1	PC2
Yield	0.070	0.661
Flowering	-0.423	-0.034
Plant height	0.406	-0.131
Panicle length	0.408	0.113
Thousand-seed weight	0.028	0.683
Hectoliter weight	-0.386	0.207
Characteristic value	5.568	2.050
Percentage of variance explained	69.6	25.6

The distribution of the four varieties is shown in Figure 3, with the Obsidiana variety characterized by the highest yields and thousand-seed weights, but it also exhibited a short plant height and intermediate maturity (Espitia et al., 2007). Karma had a lower yield but outperforms Chihuahua and Menonita, with intermediate maturity, similar to that of Obsidiana, but earlier than Chihuahua and lower hectoliter weight than Obsidiana. Compared with the other evaluated genotypes, Menonita had the earliest flowering and the highest hectoliter weight, but its yield was low, similar to that of Chihuahua. Chihuahua was the latest to flower and had the lowest hectoliter weight, which puts it at a disadvantage compared with the other varieties since Obsidiana and Karma outperform it in yield and have fewer days to flowering; Menonita is the best option for environments (cycles and planting dates) where the production cycle is short because of its earlier maturity. In this context, Zamudio-Colunga et al. (2023) reported that the Chihuahua variety is susceptible to leaf and stem rust and is largely surpassed by newly released varieties.

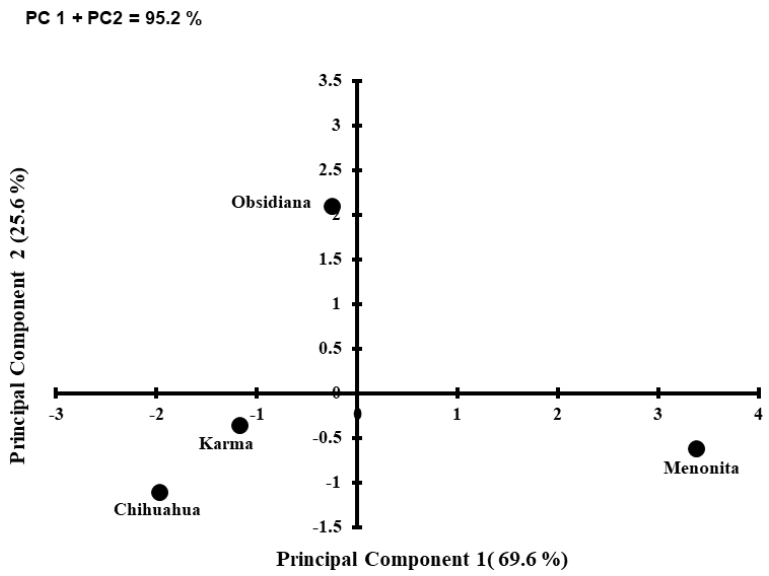


Figure 3. Variability of four oat varieties based on principal component analysis, evaluated over two fall–winter growing seasons and three planting dates under irrigated conditions, Celaya, Guanajuato.

Group II

Analysis of variance

The combined analysis (Table 6) revealed that the varieties accounted for the greatest proportion of variance in the expression of the variable's hectoliter weight, plant height, thousand-seed weight, days to flowering, and panicle length: 81.4%, 69.1%, 66.7%, 64.8%, and 45% of the total, respectively. Only in terms of yield was the contribution of variance due to cycles greater (71.2% of the total), and for days to flowering, it contributed 26.7% of the variance. The planting date was the second-largest source of variation, contributing 39.1% of the total variance in panicle length and, similarly, 17.3% of the total variance in plant height; these variables were surpassed in importance only by the varieties for the latter variable.

With respect to the interactions, the one with the greatest impact was cycles by variety on the yield variable, accounting for 13.4% of the total variance and 5.9% of the variance in panicle length, followed by cycles by planting date on thousand-seed weight, accounting for 10.3% of the total variance recorded and 5.3% of the total variance in plant height. Once again, the coefficients of variation confirm that the experimental error due to factors outside the statistical model used was low, which ensures the reliability of data collection and the uniform application of the evaluated treatments: cycles, planting dates, and varieties.

Table 6. Mean squares from the randomized complete block design with a split-plot arrangement (divided by time and space) in the combined analysis of two oat varieties over two fall-winter growing seasons in Celaya, Guanajuato

Sources of Variation	GL	Yield	Flowering	Plant Height	Panicle Length	Weight of a Thousand Seeds	Hectoliter Weight
Cicles (C)	1	33.33 *	693.44 **	5.44	0.0008	6.17	16.851
Blocks	2	1.84	0.33	180.19	0.0002	0.60	74.42
Planting Dates (PD)	2	2.27 *	110.58 **	529.19	0.0053	18.78	74.42 **
C* PD	2	1.26	64.36 **	162.03	0.0002	23.86	9.67 **
Varieties (V)	1	2.91 *	1681.0 **	2116.0 **	0.0061 *	155.0 **	460.89 **
C*V	1	6.28 **	1.78	128.44	0.0008	11.00	0.07
PD *V	2	0.38	18.58 *	78.08	0.0003	13.35	2.85
C* PD *V	2	0.37	24.36 **	41.03	0.00005	4.24	1.63
C. V. (C, %)		23.4	1.8	10.4	20.2	9.6	6.2
C. V. (PD, %)		12.4	1.9	8.6	14.1	9.8	3.2
C. V. (V, %)		12.4	1.6	10.7	10.2	8.9	3.3

GL: degrees of freedom; *, ** indicate significance at the 0.05 and 0.01 probability levels, respectively; CV: coefficient of variation

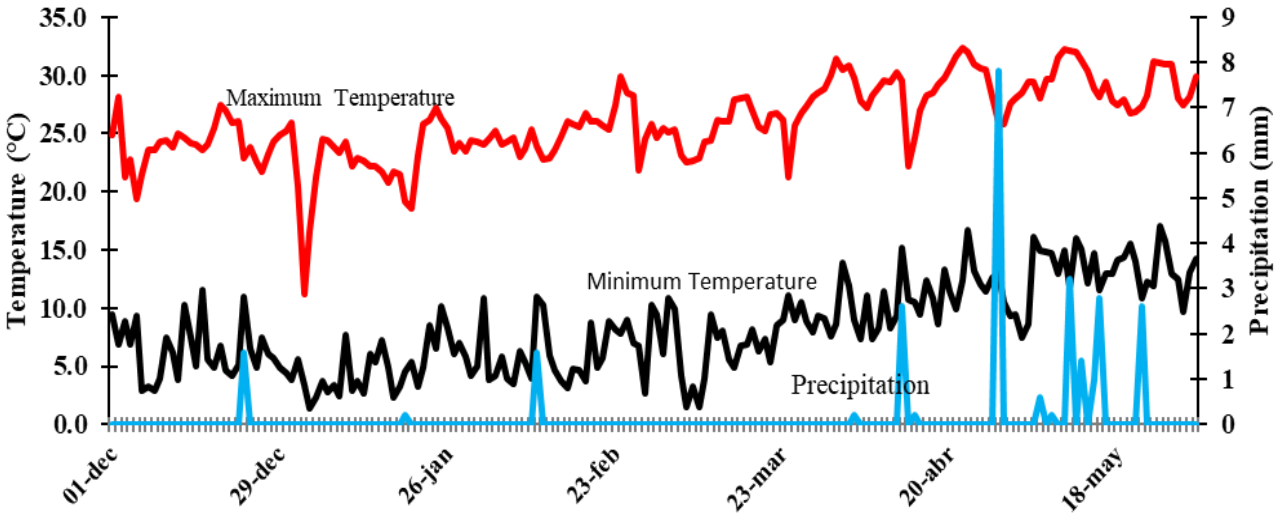


Figure 4. Minimum and maximum temperatures and precipitation in the town of Celaya, Guanajuato, during the first evaluation cycle.

Mean test

With respect to the varieties evaluated, the data in Table 7 show that, as in Group I, Variety 3 outperforms Variety 1, surpassing it statistically ($p \leq 0.05$), with 1.9 t ha^{-1} of seed and 8.8 more days of flowering; although no significant differences were detected, a trend toward higher seed hectoliter weight was observed in cycle 3. The environmental differences are likely associated with higher precipitation during the vegetative phase of the third evaluation cycle and the presence of rain during the harvest stage in cycle 1 (Figures 2 and 4, respectively); this pattern is consistent with that reported by Rodríguez-Herrera et al. (2020).

Table 7. Mean test for two fall-winter planting cycles, using two oat varieties, evaluated in Celaya, Guanajuato.

Cycles	Yield (t ha^{-1})	Flowering (days)	Plant Height (cm)	Panicle Length (m)	Weight of a Thousand Seeds (g)	Hectoliter Weight (kg hL^{-1})
Cycle 3	5.256 a	96.0 a	114.8a	0.245a	31.400a	42.312a
Cycle 1	3.332 b	87.2 b	115.6a	0.254a	32.228a	40.944a
MSD (p ≤ 0.05)	1.442	2.390	17.192	0.072	4.407	3.712

MSD: Minimally significant difference

Among the planting dates (Table 8), and similar to the response of the first group (Table 3), the early dates (December 1 and 15) presented better environmental conditions ($p \leq 0.05$) for obtaining higher yields, outperforming the third planting date (December 31), with a similar response in plant height and days to flowering between the first two dates. Among the evaluated varieties, December 1 offered the best environmental conditions ($p \leq 0.05$) for achieving the highest hectoliter weight. In other words, as the growing cycle lengthened, yield, days to flowering, plant height, and hectoliter weight increased; this was true regardless of the cycles and varieties evaluated.

Table 8. Test of means for planting dates for two oat varieties evaluated over two fall–winter growing seasons in Celaya, Guanajuato.

Planting dates	Yield (t ha^{-1})	Flowering (days)	Plant Height (cm)	Panicle Length (m)	Weight of a Thousand Seeds (g)	Hectoliter Weight (kg hL^{-1})
December 01	4.546 a	93.2 a	114.7 ab	0.236 a	33.033 a	44.446 a
December 15	4.541 a	93.5 a	122.1 a	0.274 a	30.533 a	40.715 b
December 31	3.792 b	88.1 b	108.9 b	0.238 a	31.875 a	39.723 b
MSD (p ≤ 0.05)	0.607	2.017	11.284	0.039	3.568	1.510

MSD: Minimally significant difference

Among the varieties evaluated in this group (Table 9), Turquesa statistically outperformed ($p \leq 0.05$) Chihuahua in terms of yield, with 569 kg ha^{-1}; this was likely due to a higher thousand-seed weight (4.15 g) and a higher hectoliter weight (7.156 kg hL^{-1}) than Chihuahua. The latter, however, has a longer growing season (days to flowering), greater plant height, and longer panicle length; it did not exhibit sufficient physiological efficiency to outperform Turquesa in yield.

Table 9. Analysis of means for two oat varieties based on agronomic variables and seed quality, evaluated over two fall-winter growing seasons in Celaya, Guanajuato.

Varieties	Yield (tha^{-1})	Flowering (days)	Plant Height (cm)	Panicle Length (m)	Weight of a Thousand Seeds (g)	Hectoliter Weight ($kg\ hL^{-1}$)
Turquesa	4.579 a	84.3 b	107.6 b	0.236 b	33.888 a	45.206 a
Chihuahua	4.010 b	98.5 a	122.9 a	0.262 a	29.738 b	38.050 b
MSD ($p \leq 0.05$)	0.435	1.206	10.099	0.020	2.330	1.145

MSD: Minimally significant difference

Genotype–Environment Interaction Analysis (SREG biplot) Group I

On the basis of the results of the genotype analysis and the genotype–environment interaction (Figure 5), it can be concluded that compared with those in cycle 2, the response in cycle 3 was very different. The planting dates of December 15 and 31 in cycle 3 were similar in terms of yield expression among the oat varieties, with the former yielding the highest average. In the cycle 2 trials, the highest yield was obtained on December 1, and the date with the greatest environmental stress for the expression of this trait was December 31.

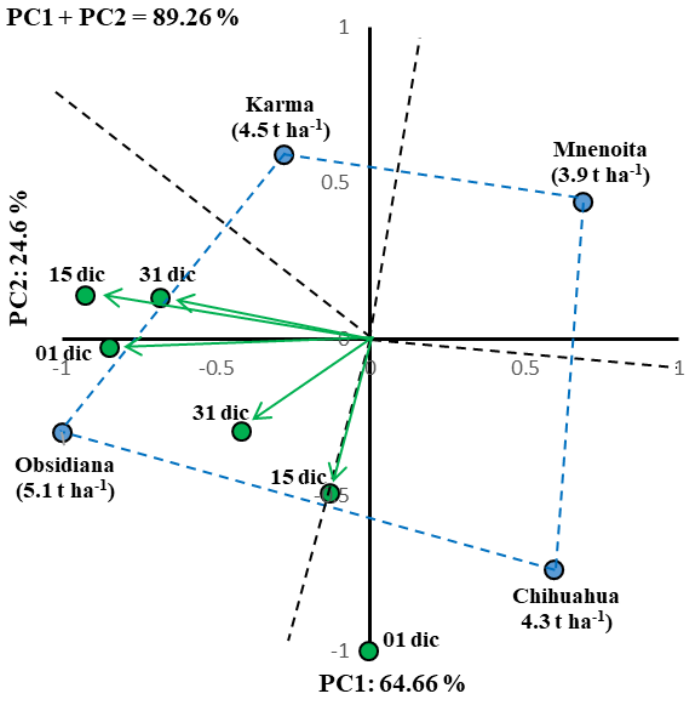


Figure 5. Genotype–environment interactions of four oat varieties across three planting dates and two fall–winter growing seasons, using the SREG model and the "which variety performed best where" framework; Celaya, Guanajuato.

Among the varieties, Obsidiana had the highest yield on the basis of the projection of the vector from the origin to the point representing each variety, followed by Karma, Chihuahua, and Menonita. Notably, Obsidiana achieved the highest yields on all the planting dates, except for December 1 of cycle 2, which is why it was located at the vertex of the "which-won-where" polygon among the genotypes

with respect to the environments, followed by Karma; at the bottom, without having stood out in yield on any of the planting dates, Chihuahua and Menonita were. On the basis of the above, it can be concluded that the highest yields were achieved during early planting dates (December 1 and 15), regardless of whether the growing seasons were favorable, such as in cycle 3, or stressful, such as in cycle 2. On the basis of the experimental data, among the early planting dates, there is a tendency to obtain higher yields in favorable environments (cycles or years) on December 15 and in less favorable environments on December 1.

Performance Stability Analysis

On the basis of the rotation of the x-axis, the definition of the average environment, and the drawing of a line perpendicular to the x-axis, the stability of the genotypes was determined (Figure 6). On the basis of the deviations from the x-axis of the average environment, compared with Menonita, Karma, and Chihuahua, the Obsidiana variety presented the highest agronomic stability (Becker, 1981) and yield; this makes it the most promising variety according to Yan and Kang (2003); the stability of Menonita and Karma was lower, with Chihuahua being the least stable. In other words, the yield of Chihuahua was difficult to predict on the basis of the evaluation cycles. The same figure shows that the y-axis allows the varieties' yields to be classified into two major groups: First, Chihuahua and Menonita had seed yields below the overall average, and in the second group, Obsidiana and Karma exceeded that average yield. The model's coefficient of determination is considered acceptable (89.26%), according to Yan and Rajcan (2002); thus, the interpretation of the figure is reliable.

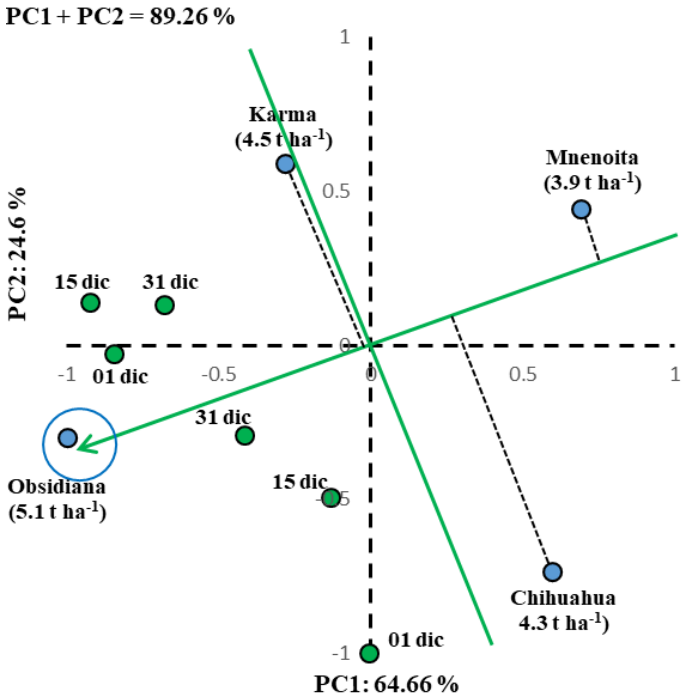


Figure 6. Yield stability of four oat varieties across six environments (with three planting dates and two growing seasons): Celaya and Guanajuato.

CONCLUSIONS

Growing conditions (cycles) and varieties are the most important factors for determining seed yield and physical quality and are closely related to a crop's phenological stage, as well as whether the variety is early-maturing or late-maturing. Among the six varieties evaluated, Obsidiana had the highest yield, and Menonita had the lowest yield because it is an early-maturing variety. Chihuahua was used as a control and had lower yields than Obsidiana did. The greatest difference among the environments was the total accumulated precipitation and its distribution during the crop development phase. Obsidiana is considered the most desirable variety because of its high and stable seed yield; it was the best variety in five of the nine evaluation environments, which was justified by its higher hectoliter weight and thousand-seed weight, as well as its smaller plant size.

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Chapter 9

Risk factors in loading and unloading operations of agricultural products in land transport units at a company in Veracruz, Mexico

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ABSTRACT

The present study aimed to identify risk factors in the loading and unloading areas of land transport units at a company located in Veracruz, Mexico. A descriptive approach was applied through the use of validated semistructured instruments to obtain information on operational, organizational, and occupational aspects across three stages: before, during, and after operations. The samples included 12 and 17 units for the unloading and loading of barley malt, respectively, and 5 units for the loading of corn semolina. Data were analyzed using absolute frequencies. The results revealed high levels of compliance in terms of operational procedures, equipment conditions, and documentation management. However, critical deficiencies were identified in the use of personal protective equipment (PPE), particularly the absence of safety helmets and the inconsistent use of goggles and gloves. Additionally, partial compliance was observed in cleaning activities, sealing procedures, and the verification of structural integrity in some transport units. External factors, such as railway disruptions and supply chain constraints, also influenced operational variability. The findings highlight the presence of organizational, operational, and behavioral risk factors that may compromise worker safety, product quality, and process efficiency. Strengthening preventive measures, standardizing procedures, and promoting a safety culture are essential to reduce risks and improve performance in agro-industrial logistics systems.

Keywords: Silos, training manual, industrial safety, operational efficiency

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INTRODUCTION

Transportation and logistics constitute strategic components of the global economy, as they facilitate the efficient flow of goods and services within supply chains (Hesse; Rodrigue, 2004; South Pacific Logistics, 2023). In this context, companies continuously seek to optimize their processes to provide more efficient, safe, and reliable services, thereby increasing their competitiveness in dynamic markets (Gunasekaran et al., 2017).

Within the agro-industrial sector, storage systems in silos represent a key element for the preservation and handling of bulk products, as they help maintain product quality and ensure the continuity of the supply chain. In these infrastructures, loading and unloading operations are particularly important, as they directly influence operational efficiency, process safety, and grain quality (Keklis et al., 2024; Adejumo; Haruna, 2013).

However, inadequate management of loading and unloading operations in grain storage systems can lead to significant occupational risks and product losses, thereby affecting the productivity of agro-industrial systems (Denisov et al., 2023). These operations are not risk-free, as factors such as prolonged waiting times, exposure to physical and mechanical hazards, and the absence or inadequate implementation of standardized procedures constitute recurring challenges that may compromise productivity, worker safety, and product quality (Kines et al., 2010).

In this context, it is essential to identify and evaluate the risks associated with these activities and to establish control measures to minimize their impact. The implementation of preventive strategies based on occupational health and safety management systems contributes not only to the reduction of workplace accidents but also to the improvement of operational efficiency and the strengthening of a safety culture within organizations (Fernández-Muñiz et al., 2009).

Therefore, the objective of this study was to describe the social, organizational, and operational risk factors in the silo area for barley malt and corn semolina in a company located in Veracruz, Mexico.

MATERIALS AND METHODS

Study Area

The study was conducted in the silo area for barley malt and corn semolina at a company located in Veracruz, Mexico.

Secondary Sources

The company's database was consulted to quantify the loading and unloading operations of barley malt, as well as the loading operations of corn semolina. Additionally, records of incoming railway units (railcars) were analyzed.

Sampling

The selection of sampled units was carried out randomly. A total of n = 12 and n = 17 units were evaluated for unloading and loading, respectively, of barley malt. For the corn semolina, a total of 5 loading units were selected.

Instrument Design

A validated semi structured questionnaire was designed as the data collection instrument. It was composed of the following sections: work area, transport unit, documentation, and personnel. The instrument was applied at three stages of the operational process: before the start of the operation, during the execution of activities, and after the completion of barley malt loading and unloading operations.

The questionnaire included dichotomous items (compliance/noncompliance), allowing for an objective assessment of the level of compliance with the established criteria at each stage of the process.

For the corn semolina, a specific validated questionnaire was designed, as the company operates under a different procedure in which only loading activities are performed, with no unloading processes. This second instrument included variables related to the transport unit, documentation, and personnel, with the aim of evaluating operational conditions and associated risk factors.

Data Analysis

Data were analyzed using absolute frequencies with Microsoft Excel Office 2019.

RESULTS AND DISCUSSION

The frequency of barley malt loading operations recorded during 2024 is shown in Figure 1. During the first months of the year, low operational activity was observed, with minimal records in February (1 load) and relatively low values in January, March, April, and May. This decrease in operations indicates a period of lower demand or limited logistical availability, which contrasts with the pattern observed in the second half of the year.

Starting in June, a significant increase in the number of loads was identified (49), followed by an increasing trend in July (53) and August (79), reaching a peak in September (97 loads). This increase can be partly explained by the dispatch of units to specific destinations, such as Monterrey, under double-trailer configurations, which resulted in duplicated records in the system, as each unit was registered as two tickets. Additionally, some of these operations were carried out during night shifts, contributing to the higher recorded operational volume.

In October (38) and November (53), a decrease in the frequency of loads was subsequently observed, suggesting a reduction in operational intensity following the peak reached in the third quarter of the year.

This behavior reflects significant operational variability, where periods of high demand (June–September) involve increased workload, a higher frequency of operations, and the potential execution of activities during extended or night shifts. These conditions may promote the emergence of risk factors such as worker fatigue, operational errors, procedural omissions, and reduced compliance with the use of personal protective equipment (PPE).

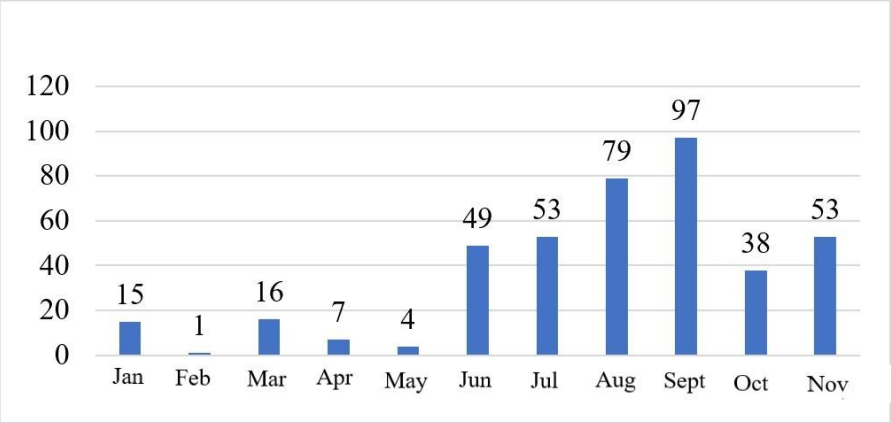


Figure 1. Absolute frequency of barley malt loading operations in 2024.

The frequency of corn semolina loading operations during 2024 is shown in Figure 2, revealing a relatively stable pattern in the first half of the year and a downward trend in the second half. However, this dynamic is not solely driven by internal operational demand but is also influenced by external factors that affect the availability of raw materials.

In particular, events such as railway blockades in the state of Puebla and protests at ports in the United States led to disruptions in the supply chain. These conditions limited the flow of the maize semolina to the company, forcing the implementation of stricter resource management strategies to prevent shortages.

As a result, a restrictive operational policy was adopted, with only one load per day carried out during certain periods. This measure allowed the continuity of the production process to be maintained; however, it directly affected the reduction in the volume of operations, particularly during the second half of the year.

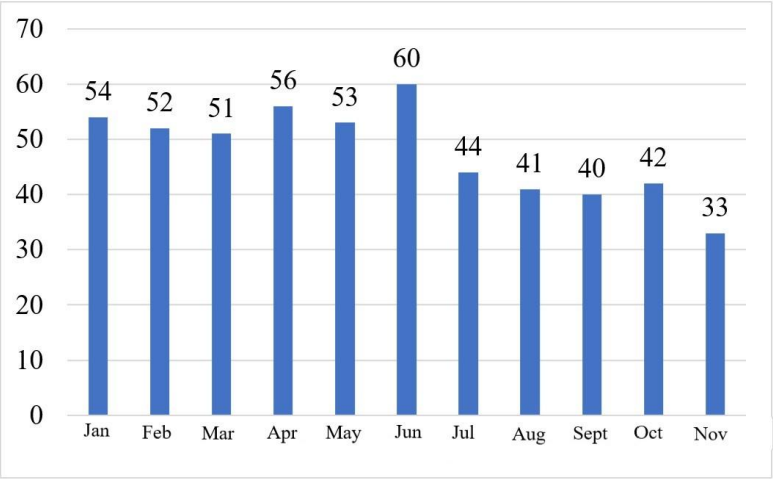


Figure 2. Absolute frequency of maize semolina loads in 2024.

The frequency of incoming railway units (railcars) with barley malt and corn semolina hoppers during 2024 is shown in Figure 3, with a total of 135 recorded units. During the first half of the year, the flow of units remained relatively stable, with values ranging between 12 and 16 hoppers per month. In particular, January (15), February (13), March (13), April (12), May (14), and June (16) reflect a consistent pattern in the supply of raw materials to the company.

However, starting in July (15), a more pronounced downward trend in the number of incoming units is observed, with significant decreases in August (8), September (9), and October (8), representing the lowest values of the analyzed period. A partial recovery is subsequently observed in November, with 12 units.

This decline in hopper flow coincides with external factors that affected the supply chain, such as railway closures operated by Ferrosur and protests at ports in the United States. These conditions limited the arrival of raw materials, creating a scenario of restricted supply.

From a risk management perspective, these results highlight the critical dependence of agro-industrial operations on railway transport infrastructure and on external, uncontrollable conditions. Disruptions in the flow of inputs not only affect operational continuity but also increase the vulnerability of the logistics system, emphasizing the need to implement contingency strategies and diversify supply channels.

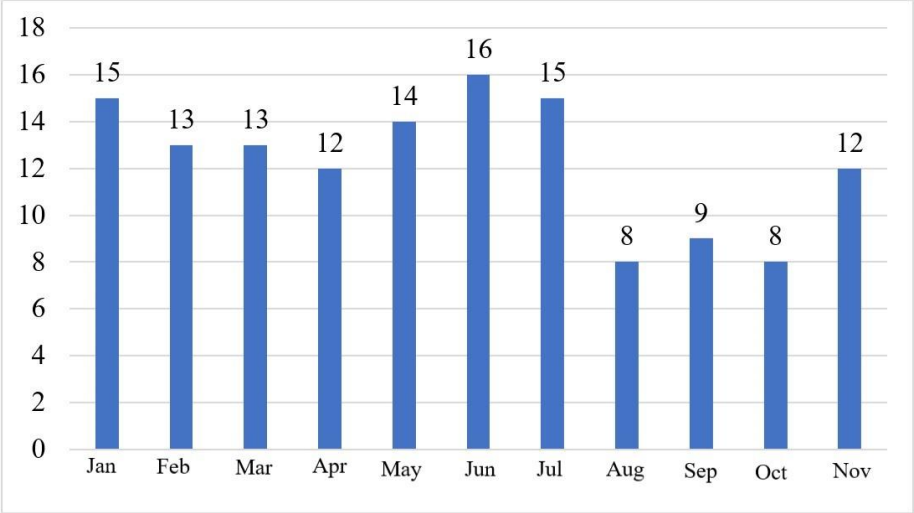


Figure 3. Number of incoming railcars carrying barley malt and maize semolina hoppers in 2024.

As shown in Figure 4, out of a total of 12 unloading operations evaluated, in 11 cases (91.7%), the area met the required conditions, whereas in 1 case (8.3%), it was not in optimal condition.

This high level of compliance suggests that, in general terms, the company maintains adequate working conditions in the area prior to the start of unloading operations, which is favorable from an operational safety perspective. A clean and obstacle-free area contributes to reducing physical risks, such as slips, trips, or interference during the handling of equipment and materials.

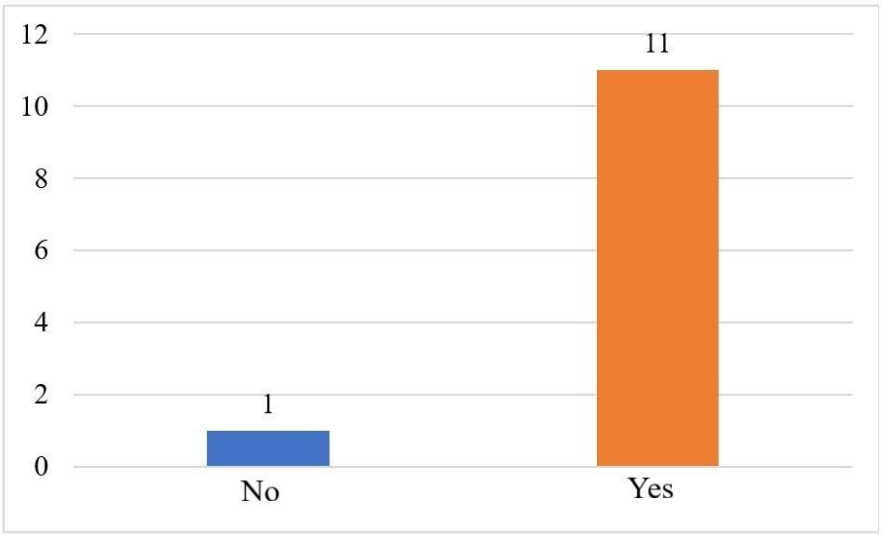


Figure 4. Evaluation of conditions in the barley malt unloading area.

The results indicate that, out of a total of 12 units evaluated, a 100% compliance rate was recorded in the presentation of the ticket, delivery notes, product quality information, and unit cleaning certificate (Figure 5). However, partial noncompliance was identified in the documentation related to the bill of lading, where 2 units (16.7%) did not have this document in full.

These findings suggest that although most documentation processes are standardized and properly implemented, specific deficiencies persist in the management of the bill of lading. According to the information collected, this situation occurred primarily in subcontracted units, in which drivers did not have a document because of administrative limitations on the part of the service provider.

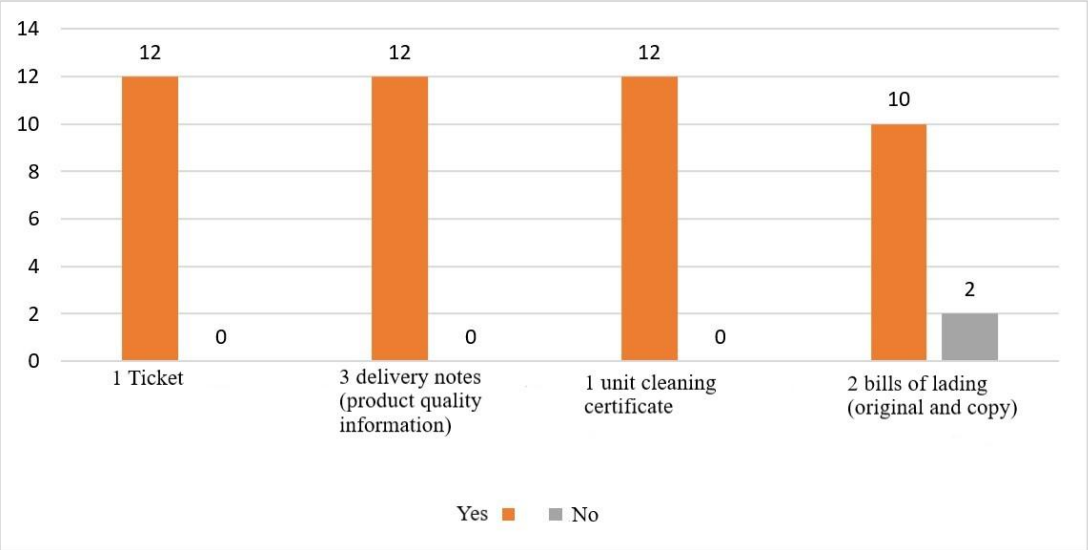


Figure 5. Assessment of source documentation for barley malt as an organizational risk factor.

As shown in Figure 6, in 100% of the units evaluated, all the equipment was in optimal operating condition prior to the start of operations, with no cases of noncompliance recorded.

These findings suggest adequate operational planning and coordination between the client company and the service provider, ensuring that equipment is properly prepared in advance through cleaning, disinfection, and technical inspection processes. Maintaining equipment under optimal conditions significantly reduces the likelihood of mechanical failure, process interruption, and potential workplace incidents during unloading operations.

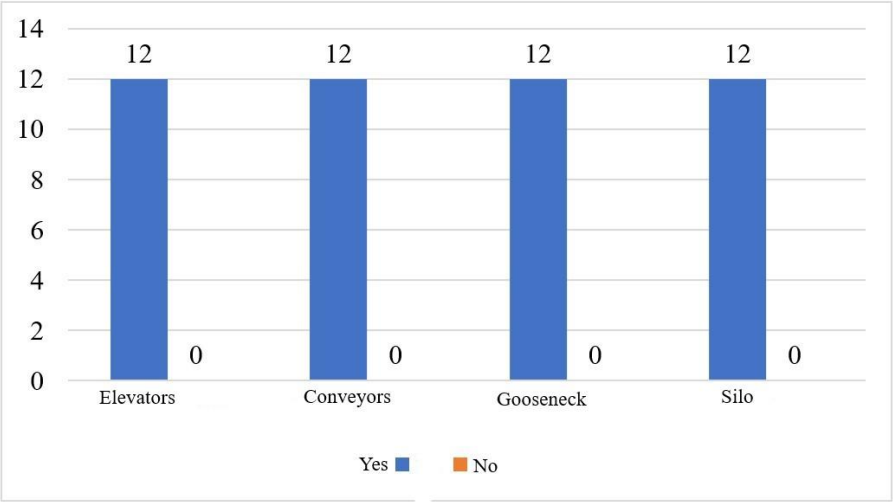


Figure 6. Assessment of the condition of receiving (unloading) equipment as an operational risk factor.

The results show full compliance (100%) in the use of gloves, safety boots, and masks across the 12 units evaluated (Figure 7). However, significant deficiencies were identified in other protective equipment. In the case of safety helmets, total noncompliance was recorded (0% usage), as none of the evaluated units had personnel equipped with this item, which is associated with a lack of provision by the company.

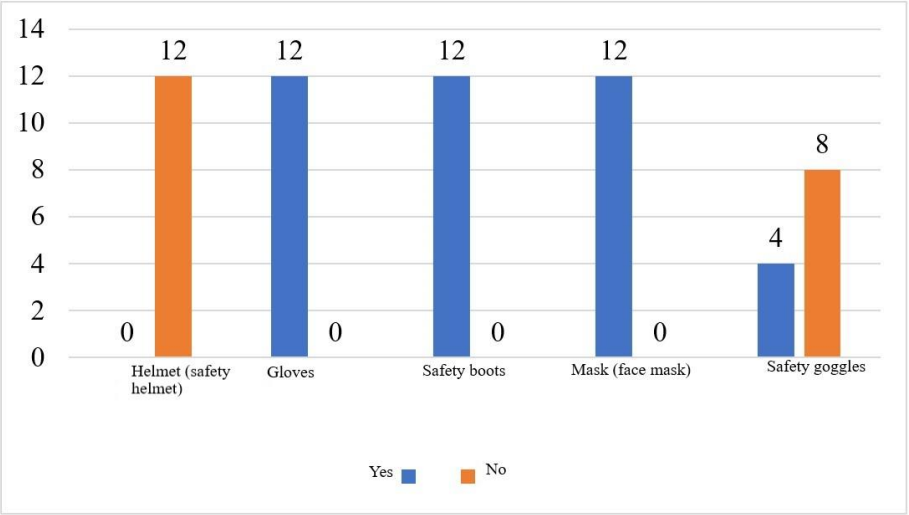


Figure 7. Level of compliance with the use of personal protective equipment (PPE) during unloading operations.

On the other hand, the use of safety goggles showed partial compliance, with only 4 out of 12 units (33.3%) using this equipment, while in 8 cases (66.7%), it was not used, despite having been provided. According to the information collected, this situation is related mainly to behavioral factors, such as perceived discomfort during task execution or voluntary omission by personnel, derived from habitual practices without the use of this type of protection.

These findings highlight the presence of organizational and behavioral risk factors, as the lack of helmet provisions and the inconsistent use of safety goggles increase exposure to physical hazards such as impacts, falling objects, and airborne particles.

The results shown in Figure 8 indicate a high level of compliance under operational conditions, as 100% of the units sampled the agricultural product, verified that it met the quality parameters, followed the established unloading procedure, ensured that the unloaded product remained free of impurities or damage, and continuously monitored the process to detect potential anomalies.

However, a specific instance of noncompliance was identified regarding the presence of security seals on the unit gates. In this case, 1 out of the 12 evaluated units (8.3%) did not have these seals, whereas 11 units (91.7%) complied with this requirement. Although minor, these findings represent a relevant issue from the perspective of operational safety and product traceability, as the absence of seals may compromise integrity control during transport.

Overall, the results demonstrate adequate control in the execution of unloading operations; however, the lack of compliance in the use of security seals highlights the need to strengthen preoperational inspection and documentation supervision mechanisms to ensure full compliance with protocols and reduce risks associated with the handling and control of barley malt.

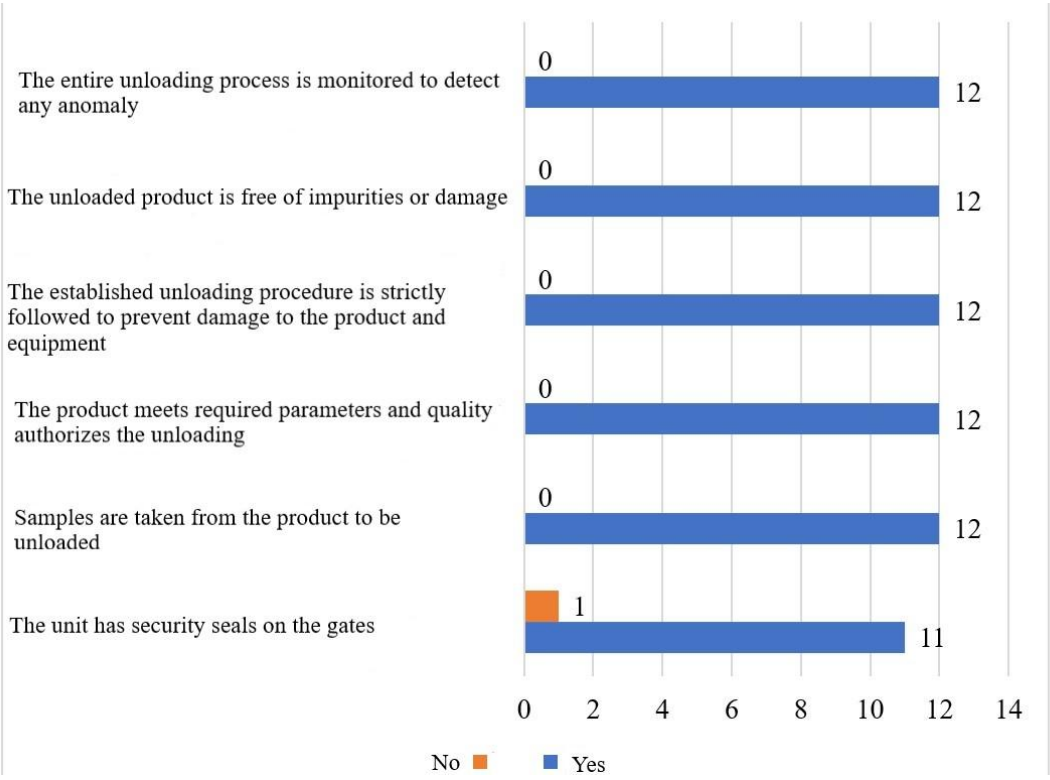


Figure 8. Verification of operational conditions during barley malt unloading as a risk control factor.

The full compliance (100%) in the recording of operations is shown in Figure 9, as all 12 evaluated units correctly completed unloading data, including date, time, unloaded quantity, and granulometric analysis results.

However, deficiencies were identified in other critical closing activities. In particular, 83.3% of the units complied with valve closure and disconnection of the unloading equipment. Similarly, a low level of compliance was observed in the thorough cleaning of the unloading area and the equipment used, with only 33.3% performing this activity, whereas in 66.7% of the cases, it was not carried out completely.

These results reveal the presence of operational and organizational risk factors, particularly in post unloading activities, where the omission of gate closure may lead to product spills, affecting both workplace safety and process efficiency. Similarly, incomplete cleaning of the area and equipment may result in residue accumulation, increasing the risk of contamination and hindering optimal conditions for subsequent unloading operations.

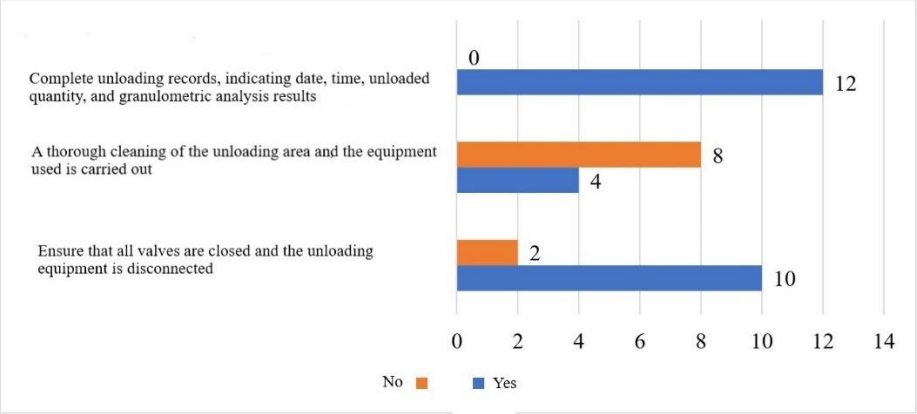


Figure 9. Verification of critical activities at the end of barley malt unloading as operational risk factors.

The level of compliance in the delivery of documentation to the carrier upon completion of unloading operations is shown in Figure 10. The results indicate 100% compliance, as in all cases, the delivery note, a copy of the weighbridge ticket, and the original bill of lading were properly provided.

These findings indicate that the documentation closing process is adequately standardized and controlled, ensuring product traceability, verification of delivered weights, and compliance with administrative and regulatory requirements in freight transport. Furthermore, the proper delivery of this documentation helps prevent inconsistencies in records and potential conflicts among the parties involved in the operation.

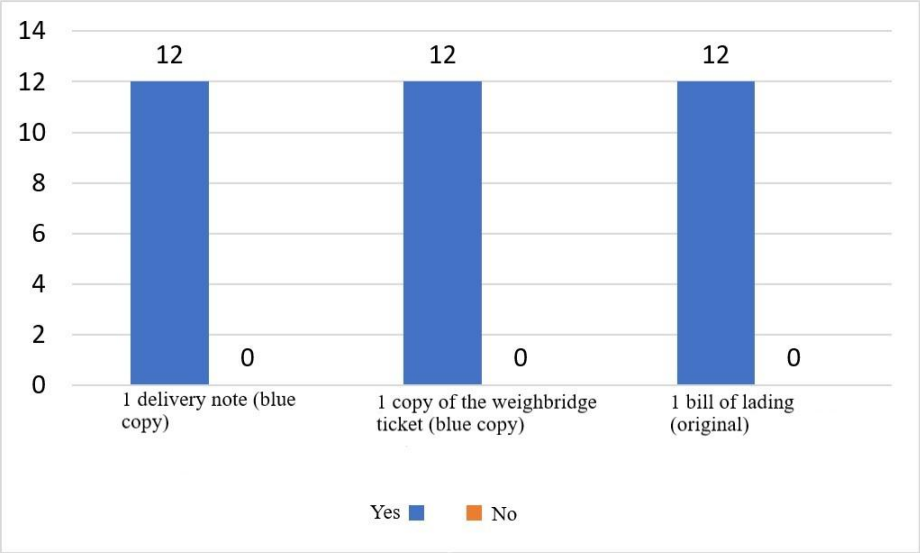


Figure 10. Compliance with the delivery of documentation to the carrier upon completion of barley malt unloading.

The evaluation of preoperational conditions prior to the start of barley malt loading operations, which is based on a checklist applied to 17 transport units, is presented in Figure 11. The results indicate a high level of compliance across most of the evaluated criteria, as in 100% of the cases, operators had a bill of lading, and it was verified that the vehicle floor was in suitable condition to prevent contamination, spillage, or damage to the bulk product.

However, partial noncompliance was identified in the cleanliness condition of the units, where 88.2% were clean, dry, and free of contaminants, while 11.8% did not meet this requirement. According to the recorded observations, residues such as branches and leaves were detected inside the cargo compartments of these units, indicating a potential risk of product contamination.

These results indicate that although documentation controls and structural inspection of the vehicles are well implemented, operational risk factors related to unit cleanliness persist. This aspect is critical, as the presence of physical contaminants may compromise product quality and affect food safety standards. Therefore, strengthening preloading inspection and cleaning procedures and enhancing supervision are recommended to ensure full compliance with the established criteria.

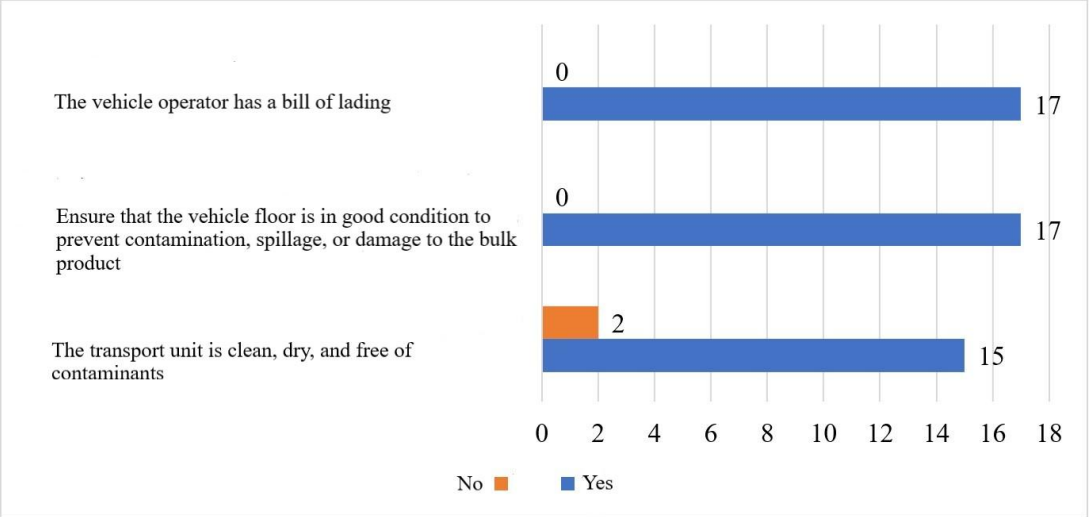


Figure 11. Verification of the initial conditions of transport units as a risk control factor.

An evaluation of the operational conditions of the loading system used during barley malt handling operations, considering components such as conveyors, elevators, dust extraction systems, and truck hoppers, is shown in Figure 12. The results show full compliance (100%) across the 17 evaluated units, as all system components were in optimal operating condition, with no cases of noncompliance recorded.

This performance reflects effective operational coordination between the client company and the service provider, where prior scheduling of activities allows for the preparation of equipment through cleaning, sanitization, and technical inspection processes. Maintaining the loading system under optimal conditions significantly reduces the likelihood of mechanical failure, dust generation, product contamination, and potential incidents during operations.

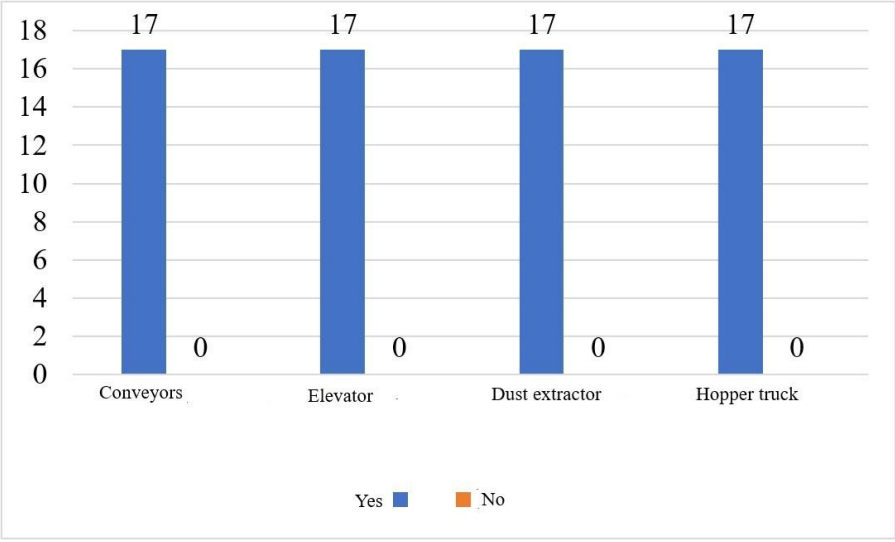


Figure 12. Verification of the loading system as an operational risk control factor.

The use of personal protective equipment (PPE) by personnel during barley malt loading operations is shown in Figure 13, considering a sample of 17 units. The results reveal significant differences in the level of compliance depending on the type of equipment.

In the case of safety helmets, total noncompliance (0% usage) was recorded, as none of the evaluated units had personnel equipped with this item, which is primarily associated with the lack of provision by the company. In contrast, full compliance (100%) was observed in the use of masks and safety boots.

With respect to safety goggles, partial compliance was identified, with 52.9% of units using this equipment and 47.1% not. Similarly, the use of gloves also resulted in partial compliance, with 47.1% of units using them compared to 52.9% that did not.

Noncompliance with the use of goggles and gloves is not related to a lack of provision but rather to behavioral factors, such as perceived discomfort or habitual practices of performing tasks without this type of protection. These findings highlight the presence of organizational and behavioral risk factors, where the absence of helmets and the inconsistent use of other protective equipment increase exposure to physical hazards such as impacts, airborne particles, and limb injuries.

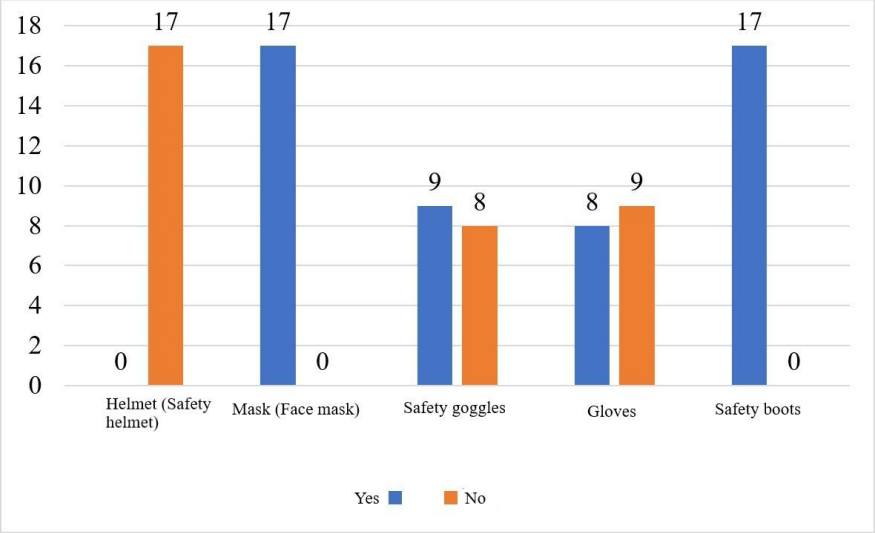


Figure 13. Level of compliance with the use of personal protective equipment (PPE) during loading operations.

The evaluation of compliance with operational procedures during the barley malt loading phase, which is based on a checklist applied to 17 units, is shown in Figure 14. The results show full compliance (100%) across all the evaluated variables, as in all the cases, constant communication was maintained with the unit operator, the loading process was continuously monitored to detect potential anomalies, and the established procedures were strictly followed to prevent damage to both the product and the equipment.

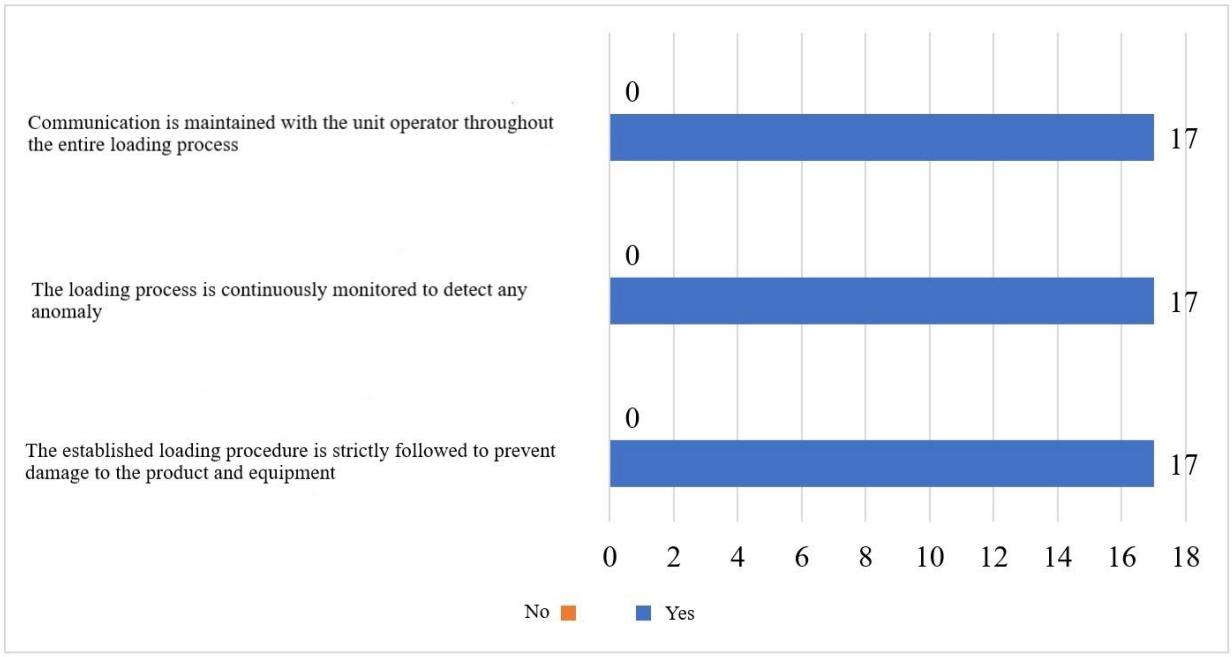


Figure 14. Verification of operational controls during loading as a risk mitigation factor.

With respect to the placement of security seals on vehicle doors and gates, 82.4% of the units complied with this requirement, whereas 17.6% did not place them properly (Figure 15). This noncompliance represents a critical issue, as it compromises product integrity and traceability during transport.

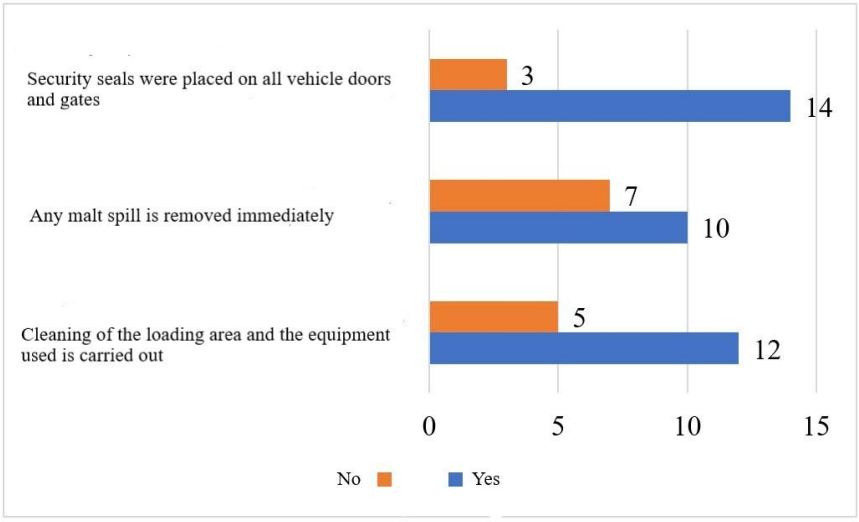


Figure 15. Verification of critical end-of-loading activities as operational risk factors.

With respect to the immediate removal of malt spills, compliance was recorded in 58.8% of the cases, whereas in 41.2%, this action was not carried out in a timely manner.

Similarly, 70.6% of the units were subjected to loading area cleaning, whereas 29.4% of the units were not adequately filled (Figure 15). These deficiencies are associated mainly with operational dynamics,

as when loading units arrive continuously, personnel tend to postpone cleaning activities until the end of the workday or until all scheduled units have been processed. However, this practice increases residue accumulation and makes it more difficult to maintain adequate working conditions in the operational area.

The results shown in Figure 16 indicate full compliance (100%) across all the evaluated variables, as in all the cases, the copy of the weighbridge ticket, the three delivery notes, the original bill of lading, and the loading inspection form were correctly provided.

These results indicate that the documentation closing process is fully standardized and controlled, ensuring that transport units can continue their journeys without administrative or legal issues. Furthermore, the proper delivery of documentation ensures product traceability, verification of transported weights, and consistency of the information recorded during the operation.

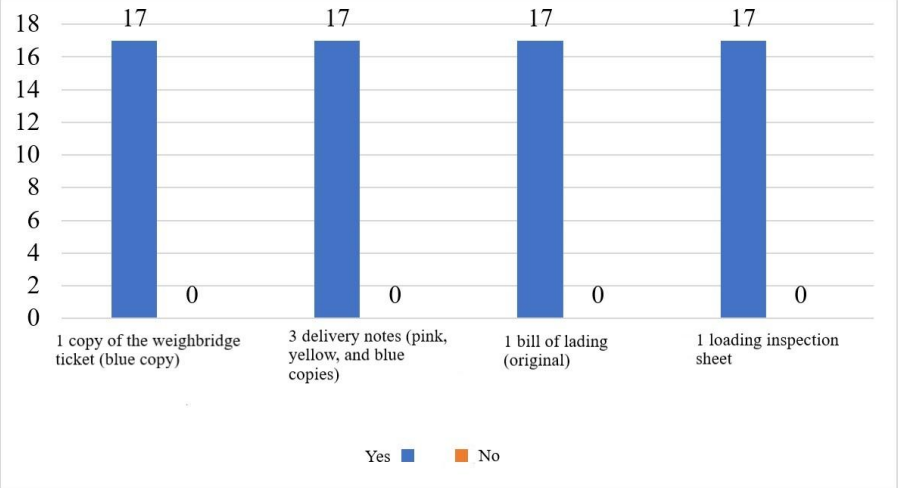


Figure 16. Verification of documentation closure as an organizational control factor in loading operations.

The evaluation of preoperational preparation conditions in units assigned for corn semolina loading, which is based on a checklist applied to 5 units, is shown in Figure 17. The results show 100% compliance for most of the evaluated variables, as in all the cases, the operator was in optimal condition, the units had valid insurance, no oil leaks were present, appropriate hoses for the operation were available, lighting and signaling systems were in good condition, and the tires met the required quality standards.

However, partial noncompliance was identified in the verification of the structural integrity of the hopper, where 80% of the units met this criterion, whereas 20% of possible deficiencies associated with cracks or signs of corrosion were detected. This finding represents a relevant issue from an operational

safety perspective, as the structural integrity of the hopper is essential for preventing material leakage, spills, or failures during the loading process.

These findings indicate that although preoperational controls are adequately implemented, an operational risk factor related to the physical condition of the hoppers persists, which may compromise both worker safety and product quality. Strengthening preoperational technical inspections and establishing stricter unit acceptance criteria are recommended to ensure full compliance with requirements and minimize risks associated with structural failure.

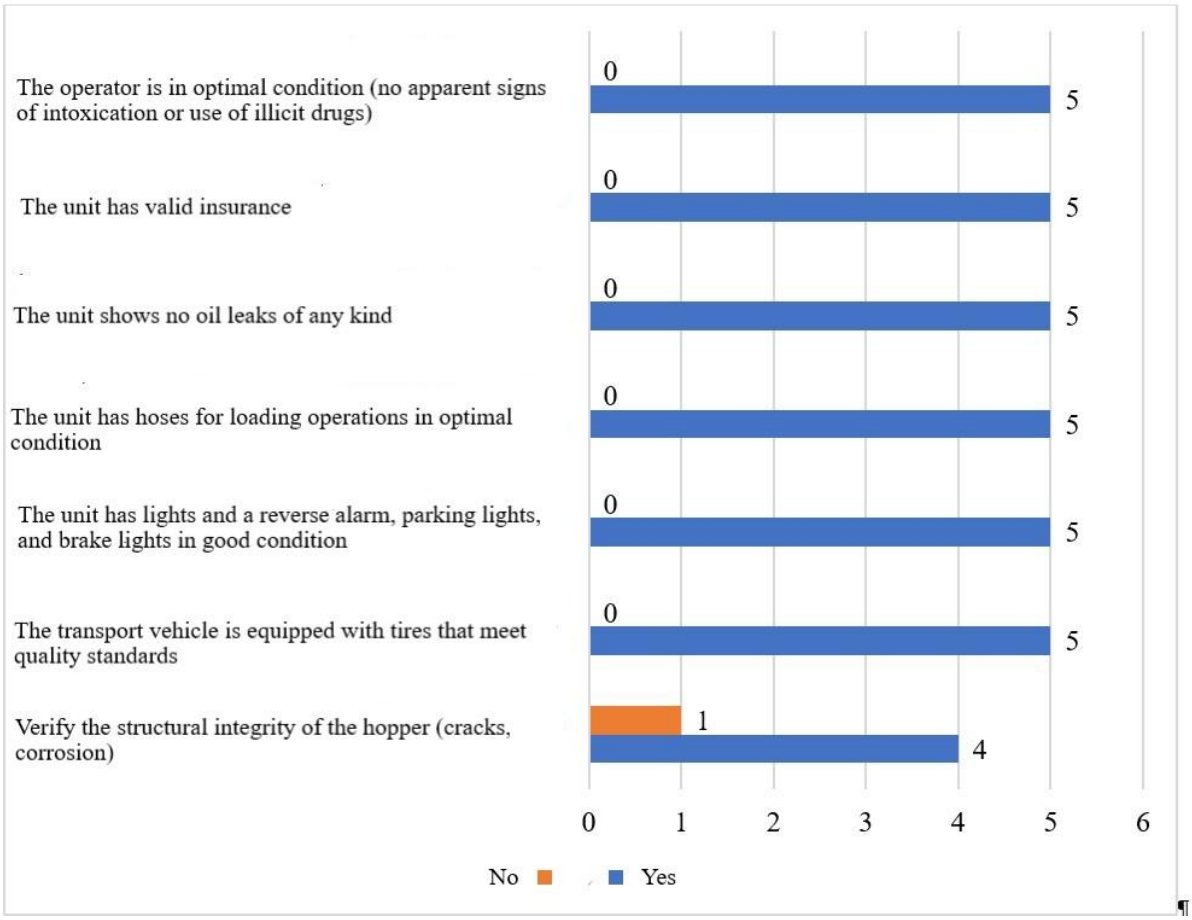


Figure 17. Verification of preoperational conditions as a risk control factor in corn semolina loading.

The evaluation of personal protective equipment (PPE) use during corn semolina loading operations is shown in Figure 18. The results show full compliance (100%) in the use of safety helmets, gloves, safety boots, and safety goggles, as personnel in all evaluated units were equipped with these protective items.

However, deficiencies were identified in other PPE components. In the case of masks, total noncompliance was recorded, as none of the evaluated units (0%) used this equipment. With respect to

hearing protection, partial compliance was observed, with only 40% of units using earplugs and 60% not using them.

The use of earplugs and masks by operators is primarily associated with specific workplace conditions, such as exposure to noise and dust; however, their use is not fully standardized among personnel. These results suggest the presence of occupational and behavioral risk factors, where the lack of mask use and the partial adoption of hearing protection increase exposure to risks associated with airborne particles and elevated noise levels.

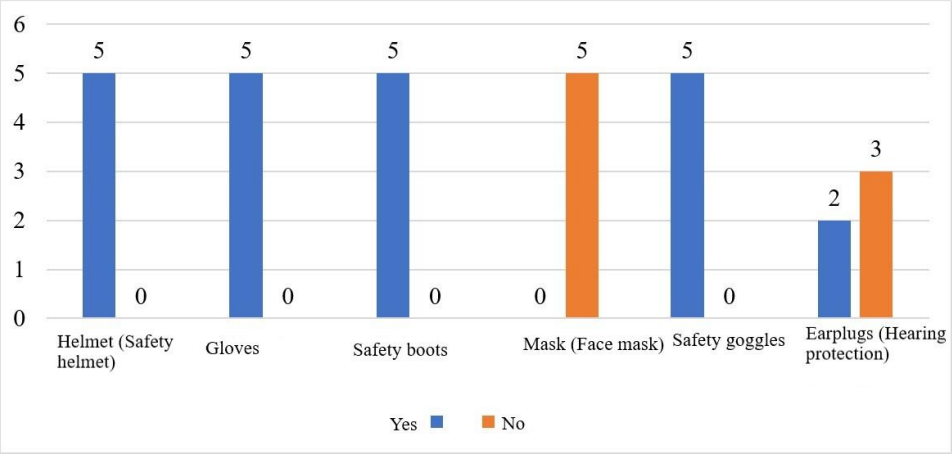


Figure 18. Verification of PPE use as an occupational risk factor in maize semolina loading.

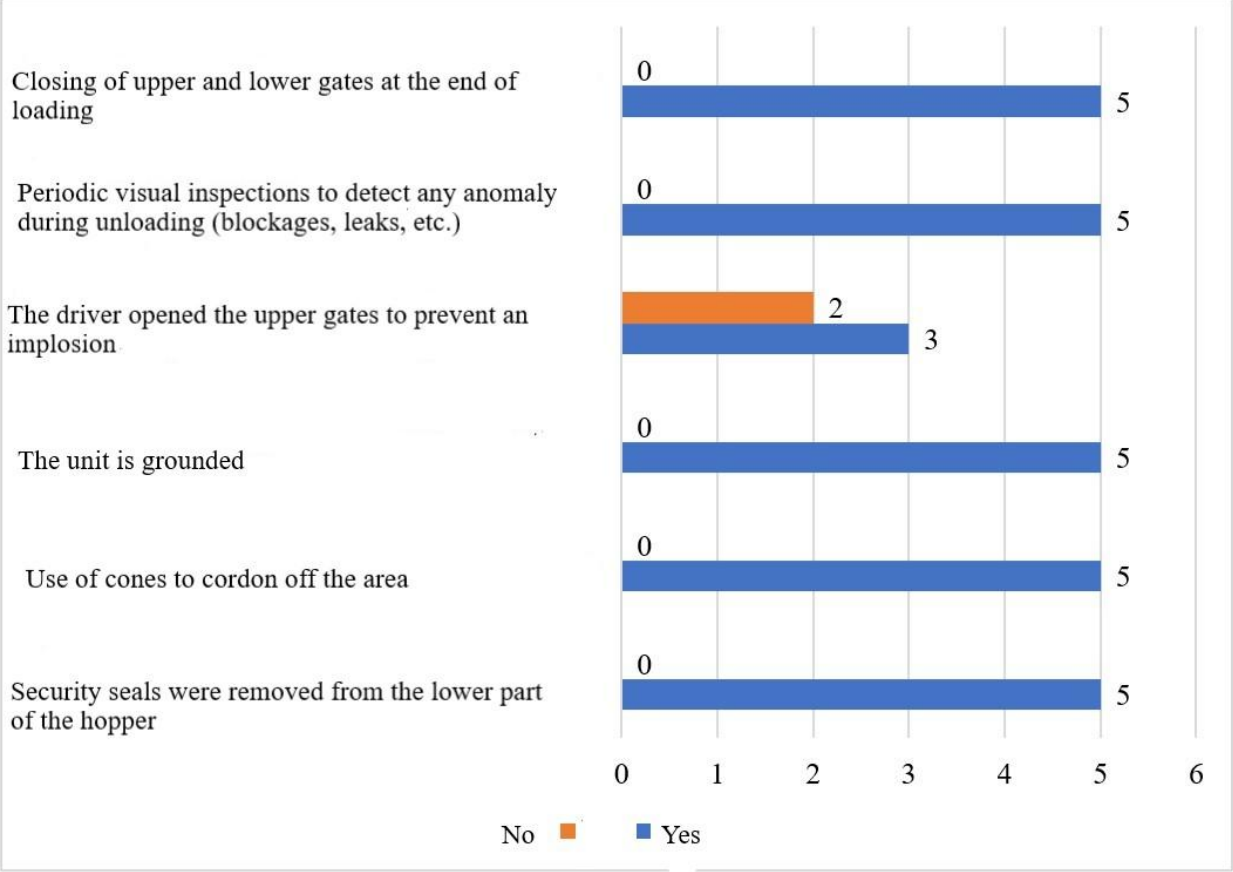


Figure 19. Verification of operational conditions during maize semolina loading as a risk control factor.

The evaluation of compliance with operational conditions during the corn semolina loading phase is shown in Figure 19. The results show a high level of compliance across most of the evaluated variables, as in 100% of the cases, periodic visual inspections were conducted to detect anomalies, gate closure was performed at the end of loading, the unit was properly grounded, safety cones were used to cordon off the area, and security seals were removed from the lower part of the hopper prior to the operation.

Partial compliance was identified in the variable related to the opening of upper hatches by the operator to prevent possible implosion during the process. In this case, 60% of the participants performed this action properly, whereas 40% did not, indicating relevant noncompliance.

The evaluation of compliance with the activities required for the release of units upon completion of the corn semolina loading process is shown in Figure 20. In 100% of the cases analyzed in this study, valve sealing was carried out using preassigned seals, as well as the final inspection of the hopper truck for corn semolina sampling.

This behavior reflects adequate standardization of closing procedures, where the involvement of responsible personnel, such as supervisors, ensures compliance with critical activities before unit departure. Proper valve sealing helps maintain the integrity of the corn semolina during transport, while final inspection and sampling allow verification of its quality and traceability.

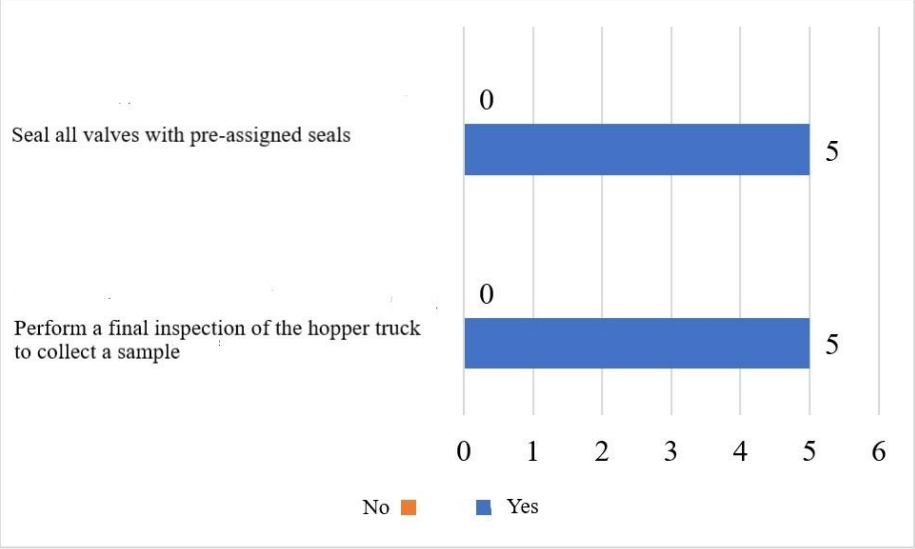


Figure 20. Verification of critical activities for unit release as a risk control factor.

The evaluation of compliance with the delivery of the documentation required for unit release upon completion of the corn semolina loading process is shown in Figure 21. A 100% compliance rate was observed across all the evaluated variables, as in all the cases, the three copies of the bill of lading, the weighbridge ticket copy, dispatch orders, the certificate of analysis (COA), and the unit cleaning certificate were correctly provided.

This behavior indicates that the documentation closing process is adequately standardized and controlled, ensuring the proper release of units without administrative or regulatory issues. Furthermore, the complete delivery of documentation guarantees product traceability, verification of transported volumes, and compliance with established quality and food safety requirements.

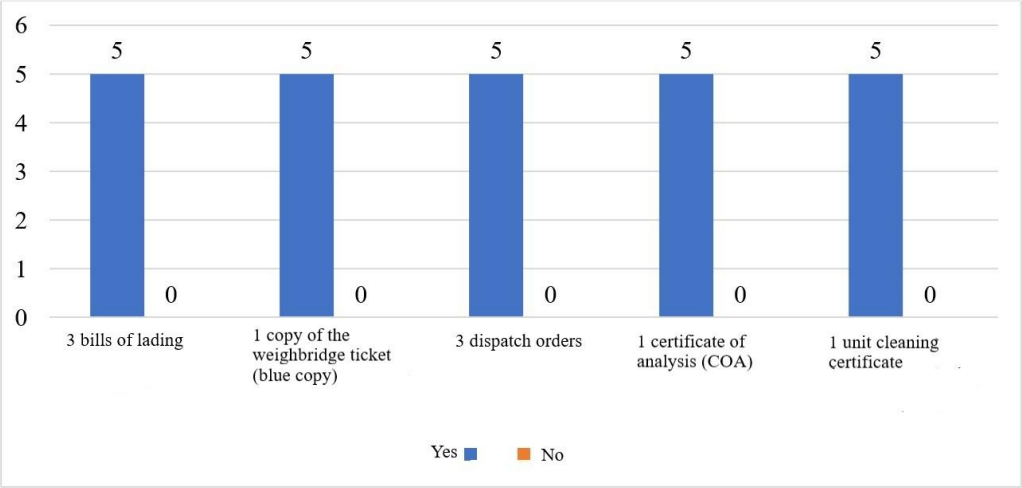


Figure 21. Verification of documentation closure as an organizational control factor in unit release.

CONCLUSIONS

Loading and unloading operations of agricultural products in land transport units represent a critical component of the agro-industrial supply chain, as they involve multiple operational, organizational, and occupational risk factors. The findings of this study indicate that although there is a high level of compliance in aspects related to documentation, equipment conditions, and operational procedures, significant deficiencies persist that may compromise worker safety and product quality.

In particular, deficiencies were identified in the use and provision of personal protective equipment (PPE), highlighting the complete absence of safety helmets and the inconsistent use of goggles and gloves, which reflects the presence of both behavioral and organizational factors. Additionally, noncompliance was detected in critical post operational activities, such as area cleaning, spill control, and the placement of security seals, increasing the likelihood of incidents and product contamination.

On the other hand, external factors, such as disruptions in railway infrastructure and limitations in raw material supply, demonstrate the vulnerability of the logistics system to uncontrollable conditions, emphasizing the need for more robust risk management strategies.

The implementation of comprehensive occupational health and safety management systems, the strengthening of personnel training, and continuous process supervision are recommended as key elements for mitigating risks, improving operational efficiency, and ensuring the sustainability of agro-industrial operations.

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Chapter 10

Physicochemical characterization of four varieties of durum wheat (*Triticum durum* Desf.) under full irrigation and reduced irrigation conditions

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ABSTRACT

In this study, the effect of reduced irrigation (RI) on durum wheat quality under semiarid conditions was assessed, considering genotype–water availability interactions. Four varieties (Isabel Oro, Don Lupe, Norocoro C2021, and Bayoyeca) were evaluated under full irrigation (FI) and RI using an integrated physicochemical, rheological, and color analysis. Moderate water deficit significantly altered grain composition: protein content increased across all varieties (e.g., Isabel Oro: 11.36→14.33%; Don Lupe: 11.24→14.33%), as did ash and lipid contents. Conversely, the hectoliter weight decreased in all the varieties (e.g., Bayoyeca: 82.96→77.83 kg hL^{-1}), whereas the thousand-kernel weight slightly increased. Rheologically, the RI increased wet and dry gluten (Isabel Oro: 28.50→32.97% and 9.63→11.27%, respectively) and dough stability (Don Lupe: 10.9→12.4 min), although the gluten index exhibited genotype-dependent responses (decreasing in Bayoyeca; stable in Isabel Oro). The color parameters showed minor changes in lightness (L*) and yellowness (b*). Multivariate analysis confirmed clear treatment–genotype differentiation. Overall, the RI improved the technological quality attributes (protein, gluten, and stability) at the expense of physical grain traits. Don Lupe and Isabel Oro demonstrated superior stability under water-limited conditions, highlighting their agronomic potential in semiarid environments.

Keywords: Reduced irrigation, water stress; wheat quality; gluten; rheological properties

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INTRODUCTION

Wheat production in arid and semiarid regions faces increasing pressure because of limited water availability, highlighting the need to optimize irrigation management without compromising yield or grain quality. In these systems, water stress not only affects productivity but also significantly alters the physicochemical composition and technological properties of wheat, which are critical for its industrial use (Buenrostro-Rodríguez et al., 2024; Yudewitz-Pohl, 2025). In particular, variables such as protein content, gluten quality, and sedimentation parameters are closely related to flour functionality, whereas characteristics such as hectoliter weight and ash content provide information on grain health, grain filling, and commercial value (Schuster et al., 2023; Yousuf et al., 2025).

Several studies have shown that the response of wheat to water stress depends largely on its genotype, highlighting the importance of evaluating specific varieties under controlled irrigation conditions. In this context, identifying materials with stable technological quality under stress conditions is a key strategy for sustaining production in scenarios of climate variability. Furthermore, the joint analysis of multiple variables allows for a better understanding of adaptation mechanisms and environment-induced changes in grain quality (Cadena-Cadena et al., 2025; Zhang et al., 2026).

In Mexico, wheat occupies a strategic position in both the food and economic spheres, as it constitutes one of the main sources of nutrients in the population's diet, second only to crops such as corn and beans (Palacios-Rojas et al., 2020). Its production is concentrated mainly in the North–Northwest and Bajío regions, where agroclimatic conditions favor its growth (Sharma et al., 2025). The state of Sonora stands out as the leading producer nationwide, accounting for more than half of the total production in recent years (SIAP, 2024). This leadership is attributed to the availability of fertile soils, suitable climatic conditions, the intensive use of agricultural technology, and the support of public policies geared toward the sector. Within this region, areas such as the Yaqui Valley represent highly significant production hubs because of their particularly favorable cultivation characteristics (Valenzuela-Antelo et al., 2018). However, despite these advantages, wheat faces significant challenges associated with increasing water scarcity, which increases its vulnerability to water stress conditions (Buenrostro-Rodríguez et al., 2022).

Wheat yield performance is typically analyzed on the basis of variables such as spike density, the number of grains per spike, and grain size. However, the evaluation of its physicochemical properties is often overlooked (Vincke et al., 2022). Wheat quality is determined by its nutritional composition and functional characteristics, including sensory attributes such as color, flavor, and aroma. Similarly, variations in protein, fiber, and mineral contents under different cropping systems are of great interest to the food industry and nutrition programs. Factors such as nutrient deficiency, soil acidity, and drought negatively affect both productivity and grain quality (Zeibig et al., 2022). Therefore, it is essential to characterize components such as proteins, carbohydrates, and minerals in grains produced under

different environmental conditions to optimize their nutritional value and health impact (Poole et al., 2021). This study posits that withholding irrigation during the final grain-filling stage causes changes in chemical composition, which could result in decreases in both yield and grain quality. Given the variability among genotypes in terms of their response to water deficit, restricted irrigation management is a useful strategy for evaluating stress tolerance and optimizing water use without compromising crop protein quality.

MATERIALS AND METHODS

Experimental site and conditions

In this study, four wheat varieties were evaluated, namely, Isabel Oro, Don Lupe, Norocoro C2021, and Bayoyeca, which were subjected to two contrasting water availability conditions: full irrigation and reduced irrigation. On the basis of this experimental design, eight treatments (M1–M8) were established, allowing for the analysis of both the individual effect of the genotype and the interaction between variety and the irrigation regime. This experimental approach facilitates the identification of distinct response patterns, as well as the detection of potential comparative advantages among varieties under stress conditions.

Proximate Chemical Analysis

Quantitative analyses were performed using standardized procedures of the Association of Official Analytical Chemists AOAC (2000).

Moisture Content

The moisture content of the food sample was determined following the protocol established in the AOAC method 950.46. Briefly, the sample was placed in a forced-air oven set at 105 °C and dried for 4–5 hours until water removal was achieved. After drying, the moisture percentage was calculated from the difference in sample weight before and after the process, and the appropriate equation was applied.

$$Moisture\ Content\ (\%) = \frac{\text{Weight loss}}{\text{Starting weight}}\ X100$$

Total ash

Approximately 3 g of the powdered sample was weighed into individual crucibles and incinerated at 550 °C for 6 hours. Following combustion, the crucibles were placed in a desiccator to cool and avoid moisture uptake and then weighed to determine the ash content.

$$\text{Total ashes (\%)} = \frac{W - Z}{N} X\ 100$$

where **W** represents the combined weight of the crucible and ash residue, **Z** corresponds to the weight of the empty crucible, and **N** indicates the initial weight of the sample.

Determination of Lipids

The crude fat content of the powdered sample was determined using the Soxhlet extraction method in accordance with AOAC Method 920.39 (2000), employing petroleum ether as the solvent. Approximately 3.0 g of sample was placed in prelabeled extraction thimbles and subjected to continuous extraction in a Soxhlet system for 24 hours. Upon completion, the solvent was evaporated, and the extraction flask was cooled prior to weighing to quantify the recovered lipid fraction.

$$\text{Lipids (\%)} = \frac{\text{Weight of lipids}}{\text{Sample weight}} X\ 100$$

Crude protein analysis

The crude protein content was determined by the micro-Kjeldahl method following AOAC 1990.04. A 0.15 g flour sample was digested with concentrated sulfuric acid and a catalyst until a clear solution was obtained. After dilution, the digest was distilled with 30% NaOH to release ammonia, which was collected in 2% boric acid with a methyl red indicator. The trapped ammonia was titrated with 0.1 N HCl to the endpoint. A blank was included, and the nitrogen content was calculated using the corresponding equation.

$$\text{Proteín (\%)} = \frac{(\text{N of HCl})(Volume\ of\ HCl\ used)(1.4\ \text{g/mol})(6.5)}{Sample\ grams}$$

Analysis of Crude Fiber

The crude fiber content was determined according to the standard procedures described by AOAC (2000). A known quantity of the sample was subjected to sequential acid and alkaline digestion to remove soluble materials. The remaining residue was filtered, thoroughly washed, dried, and weighed. The dried residue was subsequently incinerated at high temperature, and the weight loss after ashing was used to estimate the crude fiber content.

SDS-Sedimentation (mL)

Sodium dodecyl sulfate (SDS) sedimentation tests were conducted to evaluate the sedimentation capacity of flour proteins as an indicator of gluten quality. For this analysis, 1.0 g of flour sample, adjusted to a 14% moisture basis, was accurately weighed and transferred into a graduated sedimentation tube. Afterward, 10 mL of distilled water was added, and the tube was shaken vigorously to obtain a uniform suspension.

SDS Index (SDS/PROT)

After the sample was completely dispersed, 10 mL of SDS–lactic acid solution was added to the mixture. The tube was shaken for a standardized period to ensure proper interaction between the proteins and the reagent. The suspension was then allowed to stand undisturbed at room temperature for 20 minutes to allow sedimentation. At the end of the resting period, the sedimentation volume was recorded directly from the graduated scale of the tube and expressed in milliliters (mL).

The SDS sedimentation index (SDS/PROT) was calculated to standardize the sedimentation values according to the protein content of the flour samples. This index was obtained by dividing the SDS sedimentation volume (mL) by the crude protein content (%) previously determined using the micro-Kjeldahl method. The SDS index was calculated using the following equation:

$$SDS/PROT = \text{SDS sedimentation value (mL) Protein content (\%)}$$

The resulting value was expressed as millilitres per percentage of protein (mL/% protein), providing a normalized indicator of protein quality and gluten strength.

Wet gluten (%)

The wet gluten content was determined to evaluate the quantity of gluten-forming proteins present in the flour samples. For this analysis, 10 g of flour sample was accurately weighed and placed into a gluten-washing apparatus. Distilled water was added to form a dough, which was subsequently allowed to rest for a short period to ensure complete hydration of the proteins.

After the resting stage, the dough was washed under a continuous flow of water to remove starch and soluble components, leaving only the gluten network. The washing process continued until the wash water became clear, indicating the complete removal of nongluten materials. The remaining gluten mass was collected and weighed immediately. The wet gluten content was expressed as a percentage of the original sample weight using the corresponding equation (AACC, 2025; Coca Cadena, 1998).

$$\text{Wet Gluten (\%)} = \frac{\text{Weight of flour sample (g)}}{\text{Weight of wet gluten (g)}} \times 100$$

where Weight of wet gluten = weight of the wet gluten obtained (g) and Weight of flour sample = initial weight of the sample (g).

Dry gluten (%)

Metodología para Dry Gluten (%)

The dry gluten content was determined by drying the previously obtained wet gluten residue. The wet gluten sample was placed in a drying oven maintained at 105 °C and heated until a constant weight was achieved.

After drying, the sample was allowed to cool in a desiccator to prevent moisture absorption from the environment. The dried gluten residue was then weighed, and the dry gluten percentage was calculated on the basis of the initial sample weight using the appropriate equation (AACC, 2025).

$$\text{Dry Gluten (\%)} = \frac{\text{Weight of flour sample (g)}}{\text{Weight of dry gluten (g)}} \times 100$$

where Weight of dry gluten = weight of dry gluten after drying (g) and Weight of flour sample = initial weight of the sample (g).

Gluten Index (%)

The gluten index was determined to assess the strength and quality of the gluten network. The wet gluten obtained from the washing process was placed into a standardized centrifuge chamber equipped with a sieve. The sample was centrifuged under controlled conditions for a specified period.

Following centrifugation, the portion of gluten retained on the sieve was weighed, along with the total wet gluten weight. The gluten index was calculated as the percentage of gluten remaining on the sieve relative to the total wet gluten mass using the equation provided below. The results are expressed as a percentage (%), which represents the resistance of gluten to mechanical stress (AACC, 2025).

$$\text{Gluten Index (\%)} = \frac{\text{Total wet gluten weight (g)}}{\text{Weight of gluten retained on sieve (g)}} \times 100$$

where Weight of gluten retained on the sieve = gluten remaining on the sieve after centrifugation (g) and Total wet gluten weight = total weight of wet gluten before centrifugation (g).

Statical Analysis

Data were expressed as mean ± standard deviation. Statistical analyses were performed using a completely randomized factorial design (4 × 2), including four wheat varieties and two irrigation regimes. A two-way analysis of variance (ANOVA) was conducted to determine the effects of variety, irrigation regime, and their interaction. When significant differences were observed, means were compared using Tukey's test at $p \leq 0.05$. Multivariate analyses, including principal component analysis (PCA) and hierarchical clustering, were performed to explore the relationships among variables. All analyses were performed using IBM SPSS Statistics (version 22).

RESULTS AND DISCUSSION

A complete analysis of the wheat varieties evaluated under full-irrigation (RC) and reduced-irrigation (RR) conditions revealed different effects of genotype and water regime on the physical, chemical, rheological, and color properties of the grain. A consistent trend associated with moderate water stress (RR) was observed, particularly for variables related to component concentration and gluten quality.

Physicochemical properties of the grain

Thousand-kernel weight (TKW) showed moderate variation among the varieties, with values ranging from 77.8 to 80.2 g. Under RR conditions, a trend toward increased TKW was observed in all the varieties studied (Isabel Oro, 80.2 g; Don Lupe, 79.8 g) (Table 1). This behavior could be explained by reduced intraspike competition under water stress, which favors grain filling in a relatively small number of reproductive structures. However, this increase is not always consistent in the literature, where water stress frequently reduces grain size depending on its severity and timing. Pradeep et al. (2024) reported high genotypic variability in wheat varieties in terms of the contribution of spikes and awns to yield under stress.

Hectoliter weight (HW) showed the opposite trend, decreasing significantly under water stress in all the varieties. For example, Isabel Oro decreased from 81.56 to 78.53 kg hL^{-1}, whereas Bayoveca decreased from 82.96 to 77.83 kg hL^{-1}. This decrease suggests lower grain density, possibly associated with an alteration in endosperm compaction, a common phenomenon under water stress conditions. Water stress affects growth and photosynthesis through oxidative stress and physiological alterations. Morphological, osmotic, and molecular mechanisms, along with nutrients and hormonal regulators, contribute to drought tolerance. pH is a key indicator of commercial quality; therefore, this reduction may have negative implications for the industrial grading of the grain (Jain et al., 2019).

Proximate composition

The moisture content did not significantly differ among the treatments and remained within a narrow range (≈9–10%). This finding indicates that postharvest conditions or the hygroscopic equilibrium of the grain were homogeneous, allowing for reliable comparisons on a dry basis; similar results were obtained for other wheat varieties planted under similar conditions (Cadena-Cadena et al., 2025).

The ash content increased under RR in all the varieties. For example, Don Lupe increased from 1.34% to 1.64%, and Norocoro C2021 increased from 1.34% to 1.62%. This pattern suggests a higher concentration of minerals, possibly due to a reduced dilution effect under conditions of lower starch accumulation (Moreira et al., 2025).

Table 1. Grain quality parameters of durum wheat under different irrigation conditions.

Sample	Irrigation Type	Weight of 1000 grains (g)	Test WT (kg/hl)	Moisture	Ash	Fat (%)	Fiber	Protein
Isabel oro	RC	78.5±0.67	81.56±0.26^{b}	9.37±0.61^{a}	1.34±0.11^{a}	1.78±0.22^{a}	2.66±0.19^{a}	11.36±1.09^{a}
	RR	80.2±0.58	78.53±0.51^{a}	10.32±0.60^{a}	1.63±0.08^{a}	2.19±0.21^{bc}	2.9±0.65^{a}	14.33±0.54^{b}
Don lupe	RC	77.8±0.72	81.26±0.68^{b}	9.3±0.48^{a}	1.34±0.11^{a}	1.75±0.31^{a}	3.22±0.65^{a}	11.24±1.02^{a}
	RR	79.8±0.61	78.36±0.76^{a}	9.66±0.66^{a}	1.64±0.07^{a}	2.15±0.69^{c}	3.01±0.37^{a}	14.33±0.55^{b}
Bayoyeca	RC	79.2±0.85	82.96±0.54^{b}	9.03±1.50^{a}	1.55±0.16^{a}	1.85±0.28^{ab}	2.63±0.27^{a}	13.34±0.57$^{a\,b}$
	RR	80±0.77	77.83±0.17^{a}	8.8±0.87^{a}	1.45±0.08^{a}	1.8±0.33^{a}	2.76±0.36^{a}	14.56±1.47^{b}
Norocoro C2021	RC	81.54±0.59	81.86±0.69^{b}	9.41±1.82^{a}	1.34±0.03^{a}	1.75±0.66^{a}	2.48±0.31^{a}	13.02±0.59$^{a\,b}$
	RR	78.5±0.67	77.13±0.58^{a}	10.14±0.46^{a}	1.62±0.09^{a}	2.19±0.78^{bc}	3.56±0.75^{a}	14.34±1.32^{b}

* Different lowercase letters within the same column indicate significant differences according to Tukey's test ($p \leq 0.05$).

Crude fat also increased under RR conditions, with values increasing from approximately 1.75–1.85% under RC to 2.15–2.19% under RR. This increase could be related to metabolic adaptations of the grain in response to stress, where lipids play a role in the stability of cell membranes (Moreira et al., 2025). The fiber content did not clearly vary, although in some cases, such as Norocoro C2021, an increase was observed (from 2.48% to 3.56%). This could be associated with structural modifications in the cell wall under water stress. Moreover, the protein content consistently increased under RR in all the varieties. Isabel Oro increased from 11.36% to 14.33%, and Bayoveca increased from 13.34% to 14.56%. This phenomenon is attributed to lower carbohydrate accumulation, which increases the relative proportion of nitrogen in the grain. From a technological standpoint, this increase is favorable, as protein is directly related to baking quality. In the drought-tolerant and drought-susceptible wheat varieties C-306, PBW-175, NI-5439, HD-2329, and PBW-343, which were grown under controlled conditions, water stress reduced the yield and total protein content but increased the protein concentration. Factors such as precipitation, type of drought, and nitrogen management can influence yield response (Singh et al., 2008; Wan et al., 2022).

Rheological properties of gluten

The wet gluten (WG) and dry gluten (DG) contents increased under RR, particularly in Isabel Oro and Don Lupe (Table 2). For example, Isabel Oro increased from 28.50% to 32.97% for the GH and from 9.63% to 11.27% for the GS. These results are consistent with the increase in total protein, since gluten represents the functional protein fraction of wheat. However, the protein composition of wheat depends on genetic and environmental factors such as soil, temperature, drought, CO_2, and fertilization, which significantly influence gluten quality and crop yield (Wieser et al., 2023)

Table 2. Parámetros de calidad del grano en trigo duro bajo diferentes condiciones de riego.

Sample	Irrigation Type	SDS (%)	Index (mL/% protein)	Wet Gluten (%)	Dry Gluten (%)	Gluten Index (%)
Isabel oro	RC	12.04±1.2^{a}	0.72±0.025^{a}	28.50±1.25^{b}	9.63±1.25^{a}	78.00±2.87^{a}
	RR	9.92±0.70^{b}	0.90±0.037^{a}	32.97±2.42^{ab}	11.27±1.32^{ab}	78.40±3.94^{a}
Don lupe	RC	9.33±0.25^{b}	0.78±0.038^{a}	27.07±1.78^{a}	9.20±0.57^{a}	81.57±3.88^{ab}
	RR	11.00±0.98^{a}	0.80±0.098^{a}	31.67±1.71^{ab}	11.00±1.98^{ab}	80.23±2.14^{ab}
Bayoyeca	RC	10.30±0.25^{b}	0.88±0.12^{a}	30.83±1.32^{ab}	10.43±1.23^{ab}	84.17±3.87^{ab}
	RR	11.23±0.86^{a}	0.87±0.078^{a}	29.01±1.12^{a}	9.87±0.95^{a}	75.50±2.57^{ab}
Norocoro C2021	RC	9.37±0.77^{b}	0.93±0.018^{a}	30.57±1.39^{ab}	10.30±0.87^{ab}	86.23±1.31^{ab}
	RR	11.20±0.81^{a}	0.77±0.054^{a}	30.98±1.27^{ab}	10.40±0.84^{ab}	72.67±1.87^{c}

*Different lowercase letters within the same column indicate significant differences according to Tukey's test ($p \leq 0.05$).

The gluten index (GI) (Table 2), an indicator of gluten quality, showed variable responses depending on the genotype. While it remained relatively stable (~78%) in Isabel Oro, it decreased notably in Bayoveca under RR (from 84.17% to 75.50%), suggesting a possible impact on gluten strength. In contrast, Don Lupe maintained relatively high values (>80%), indicating greater stability under water stress. The gluten quality varies by wheat variety when water is scarce: some varieties maintain strong and stable gluten, whereas others weaken it under water stress (Han et al., 2025).

The results of the SDS sedimentation test shown in Table 2, associated with baking quality, revealed increases in some varieties under RR (Don Lupe, Bayoveca) but decreases in others (Isabel Oro). These findings demonstrate that the response to water stress is highly genotype dependent, particularly regarding the composition of glutenins and gliadins. However, the sedimentation index (Index) did not significantly differ between treatments and remained in the range of 0.72 to 0.93, suggesting that the overall functionality of the gluten was not drastically altered in terms of its hydration capacity (Shewry et al., 2024).

Color Properties

Color parameters (L*, a*, b*) showed subtle but consistent changes under RR. The L* value (luminance) increased slightly in all the varieties, indicating lighter grains. For example, Bayoveca increased from 79.9 to 81.2 (Table 3). This change could be associated with a lower concentration of pigments or modifications in the endosperm structure. However, flour color does not depend solely on the presence of pigment particles but also on the reflectance of the endosperm, which is influenced by factors such as protein content and variety-specific characteristics. This interaction of factors limits its usefulness as a precise indicator of quality, as it can generate variations in color without necessarily reflecting actual changes in the composition or technological properties of the flour (Barnes, 1986; Wang et al., 2025).

The b* parameter (yellowness) slightly decreased under RR, which is relevant in durum wheat, where yellow color is a quality attribute for the pasta industry. This decrease could indicate a reduction in carotenoids. However, reduced irrigation stresses wheat, activating antioxidants such as carotenoids and anthocyanins to protect the cells. Wheat color is influenced primarily by genotype, environment, and processing. The yellowness index is a key predictor of pasta color. These compounds concentrate pigments in relatively small grains, increasing their color and nutritional value (Ficco et al., 2014; Troccoli et al., 2025).

Chroma and color difference (ΔE) increased under RR, suggesting perceptible changes in grain coloration. However, the hue remained virtually constant (~1.41–1.49), indicating that the overall color tone did not change substantially. The increase in chroma under reduced irrigation (RR) reflects greater color saturation or intensity, likely because of the concentration of carotenoids (Hidalgo et al., 2014). The fact that the hue angle remains constant indicates that, although the grain is more vibrant, it still belongs to the same color family (yellow–orange) without shifting toward other shades. The ΔE confirms that these variations are visually detectable (Nkhata, 2020).

Dough stability

Dough stability (Estab) (Table 2), measured in minutes, increased under RR in all the varieties. For example, Don Lupe increased from 10.9 to 12.4 min, whereas Norocoro C2021 increased from 8.2 to 9.8 min. An increase in kneading resistance could be attributed to a higher concentration and better quality of gluten proteins (Cappelli et al., 2020; da Silva Ramos et al., 2023). This behavior is consistent with the previously reported increase in protein and gluten content (Table 1). A dough with greater strength and rheological stability improves its performance during processing and contributes to the production of higher-quality final products (Cappelli et al., 2020).

Table 3. Color (L*, a*, b*, ΔE, chroma, hue) and dough stability in wheat genotypes under different irrigation regimes.

Sample		L*	a*	b*	Stability (min)	Color difference ΔE	Croma	HUE
Isabel oro	RC	78.2 ± 0.7	2.2 ± 0.2	18.8 ± 0.5	7.3 ± 0.6	80.5 ± 0.8	78.3 ± 0.7	1.41 ± 0.02
	RR	79.8 ± 0.6	2.0 ± 0.2	18.2 ± 0.4	8.9 ± 0.7	81.7 ± 0.7	79.9 ± 0.6	1.42 ± 0.02
Don lupe	RC	76.5 ± 0.8	2.7 ± 0.3	19.7 ± 0.6	10.9 ± 0.9	79.0 ± 0.9	76.2 ± 0.8	1.43 ± 0.02
	RR	78.0 ± 0.7	2.4 ± 0.2	19.0 ± 0.5	12.4 ± 1.0	80.4 ± 0.8	77.8 ± 0.7	1.44 ± 0.02
Bayoyeca	RC	79.9 ± 0.6	1.9 ± 0.2	17.9 ± 0.4	5.6 ± 0.5	82.0 ± 0.7	80.1 ± 0.6	1.48 ± 0.02
	RR	81.2 ± 0.5	1.7 ± 0.1	17.3 ± 0.3	7.2 ± 0.6	83.1 ± 0.6	81.4 ± 0.5	1.49 ± 0.01
Norocoro C2021	RC	80.6 ± 0.5	1.8 ± 0.1	17.4 ± 0.3	8.2 ± 0.7	82.7 ± 0.6	80.6 ± 0.5	1.48 ± 0.01
	RR	81.7 ± 0.4	1.6 ± 0.1	16.9 ± 0.3	9.8 ± 0.8	83.5 ± 0.5	81.7 ± 0.4	1.49 ± 0.01

**Different lowercase letters within the same column indicate significant differences according to Tukey's test ($p \leq 0.05$).

Multivariate Analysis (Clustering) and PCA

Hierarchical cluster analysis revealed clear differentiation between treatments (RC vs. RR) (Figure 1), as well as between genotypes. Two major clusters were identified: one associated primarily with full irrigation conditions and the other with reduced irrigation. Within these clusters, varieties were grouped according to similarities in chemical composition and rheological properties.

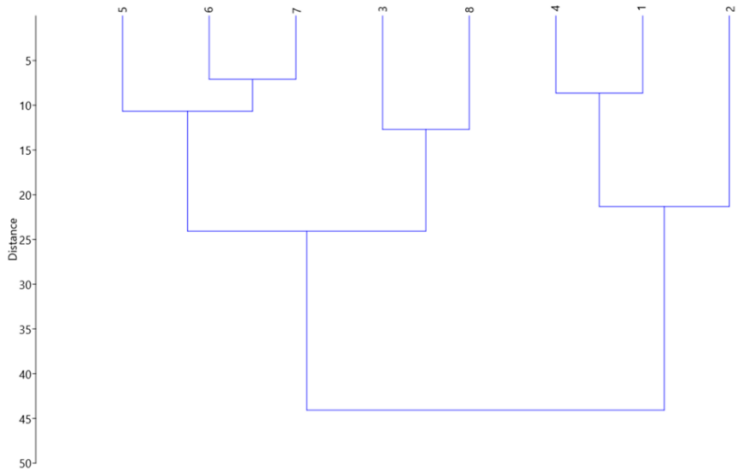


Figure 1. Hierarchical clustering dendrogram of wheat genotypes under reduced-irrigation conditions based on quality variables. The distance on the vertical axis represents dissimilarity: low values indicate greater similarity, whereas high values reflect greater difference. The clusters group genotypes with similar performance. The numbers correspond to the varieties evaluated under full irrigation (1,3,5,7) and reduced irrigation (2,4,6,8).

In particular, Isabel Oro and Don Lupe were similar in both regimes, suggesting similar physiological responses. In contrast, Bayoveca and Norocoro C2021 exhibited greater variability, indicating a more complex interaction with water stress.

The observed separation between clusters indicates that the water regime significantly affects the overall quality of wheat, with a magnitude comparable to that of the genotype. Under reduced-irrigation conditions, this behavior is consistent with what has been reported in the literature, where water deficit significantly affects agronomic, physiological, and productive traits, indicating a strong genotype × environment interaction. In particular, water stress can induce changes in water use efficiency, stomatal conductance, and biomass allocation, which directly impact crop quality and yield. Therefore, the results highlight the need to simultaneously consider the effects of reduced irrigation and genetic variability in breeding programs and in the agronomic management of the crop (Li et al., 2024).

Reduced irrigation, far from being solely a limiting factor, can induce improvements in certain quality attributes, particularly protein content and rheological properties. However, these benefits are accompanied by reductions in physical parameters such as hectoliter weight, which can affect marketability.

From a genetic breeding perspective, varieties such as Don Lupe and Isabel Oro show greater quality stability under stress conditions, positioning them as promising candidates for production systems with limited water availability.

In contrast, varieties such as Bayoveca exhibit greater sensitivity to key parameters such as the gluten index, which could limit their use under water stress conditions if specific industrial standards are to be maintained.

The distribution of genotypes under reduced irrigation based on the evaluated quality variables is shown in Figure 2, revealing a clear differentiation in their performance. The observed dispersion indicates that water stress generates variability in technological attributes, allowing for the identification of groups with similar characteristics. Genotypes located in the same direction as the vectors show a positive association with those variables, suggesting a greater value or influence of these attributes on their performance.

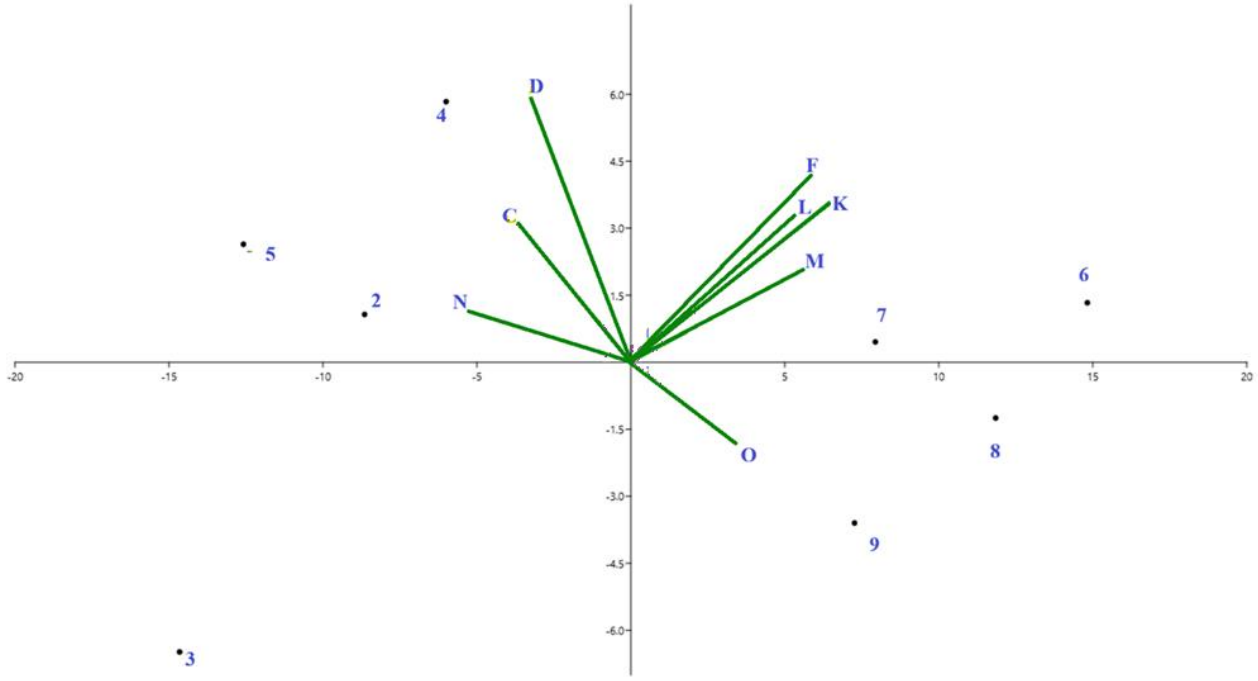


Figure 2. Biplot of the principal component analysis (PCA) of wheat genotypes under reduced-irrigation conditions, based on quality variables. The points represent the genotypes, while the arrows indicate the evaluated variables and their contribution to total variability. The direction and length of the vectors reflect the relationship and magnitude of each variable, respectively. Proximity between genotypes indicates similarity in their behavior, whereas their dispersion highlights differences in quality response under water stress. The numbers correspond to the evaluated varieties. The numbers correspond to the varieties evaluated under full irrigation (2,3,5,8) and reduced irrigation (3,5,7,9). The letters represent the vectors corresponding to the physicochemical and rheological variables analyzed: C: pH, D: Grain weight (g), F: Protein (%), K: Moisture (%), L: SDS, M: SDS Index, N: GH (%), O: GS (%)

On the other hand, genotypes situated in positions opposite the vectors show a negative relationship, indicating a weaker contribution from those variables. The length of the vectors suggests that some variables have greater weight in explaining total variability and are decisive in differentiating genotypes under reduced-irrigation conditions. Similarly, the proximity between certain vectors indicates correlations among variables, suggesting that some quality attributes are closely related to one another.

Reduced irrigation does not affect all genotypes uniformly but induces differentiated responses depending on their intrinsic characteristics. This allows for the identification of materials with greater stability or better-quality performance under water stress, which is relevant for breeding programs and for the selection of varieties under conditions of limited water availability.

CONCLUSIONS

Overall, the results show that moderate water stress (RR) induces significant changes in wheat quality, with positive effects on protein concentration and rheological properties but negative effects on the physical characteristics of the grain. The magnitude and direction of these changes depend largely on

the genotype, underscoring the importance of variety selection in scenarios of climate change and water availability. This study provides relevant evidence for optimizing management strategies and variety selection in semiarid agricultural systems, where balancing yield and quality is a constant challenge.

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Chapter 11

Antifungal activity of edible chitosan coatings and *Coriandrum sativum* L. essential oil against *Colletotrichum gloeosporioides* and their application on mango fruits

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ABSTRACT

Anthracnose caused by *Colletotrichum gloeosporioides* is a major postharvest disease of mango, leading to significant economic losses. The use of synthetic fungicides has limitations because of resistance strains and safety concerns. In this study, the antifungal activity of edible chitosan coatings and *Coriandrum sativum* L. (coriander) leaf essential oil, alone and in combination, against *C. gloeosporioides* was evaluated through *in vitro* and *in situ* assays on mango fruits. The essential oil was obtained by hydrodistillation (yield of 0.2% w/w). *In vitro* tests revealed that the oil at 5 mg mL-1 inhibited mycelial growth (71.5%), conidial germination (64.5%) and sporulation (64.9%) in a concentration-dependent manner. Chitosan coating (1%) alone achieved 42.6% mycelial inhibition. The combination of chitosan with 5 mg mL-1 (QAE5) essential oil significantly improved antifungal activity, reaching 62.5% mycelial inhibition and 72.1% germination inhibition *in vitro*. In mango fruits (cv. manila), QAE5 reduced anthracnose severity to 42.7% after 18 days of storage at 13 °C, compared with 98.8% in the control and 70.9% with chitosan alone. The results demonstrate a synergistic effect between chitosan and coriander essential oil, providing an effective, safe and sustainable alternative for the postharvest management of mango anthracnose.

Keywords: antifungal; chitosan; edible coatings; Coriandrum sativum; anthracnose.

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INTRODUCTION

Fungal diseases represent a significant cause of economic losses in high-value horticultural products (Ramírez-Salcedo et al., 2019). Among the most prevalent phytopathogens are *Colletotrichum gloeosporioides*, the causal agent of anthracnose, a disease affecting leaves, young branches, inflorescences and fruits. In the particular case of mango (*Mangifera indica* L.), anthracnose is among the main postharvest diseases, with yield losses that can reach 100% in plantations without proper management (Dofuor et al., 2023). Infection of the fruits induces their premature drop, and during ripening, irregular dark brown to black lesions develops that compromise both the sensory quality and the commercial value of the product, generating substantial economic losses for producers.

The control of this disease has traditionally been based on the application of synthetic fungicides such as benomyl, thiabendazole and imazalil. However, the intensive use of these compounds has led to the emergence of resistant strains of the pathogen, which has resulted in increased application concentrations and frequency (Zhang et al., 2025). This practice not only increases production costs but also carries the risk of exceeding the maximum permitted residue limits, thus compromising fruit safety and consumer health. In this context, natural products, such as essential oils, organic acids and polyphenolic compounds, are promising alternatives for the control of postharvest diseases because of their biodegradability and efficacy against various fungal species (Tubay-Bermúdez et al., 2024).

Chitosan coatings are promising because they possess broad-spectrum antimicrobial activity. López-Mora et al. (2013) reported that 1% chitosan inhibited the germination of Alternaria alternate and reduced sporulation. Similarly, Correa-Pacheco et al. (2017) reported that nanostructured chitosan films inhibited the mycelial growth (0.2%) and spore germination (96%) of *C. gloeosporioides*. Rayón-Díaz et al. (2021) also demonstrated that treatment with chitosan (0.1–1.5%) resulted in up to 99% inhibition of the mycelial development of *C. gloeosporioides*. Therefore, the incorporation of essential oils into chitosan coatings could enhance the control of this pathogen on fruits.

Essential oils are complex mixtures of polar and nonpolar compounds of plant origin that exert synergistic antifungal activity and play a relevant role in protecting crops against biotic enemies. They therefore constitute a viable alternative for extending the shelf life of horticultural products (Alonso-Gato et al., 2021). Coriander (*Coriandrum sativum* L.), an annual herbaceous plant of the Apiaceae family, is widely cultivated in regions such as Morocco, France, Spain, India and Mexico (Leal-Torres et al., 2013). Its essential oil has been recognized as safe (GRAS, generally recognized as safe) by the United States Food and Drug Administration (FDA). The major volatile compounds in seed oil are linalool and γ-terpinene, while in fresh leaves, decanal and trans-2-decenal predominate (Mandal & Mandal, 2015). This oil exhibits a wide range of biological activities, such as antioxidant, antidiabetic, anxiolytic, anti-inflammatory, antihypertensive, antidySlipidemic, diuretic and antimicrobial activities (Bogavac et al., 2015; Al-Khayri et al., 2023). Bogavac et al. (2015) reported that a minimum inhibitory concentration (MIC) of 0.4 μL mL^{-1} of coriander leaf essential oil was effective against *Escherichia coli*. Moreover, Yildiz

(2016) evaluated the antifungal activity of this oil against *Candida albicans* and *Aspergillus niger* and reported inhibition zones of 18 mm (MIC of 7.8 μg mL-1) and 14 mm (MIC of 250 μg mL-1), respectively. Despite this background, most studies have focused on coriander seeds (Darughe et al., 2012; Mandal; Mandal, 2015), and research on the antifungal activity of leaf oil against phytopathogenic fungi is scarce (Sobhani et al., 2022).

Considering the need to develop sustainable alternatives for the management of anthracnose in mango and given the lack of information on the combined effect of *C. sativum* L. (leaf) essential oil with chitosan coatings on *C. gloeosporioides*, the present study aimed to evaluate the antifungal activity of edible chitosan coatings and *Coriandrum sativum* L. essential oil, as well as their combination, on the development of *C. gloeosporioides* through in vitro and in situ assays on mango fruits.

MATERIALS AND METHODS

Plant material

Coriander leaves (*Coriandrum sativum* L.) were collected during September 2023 from an orchard located in Ixil, Yucatán, Mexico (21° 9.8' N, 89° 28.56' W; 5 m a.s.l.). Healthy, well-developed plants at 90 days after sowing were selected. Leaves were taken at random and immediately transported to the Fruit and Vegetable Workshop of the Instituto Tecnológico de Chiná, where they were processed on the same day.

Extraction of essential oil from C. sativum L.

The essential oil was obtained from fresh leaves and cut into fragments of approximately 0.5 cm. Samples of 50 g of leaves were subjected to hydrodistillation in 200 mL of distilled water for 4 hours using a Clevenger-type apparatus. The process was repeated several times until a sufficient amount of oil was obtained (1500 g of leaves in total). The hydrodistilled extract was dried over anhydrous sodium sulfate (Na_2SO_4; J. T. Baker®, Mexico) and stored in amber glass vials at 4 °C until analysis. The average yield of the essential oil was 0.2% w/w (wet basis).

Obtaining the C. gloeosporioides strain.

Monosporic isolates of *Colletotrichum gloeosporioides* from the Microbiology Laboratory Collection of the Universidad de la Sierra Sur were used. The strains, previously identified on the basis of their morphological and molecular characteristics, were reactivated on potato dextrose agar (PDA; Bioxon®, Mexico) and incubated at 25 ± 2 °C for five days.

Antifungal effect of C. sativum L. essential oil.

The assay was performed according to the methods of Chen et al. (2013) with modifications. Finally, essential oils were prepared at concentrations of 0.5, 1, 3 and 5 mg mL-1 in PDA medium. For

this purpose, the oil was mixed with Tween® 20 (0.1% v/v; Hycel, Mexico) and added to 100 mL of molten PDA (45 °C). The mixture was poured (20 mL per plate) into 90 mm diameter Petri dishes (five replicates per treatment) and allowed to solidify at room temperature (25 ± 2 °C). On the surface of each plate, a 5 mm diameter agar disc with active mycelium (5- day-old culture) was placed. PDA without essential oil was used as a negative control, and PDA with Tween® 20 (0.1%) was used as a solvent control. The plates were incubated at 25 ± 2 °C, and the mycelial growth diameter was measured daily (in two perpendicular directions) using a Vernier caliper. The experiment ended when the mycelium of the control completely covered the plate.

The percentage of mycelial growth inhibition for each treatment was calculated using the following formula:

$$\mathbf{MGI\ (\%) = (DMCc - DMCt)/DMCc \times 100}$$

where MGI = mycelial growth inhibition; DMCc = diameter of mycelial growth of the control; and DMCt = diameter of mycelial growth of the treatment.

At the end of the incubation period, the spores produced on each plate were collected by adding 10 mL of sterile distilled water and gently scraping the surface with a sterile glass rod. The suspension was filtered through sterile gauze, and the number of conidia was quantified in a Neubauer chamber (BRAND®, Mexico) under an optical microscope (ZEISS® Axio Lab. A1, Oberkochen, Germany). The percentage of sporulation inhibition (SI) was calculated as follows:

$$\mathbf{SI\ (\%) = (NCc - NCt)/NCc \times 100}$$

where SI = sporulation inhibition; NCc = number of conidia of the control; and NCt = number of conidia of the treatment.

From a 5--day--old sporulated culture, a suspension of 10^6 conidia mL^{-1} in sterile distilled water was prepared. Fifty microliters of this suspension were placed on the surface of PDA plates containing different essential oil concentrations (0.5–5 mg mL^{-1}). The plates were incubated for 8 h at 25 ± 2 °C. One hundred conidia per treatment were examined under a microscope; a conidium was considered to have germinated when the length of the germ tube was at least twice the major diameter of the conidium. The percentage of germination inhibition (GI) was determined using the following equation:

$$\mathbf{GI\ (\%) = (NGc - NGt)/NGc \times 100}$$

where GI = germination inhibition; NGc = number of germinated conidia of the control; and NGt = number of germinated conidia of the treatment.

Preparation of coatings based on chitosan and coriander (C. sativum L.) essential oil.

Medium-molecular-weight chitosan (75–85% degree of deacetylation, viscosity 200–800 cps; Sigma Aldrich®, Missouri, USA) was used. On the basis of the mycelial growth inhibition results, three concentrations of coriander leaf essential oil were selected: 1, 3 and 5 mg mL-1. A 1% (w/v) chitosan solution was prepared by dissolving the polymer in 1% (v/v) acetic acid with constant stirring for 24 hours at room temperature. The solution was adjusted to pH 5.6 with 1 N NaOH and homogenized for 15 minutes until a stable emulsion was obtained. The emulsion was subsequently degassed under vacuum to remove air bubbles and divided into three equal portions. To each portion, the corresponding concentration of essential oil was added, and the mixture was homogenized for 10 minutes and allowed to rest for 24 hours before use (Sebti et al., 2005; Ramos et al., 2012).

Antifungal effect of the coatings

Molten PDA (45 °C) was mixed with each of the chitosan- essential oil emulsions (final oil concentrations: 1, 3 and 5 mg mL^{-1}) in a 1:1 (v/v) ratio, and 20 mL was poured into Petri dishes. PDA with 1% chitosan solution (without essential oil) was used as a positive control, and PDA alone was used as an absolute control. On each plate, a 5 mm disc of fungal mycelium (5 days old) was placed. The plates were incubated at 25 ± 2 °C for 7 days. The mycelial diameter was measured daily, and the percentage of growth inhibition was calculated using the same formula as in the previous section. To evaluate conidial germination in the presence of the coatings, a suspension of 10^6 conidia mL^{-1} was prepared, and 50 µL was inoculated onto the surface of the PDA plates. After 20 minutes in a laminar flow cabinet (to absorb excess moisture), 5 mL of each coating emulsion was poured over the inoculum and allowed to dry for 90 minutes at room temperature. The plates were incubated for 7 hours at 25 ± 2 °C, after which the germination of 100 conidia per treatment was determined. The percentage of germination inhibition (GI) was calculated as previously described.

In vivo evaluation of the coatings

Mango fruits (*Mangifera indica* L.) cv. Manila, harvested in May 2024 from a commercial orchard in Chiná, Campeche, Mexico, were used. Fruits were selected for their homogeneous mass (272 ± 11 g), physiological maturity and absence of physical damage or pathological symptoms. After the samples were washed and disinfected with 2% (v/v) sodium hypochlorite, three wounds were made in the equatorial zone of each fruit (2 mm diameter × 2 mm depth) with a sterile dissecting needle. Each wound was inoculated with 10 µL of a suspension of 10^6 conidia mL^{-1} of *C. gloeosporioides*. The inoculated fruits were kept for 24 hours at room temperature (25 ± 2 °C) to allow infection and then immersed for 20 seconds in the corresponding coating emulsions (chitosan with 1, 3 or 5 mg mL^{-1} of essential oil). Inoculated

fruits without a coating were used as a negative control. The fruits were subsequently stored at 13 °C for 18 days.

Determination of severity by digital image analysis

Anthracnose severity was quantified as the percentage of necrotic area on the fruit surface. Three digital images per fruit (one per wound) were captured using a FUJIFILM® FinePix S1000fd camera (JPG format, 2736 × 3648 pixels, RGB color space, 24 bits per pixel, 300 dpi resolution). The images were calibrated such that 175.09 pixels corresponded to 1 cm^2. Analysis was performed with ImageJ software v1.51e (National Institutes of Health, Bethesda, MD, USA).
The determination of the severity percentage was carried out in two stages: In the first stage, image binarization (Process > Make binary), selection of the mango region using the "Wand" tool (Tolerance = 0 and Legacy mode) and determination of the total mango area (TMA) using the "Analyze particle" function were performed. In the second stage, the "color threshold" tool was used to segment the images on the basis of thresholds for channels of a color space. The RGB color space was converted to the HSB (hue, saturation, and brightness) color space to obtain an adequate representation of the pixels and thereby facilitate the analysis. To segment each image, the following values were used: Hue = 0–255, Saturation = 0–255 and Brightness = 0–140. The value of the damaged area of each mango (DAM) was obtained using the sequence of commands "Analyze/Analyze particle". The severity percentage (S) was calculated using the following equation:

$$\mathbf{S\ (\%) = DAM/TMA \times 100}$$

where DAM = damaged area of the mango in cm^2 and TMA = total mango area in cm^2. Digital image processing was performed with ImageJ version 1.51e (National Institute of Health, Bethesda, MD, USA; this software is available at https://imagej.nih.gov/ij/download.html

Experimental design and statistical analysis

For all *in vitro* and *in vivo* assays, treatments were distributed in a completely randomized design. Each experiment was performed twice independently. In the *in vitro* assays, the experimental unit was a Petri dish, with five replicates per treatment in each repetition of the experiment (total n = 10 per treatment). In the *in vivo* assay, 10 fruits per treatment (three wounds per fruit) were used in each of the two repetitions.

The percentages of growth inhibition, sporulation and germination were transformed using arcsine √(percentage/100) to meet the assumptions of normality and homoscedasticity. The transformed data were subjected to two-way analysis of variance (ANOVA) (experiment × treatment), and as no significant effect of the experiment was found, the data were combined. Differences between means were

evaluated with Tukey's test ($p \leq 0.05$) using Statgraphics Centurion XVI software (Statistical Graphics Corp., USA).

RESULTS AND DISCUSSION

Antifungal effect of Coriandrum sativum L. essential oil in vitro.

Coriander essential oil had a concentration-dependent inhibitory effect on the mycelial growth, conidial germination and sporulation of *C. gloeosporioides* (Table 1). The concentration of 5 mg mL^{-1} resulted in the greatest mycelial growth inhibition (71.5%), followed by 3 mg mL^{-1} (61.2%), with significant differences between all the treatments ($p \leq 0.05$). The inhibition of mycelial growth can be attributed to the ability of the major terpenic compounds (decanal and trans-2-decenal) to interact with the fungal cell membrane. Recent work has shown that essential oils can damage the integrity and permeability of the plasma membrane, decrease intracellular ATP levels and increase the production of reactive oxygen species (Xia et al., 2025).

Table 1. Effects of *Coriandrum sativum* L. essential oil concentration on the percentage of mycelial growth inhibition (MGI), germination inhibition (GI) and sporulation inhibition (SI) of *C. gloeosporioides*.

Essential Oil Concentrations (mg mL^{-1})	MGI[z]	GI[z]	SI[z]
0.5	41.2d	15.3c	9.7c
1	56.7c	15.8c	5.3d
3	61.2b	55.1b	33.6b
5	71.5a	64.5a	64.9a
Testigo	0 e	0 d	0 e

[z]Different letters in the same column indicate a significant difference according to Tukey's test ($n = 10$, $p \leq 0.05$).

Similarly, germination inhibition reached its maximum value (64.5%) at 5 mg mL^{-1}, whereas concentrations of 0.5 and 1 mg mL^{-1} were ineffective (15.3 and 15.8%, respectively). Sporulation was inhibited by 64.9% at the highest concentration but was practically zero (5.3%) at 1 mg mL^{-1} (Table 1). The progressive reduction in mycelial growth as a function of the essential oil concentration is shown in Figure 1. This response pattern is consistent with that reported in the literature for various essential oils against phytopathogenic fungi (Atnafu et al., 2024). For example, Mutlu Ingök et al. (2020) reported in an updated review that the antifungal efficacy of essential oils is directly related to their concentration, attributing this behavior to the lipophilic nature of their bioactive compounds, which require them to reach a critical threshold to interact effectively with fungal membranes. Similarly, Raveau et al. (2020) reported that the inhibitory activity of essential oils on phytopathogens is clearly concentration-dependent, showing that sublethal concentrations can induce fungistatic effects, while higher concentrations achieve complete fungicidal control. This dose-response relationship is fundamental for the development of practical formulations, as it allows identification of the optimal

concentration that maximizes efficacy without compromising economic viability or the quality of the treated product.

Mohanapriya et al. (2024) evaluated essential oils of citronella, clove, ginger, basil, lemongrass, garlic, cinnamon, black cumin, black pepper and onion against *C. gloeosporioides* and reported complete mycelial growth inhibition at concentrations as low as 0.050% for citronella and cinnamon and 0.075% for lemongrass and clove. That study emphasized that such inhibition follows a concentration-dependent pattern, attributable to the presence of specific bioactive compounds such as citronellal, eugenol, cinnamaldehyde and geranial, which act on fungal membranes and enzymatic functions.

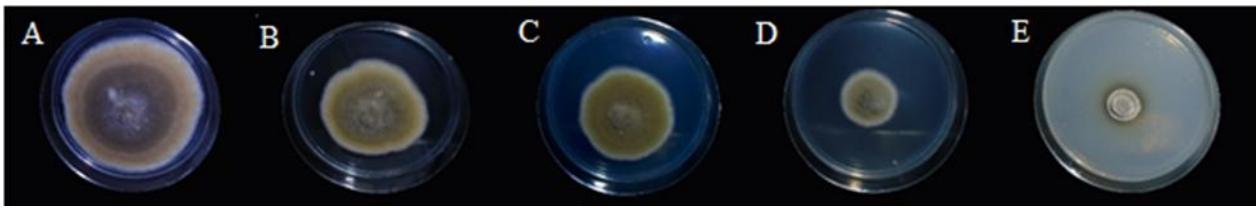


Figure 1. *In vitro* effectiveness of the essential oils on the mycelial growth of *C. gloeosporioides*: A) Control, B) 0.5 mg mL^{-1}, C) 1 mg mL^{-1}, D) 3 mg mL^{-1} and E) 5 mg mL^{-1}.

In vitro antifungal effect of chitosan essential oil coatings

Chitosan coatings at 1% (C) showed moderate activity (42.6% mycelial inhibition) (Table 2). Recent studies have confirmed that 0.8% (w/v) chitosan can inhibit up to 78% of the mycelial growth of *C. gloeosporioides* (Gumede et al., 2025). The antifungal mechanism of chitosan is related to its polycationic character at acidic pH (5.6), which favors electrostatic interactions with anionic groups on the fungal surface, causing structural disorganization and leakage of ions and nutrients (Yang et al., 2005; Aloui et al., 2014). Current research has shown that chitosan nanocomposites induce severe damage to hyphae and spore collapse, which is attributed to the synergistic generation of reactive oxygen species and chitosan-mediated membrane disruption (Hassan et al., 2026).

The incorporation of essential oil significantly improved the antifungal activity of the coatings, especially in terms of the inhibition of germination (72.1% in C-EO5 vs. 36.5% in C). This synergistic effect can be explained by the complementary action of both compounds: while chitosan alters the cell wall, the hydrophobic compounds of the essential oil easily penetrate the destabilized membrane, enhancing cell integrity loss (Aloui et al., 2014; Minova et al., 2015). Moreover, the chitosan matrix can act as a protective vehicle that gradually releases volatile compounds, prolonging their antifungal activity (Ramos et al., 2012). Recently, chitosan coatings with cinnamon or lemongrass essential oil completely inhibited mycelial growth of *C. gloeosporioides* in in vitro assays and significantly reduced anthracnose severity in mangoes (Hassam et al., 2026).

Table 2. Effect of chitosan essential oil on the percentage of *C. gloeosporioides* mycelial growth inhibition (MGI) and germination inhibition (GI).

Treatment [y]	MGI[z]	GI[z]
Q	42.6c	36.5d
QAE1	53.7b	44.8c
QAE3	61.0a	59.5b
QAE5	62.5a	72.1a
Control (PDA)	0 d	0 e

[y]Q: 1% chitosan coating; QAE1: 1% chitosan coating + 1 mg mL^{-1} essential oil; QAE3: 1% chitosan coating + 3 mg mL^{-1} essential oil; QAE5: 1% chitosan coating + 5 mg mL^{-1} essential oil.
[z]Different letters in the same column indicate a significant difference according to Tukey's test (n = 10, $p \leq 0.05$).

In vivo effects of the coatings on anthracnose severity in mango

There were significant differences in the severity percentage of *C. gloeosporioides* on fruits among the treatments ($p \leq 0.05$) (Figure 2). The control had the highest severity percentage (98.8%), followed by the C treatment (70.9%). The most effective treatment was C-EO5 (chitosan + essential oil at 5 mg mL^{-1}), with 42.7% severity, followed by C-EO3 (3 mg mL^{-1}), with 53.9%. The reduction in the number of necrotic lesions on fruits treated with C-EO5 compared with that on the control and C.

These results are comparable to those of recent research. For example, an active chitosan coating with ZnO nanoparticles and Eucalyptus radiata essential oil reduced postharvest rot severity in mangos by 64.3% (Oliveira et al., 2025). Similarly, a thyme essential oil nanoemulsions encapsulated in chitosan reduced anthracnose incidence in tomatoes by 50% (Gumede et al., 2025). The lower severity observed in fruits treated with C-EO5 can be attributed not only to direct inhibition of fungal growth and germination (as demonstrated in vitro) but also to the induction of defense mechanisms in the fruit, such as the activation of cell-wall-related enzymes (chitinases, glucanases) or the accumulation of phytoalexins, as suggested by Bautista et al. (2003) and more recent studies (Parente et al., 2025). Although the assay conditions differ, these results underscore the potential of essential oils as effective tools for anthracnose management.

Despite the efficacy demonstrated by the C-EO5 treatment, the residual severity of 42.7% indicates that there is still room for improvement. High doses of essential oils could increase control, but the possible effects on the sensory properties of mangos must be carefully evaluated, since high concentrations of essential oils may result in the transfer of unwanted odors or flavors. Furthermore, the stability of the coating during prolonged storage periods and its behavior under real commercial conditions require additional investigation.

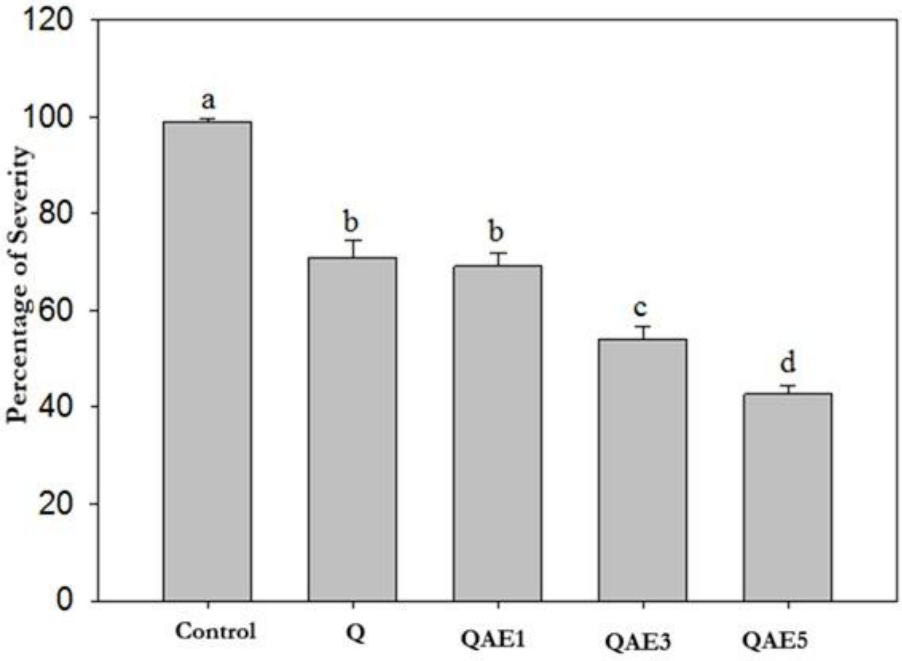


Figure 2. Percentage of anthracnose severity on mango fruits with chitosan and essential oil coatings. Data represent the mean ± standard deviation. Bars with the same letters are not significantly different (n = 10, p ≥ 0.05). Q: 1% chitosan coating; QAE1: 1% chitosan coating + 1 mg mL^{-1} essential oil; QAE3: 1% chitosan coating + 3 mg mL^{-1} essential oil; QAE5: 1% chitosan coating + 5 mg mL^{-1} essential oil.



Figure 3. Expression and severity of anthracnose symptoms 18 days after inoculation in mangoes with chitosan and essential oil coatings, as determined by digital image analysis. Q: 1% chitosan coating; QAE1: 1% chitosan coating + 1 mg mL^{-1} essential oil; QAE3: 1% chitosan coating + 3 mg mL^{-1} essential oil; QAE5: 1% chitosan coating + 5 mg mL^{-1} essential oil.

CONCLUSIONS

The essential oil of *Coriandrum sativum* L. leaves presents concentration dependent antifungal activity against *Colletotrichum gloeosporioides*, inhibiting mycelial growth, conidial germination and sporulation. Edible chitosan coatings at 1% enriched with 5 mg mL^{-1} of this essential oil (C-EO5) significantly improved antifungal efficacy compared with chitosan alone, reducing anthracnose severity in mango by 56.8%. The correlation between in vitro germination inhibition and in vivo severity emphasizes the importance of evaluating early infection processes. This study provides a solid foundation for the development of a sustainable, safe and effective postharvest coating, with the potential to reduce the use of synthetic fungicides in the management of mango anthracnose.

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Chapter 12

Agricultural gypsum with micronutrients in citrus cultivation

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ABSTRACT

Citrus production in the state of Sonora represents one of the most relevant and important sectors in the region, especially for oranges, lemons, grapefruits, and tangerines. Our soils are predominantly alkaline, with a pH of approximately 7.7, resulting in serious problems with the availability of micronutrients, specifically iron, followed by zinc, copper, and manganese. The use of agricultural gypsum in these soils helps improve their structure and induces temporary acidification, facilitating the absorption of these elements. Furthermore, the addition of humic and fulvic acids promotes the natural chelation of these minerals. This research involved the application of agricultural gypsum with micronutrients and humic and fulvic acids to orange, lemon, and mandarin trees in the soils of Yaqui Valley, Sonora, at two doses of this fertilizer formula: 25 and 50 kg per hectare. The results revealed more than a 30% increase in micronutrient accumulation and more than a 25% increase in fruit yield and quality.

Keywords: acidification, micronutrients, humic acids, fulvic acids, citrus.

INTRODUCTION

The agri-food sector is considered one of the most important for the global economy, and growing fruit is among its most profitable activities (Rinconada et al., 2022). In Mexico, the production and marketing of citrus fruits represents an attractive economic activity, both for the domestic and export markets. Among this group of fruits, oranges, lemons, tangerines, and grapefruits stand out. They are considered fruits of great importance because of their nutritional and health properties, including vitamin C, antioxidants, and pectin (Valencia; Duana, 2019).

Citrus fruits are moderate to large trees or shrubs belonging to the Rutaceae family. They have evergreen, simple, and generally oval or elliptical leaves. Their flowers are fragrant and have petals that are generally white or slightly tinged with shades of pink or purple. They produce fleshy, juicy fruits with

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thick rinds, containing numerous juice-filled vesicles, characterized by their high vitamin C content and acidic flavor.

According to the SIAP, 2023, Mexico ranks fourth globally as the largest producer of oranges, with a production of 4.8 million tons (MT). Veracruz, Tamaulipas, and Puebla are the main producing states, while Sonora ranks seventh, with a production of 165,475 tons (Lara et al., 2022).

With respect to lemon cultivation, Mexico ranks second globally, with a production of 3.1 MT, with Veracruz, Michoacán, and Colima standing out as the states with the highest production volume. Mexico is the third-largest producer of grapefruit worldwide, with 489,102 tons. In addition, mandarin production places Mexico 14th worldwide with more than 300,000 tons (SIAP, 2023).

Agricultural activities are negatively impacted by salinity problems and high levels of exchangeable sodium. One practice to recover degraded sodic soils and improve various physical soil properties is the application of agricultural gypsum (Trasviña et al., 2018).

Agricultural gypsum is a very soft mineral composed of calcium sulfate dihydrate ($CaSO_4 \bullet 2H_2O$) and is used as a soil amendment and fertilizer because it provides calcium and sulfur. The percentage of calcium in agricultural gypsum ranges from 18 to 22%, while the sulfur content ranges from 16 to 18%.

The results obtained from the application of agricultural gypsum to soils consistently show increases in Ca^{2+} and SO_4^{-2} (Beltrán et al., 2019). Its application improves soil properties by displacing Na+ and occupying these exchange sites with Ca^{2+}, resulting in the formation of Na_2SO_4, which is leached. Consequently, excess salts are eliminated, and the pH is reduced (Zenteno et al., 2020).

This compound is considered an economically viable alternative for use as a fertilizer to meet sulfur requirements and address soils deficient in this nutrient. It is also an important source of calcium (Beltrán et al., 2019).

These micronutrients are elements that are required in small quantities. However, their absence or deficiency can negatively affect crop growth, health, and yield (Ronen, 2016). Iron (Fe): Fe is considered a highly important micronutrient for plant development. It is absorbed in ferrous form (Fe^{2+}) and in organic form. It is involved in physiological processes such as respiration, chlorophyll biosynthesis, and redox reactions (Santis-Santis et al., 2019). This element functions as a structural component and as an enzyme cofactor. It is not available in plants growing in saline and calcareous soils since Fe^{3+} is insoluble at neutral and high pH. Its deficiency causes a loss of green color in leaves, known as iron chlorosis (Puente-Ramírez et al., 2022).

Copper (Cu): Cu is an important element for plants and is required at low concentrations. It acts as a catalyst in oxidation–reduction reactions. It also plays important roles in respiration and photosynthesis, in addition to its participation in plant physiological processes such as protein metabolism, nitrogen fixation, and carbohydrate distribution (León-Morales; Sepúlveda-Jiménez, 2012). It is absorbed by plants in two different forms: as a divalent ion (Cu^{+2}) in well-aerated soils and as a monovalent ion (Cu^{+}) in moist soils with low oxygen concentrations. At concentrations higher than those

required by plants, it can produce toxic effects, such as inhibiting root and shoot growth (Villegas-Torres et al., 2015). Zinc (Zn): Zn is a very important element for plants and is absorbed as Zn^{2+}. Among its functions. Manganese (Mn) in the Earth's crust is found in the form of oxides, carbonates, and silicates. Its total content in the soil generally ranges from 200 to 3,000 $mg \cdot kg^{-1}$ and can be found in divalent, trivalent, or tetravalent forms. However, the more oxidized forms are less readily available to plants (Franco, 2023; Gomez et al., 2006).

The present study aimed to evaluate the effects of applying agricultural gypsum to soil supplemented with micronutrients (Fe, Cu, Zn and Mn) and humic and fulvic acids through nutrient analysis and physicochemical measurements to determine its effects on the physiological, productive and quality development of citrus crops.

MATERIALS AND METHODS

The experiment was conducted in a citrus orchard (block 902) in Yaqui Valley, Sonora, Mexico, during the spring–summer cycle of 2024. A completely randomized block design was used for the citrus crops, with five treatments and ten replicates per treatment.

The treatments (Table 1) were applied at different stages of the phenological development of each fruit tree.

- Orange: (1) flowering, (2) fruit set, (3) 50% of its final size (Figure 2).
- Lemon: (1) flowering, (2) fruit set, (3) 50% of its final size (Figure 1).
- Mandarin: (1) flowering, (2) fruit set, (3) fruit at 50% of its final size (Figure 3).

Table 1. Treatments to apply to fruit trees.

Treatments	Formulation
T1	Without treatment
T2	25 kg ha^{-1} [agricultural plaster + Micronutrients (Fe, Cu, Zn y Mn)]
T3	50 kg ha^{-1} [agricultural plaster + Micronutrients (Fe, Cu, Zn y Mn)]
T4	25 kg ha^{-1} [agricultural plaster + Micronutrients (Fe, Cu, Zn y Mn) + fulvic/humic acids]
T5	50 kg ha^{-1} [agricultural plaster + Micronutrients (Fe, Cu, Zn y Mn) + fulvic/humic acids]

VARIABLES EVALUATED AND EQUIPMENT USED

- Chlorophyll index, measured with a Minolta SPAD 502 instrument.
- Nutrient concentration in leaf tissue measured with a HACH DR-3900 spectrophotometer
- Number and weight of fruits, measured with a balance scale
- Yield, measured with a balance scale

- Polar and equatorial diameters of the fruit, measured with a caliper
- Juice percentage, measured with a juicer
- Total soluble solids (°Brix), measured with a refractometer
- Titratable acidity, measured by the percentage of citric acid by titration with NaOH and phenolphthalein as indicators
- Fruit firmness, measured with a penetrometer
- Shelf life (weight loss), measured with a balance scale

The sample consisted of groups of 10 fruits per treatment per block. All harvest and postharvest measurements were taken in the morning. An analysis of variance was performed, using the LSD test ($p \leq 0.05$), with the statistical program Statgraphics Plus 5.1.

RESULTS AND DISCUSSION

Lemon cultivation

The application of the treatments increased the chlorophyll concentration during most of the measurement weeks. Compared with the other treatments, treatments T4 and T5 resulted in the highest concentrations. Compared with T1, T4 significantly differed from the control, with increases of 6.23%, 6.05%, and 4.51% at weeks 4, 7, and 8, respectively. Similarly, compared with T1, T5 was significantly different, with an increase of 4.54% for week 8 (Figure 1).

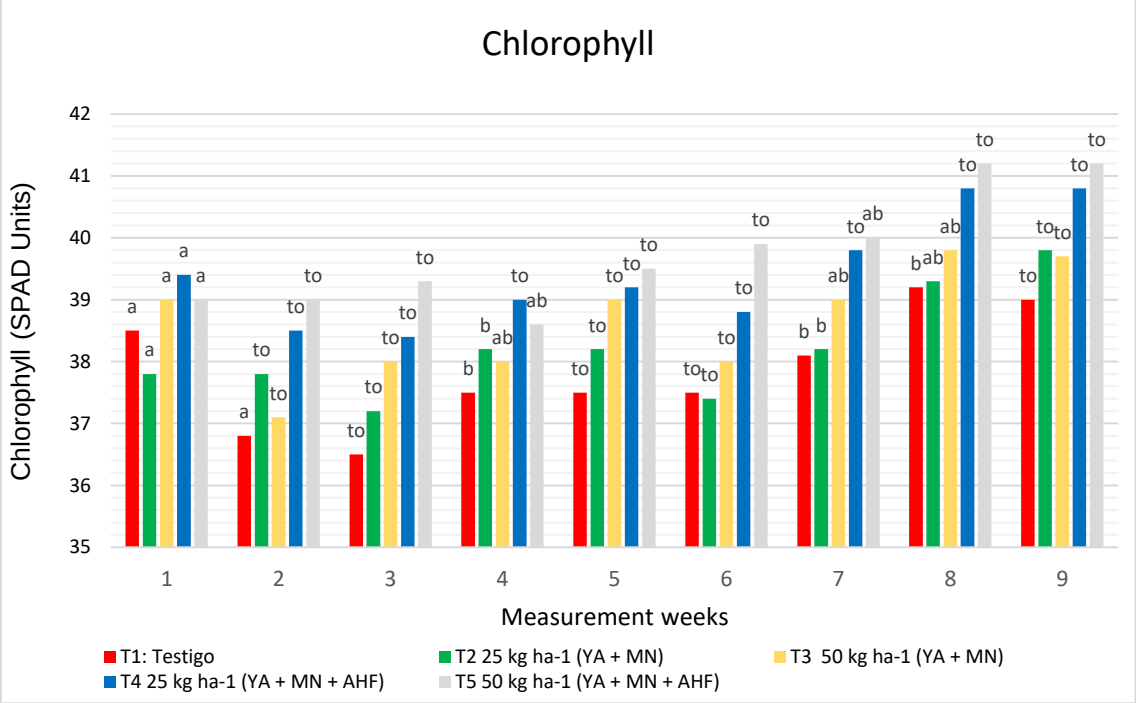


Figure 1. Chlorophyll concentration in lemon trees after application of agricultural gypsum supplemented with micronutrients.

With respect to the nutrient concentration of the leaf tissue, increases in macro- and micronutrients were detected (Table 2), with increases of up to 12.38% N, 12.28% P, 31.13% K, 24.43% Ca, 35.21% Mg, 17.27% Fe, 18% Cu, 6.66% Zn and 9.52% Mn, with treatments T3, T4 and T5 showing the greatest results.

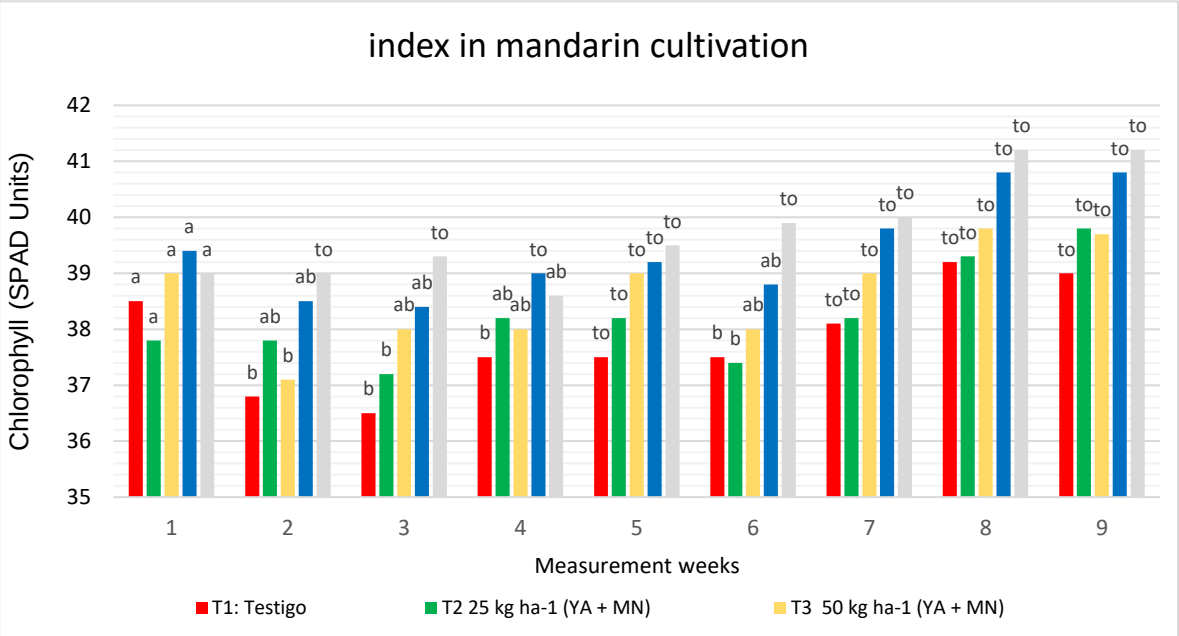


Figure 2. Chlorophyll concentration in mandarin trees after application of agricultural gypsum with micronutrients

Table 2. Nutrient concentration of leaf tissue in lemon trees after application of agricultural gypsum supplemented with micronutrients.

Cultivation stage	Treatments	N	P	K	AC	Mg	Faith	Cu	Mn	Zn
				%				ppm		
Start of flowering	T1	2.11	0.38	2.35	2.12	0.55	78	25	25	23
	T2	2.33	0.44	3.20	2.84	0.69	94	54	49	30
	T3	3.09	0.49	3.30	2.23	0.69	119	53	33	33
	T4	2.81	0.48	3.30	2.48	0.46	128	57	44	38
	T5	2.22	0.52	3.10	2.21	0.55	93	52	36	29
Fruit set	T1	2.40	0.89	2.70	2.72	0.95	99	31	38	34
	T2	3.03	1.05	3.40	2.96	0.91	122	43	48	39
	T3	2.89	1.10	3.20	3.11	1.08	125	36	44	40
	T4	2.39	1.36	3.30	3.16	1.01	145	43	40	46
	T5	2.54	1.35	2.94	2.81	0.99	108	41	42	49
Fruit formation	T1	2.64	0.58	1.62	2.10	0.64	154	23	30	25
	T2	2.88	0.64	1.94	2.55	0.79	184	29	34	30
	T3	3.18	0.73	1.99	2.84	0.80	193	28	33	37
	T4	3.29	0.81	2.40	2.83	0.79	195	30	35	39
	T5	2.75	0.99	1.88	2.80	0.88	196	33	40	38
Reference: Plant Analysis Handbook		2.20-2.70	0.10-0.30	1.00-2.00	1.50-4.00	0.20-0.50	50-300	8-40	20-500	50-100

Tangerine cultivation

The application of the treatments increased the chlorophyll concentration during most of the measurement weeks (Figure 2). Compared with T1, T2, and T3, T4 and T5 presented the highest concentrations. Compared with the control treatment, treatment T4 resulted in a statistically significant difference, with an increase of 4.31% by week 4. Moreover, compared with T1, T5 showed statistically significant differences, with increases of 5.22%, 7.22%, 6.64%, and 5.91% for weeks 2, 3, 6, and 9, respectively.

With respect to the nutrient concentration of the leaf tissue, increases in macro- and micronutrients were detected (Table 3), with increases of up to 31.82% N, 10.76% P, 14.06% K, 25.57% Ca, 19.35% Mg, 39.50% Fe, 40.00% Cu, 50% Zn and 21.74% Mn, with treatments T3, T4 and T5 showing the greatest results.

Table 3. Nutrient concentration of leaf tissue in mandarin trees after application of agricultural gypsum with micronutrients.

Cultivation stage	Treatment	N	P	K	AC	Mg	Faith	Cu	Zn	Mn
				%				ppm		
Bloom	T1	2.0c	0.5b	2.32b	2.1c	0.5c	110c	14c	15b	23c
	T2	2.4b	0.5b	2.61ab	2.3bc	0.6bc	118c	16bc	16b	26bc
	T3	2.4b	0.5b	2.68a	2.5a	0.7ab	139b	18ab	17b	25bc
	T4	2.7a	0.7a	2.63ab	2.5ab	0.7ab	160a	18ab	18ab	29ab
	T5	2.7a	0.7a	2.76a	2.6a	0.7a	155ab	19a	21a	31a
Fruit set	T1	2.4ab	0.5a	2.01b	1.2b	0.5b	117c	15b	32ab	46b
	T2	2.3b	0.5a	2.29a	2.3a	0.6ab	122c	19a	34ab	58ab
	T3	2.4ab	0.5a	2.38a	2.1a	0.7a	184b	18ab	30b	56ab
	T4	2.6a	0.6a	2.46a	2.3a	0.7a	190ab	19a	38ab	66a
	T5	2.6a	0.7a	2.46a	2.5a	0.7a	208a	20a	44a	68a
Fruits at 50% of their development	T1	2.4a	0.6a	0.99a	1.9b	0.5a	119b	16a	42b	50b
	T2	2.4a	0.5a	1.11a	2.1ab	0.6a	139ab	15a	59a	56b
	T3	2.4a	0.6a	1.21a	2.5a	0.6a	153a	15a	52ab	54b
	T4	2.5a	0.5a	1.31a	2.2ab	0.6a	163a	16a	56ab	57b
	T5	2.61a	0.58a	1.0a	2.5ab	0.5a	148ab	16a	63a	75a
Reference		3.00–3.40	0.15–0.25	0.90–1.10	1.50–4.00	0.17–0.44	60–150	5–35	20–75	20–200

Orange Cultivation

The chlorophyll levels significantly differed between the treated trees and the control. During week one, the control had 42 SPAD units, whereas treatment 4 had 44 SPAD units. Similarly, at week two, treatments 4 and 5 yielded the best results, with increases of more than 12%. In week 10, all the treated trees outperformed the control, with 46 SPAD units versus 42 SPAD units. In week 11, there were no differences, but the treated trees still outperformed the control. Week 16 showed the greatest

improvement, with treatment 5 having more than 48 SPAD units compared with 42 SPAD units in the control, representing a 15% increase (Figure 3, Figure 4).



Figure 3. Orange plants at 50% fruit development.

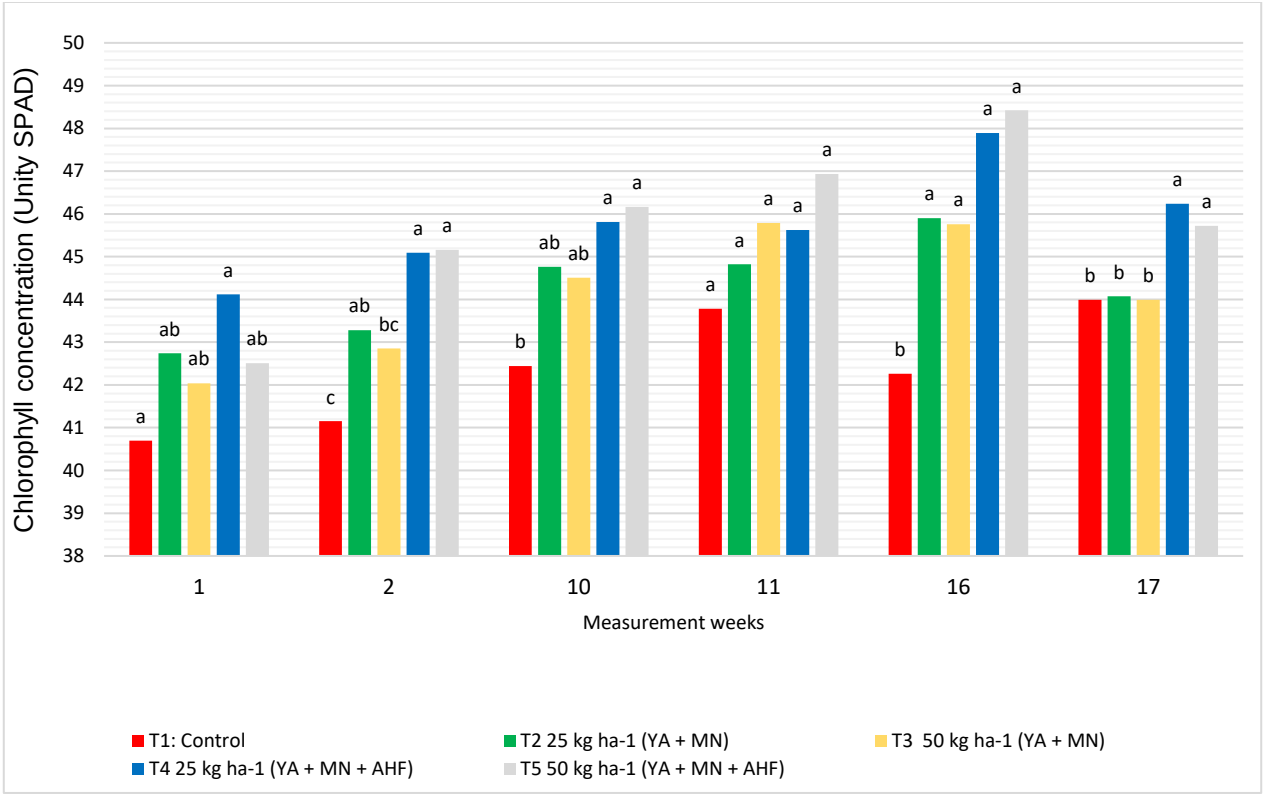


Figure 4. Chlorophyll levels in orange trees treated with agricultural gypsum plus micronutrients and humic and fulvic acids. Means with the same letter within each column are not significantly different (Tukey, $P \leq 0.05$). ns = not significant.

Table 4. Response of the application of agricultural gypsum with micronutrients and humic and fulvic acids to the components of orange yield and quality.

Treatments	Numer of fruits	Fruit weight (g)	Polar diameter (mm)	Ecuatorial diamater (mm)	Yield per tree (kg tree-^{1})
T1: Control	243 a	216.71 b	76.88 a	73.64 a	50.96 a
T2: 25 kg ha-^{1} (YA + MN)	189 a	226.99 ab	77.80 a	74.28 a	46.08 a
T3: 50 kg ha-^{1} (YA + MN)	216 a	238.42 a	79.20 a	75.44 a	46.78 a
T4: 25 kg ha-^{1} (YA + MN + AHF)	362 a	227.65 ab	78.08 a	74.60 a	63.13 a
T5: 50 kg ha-^{1} (YA + MN + AHF)	238 a	224.99 ab	78.28 a	74.28 a	55.08 a

Means with the same letter within each column do not differ statistically (Tukey, P ≤ 0.05). ns = not significant.

With respect to yield components (Table 4), a 50% increase in the number of fruits was found in treatment 4, with 25 kg of gypsum with acids, a 12% increase in fruit weight, and a 25% increase in fruit yield, with 63 tons per hectare compared to 50 tons per hectare for the control, which was also highly significant in treatment 4.

With respect to fruit quality, in terms of dimensions, there were no statistically significant differences, although the fruit in the treated trees was slightly larger, while the shape and quality of the fruit remained the same.

In terms of the fruit quality parameters (Table 5), the juice percentage was greater in treatment 5, which was approximately 20% greater, although all the treatments significantly outperformed the control. The total soluble solids content did not differ, with slightly higher levels in the treated fruit. Acidity remained within normal ranges, and the Brix/acidity ratio was also within normal limits. With respect to firmness, the fruit showed no problems and was within normal limits, with values ranging from 91 to 96 N, with the treated fruit exhibiting the best firmness.

Table 5. Response of the application of agricultural gypsum with micronutrients and humic and fulvic acids on postharvest fruit parameters: juice, acidity, Brix, and firmness in oranges.

Treatments	Percentage of juice (%)	TSS (% °Brix)	Titulable acidity (%)	Firmeza (N)
T1: Control	42.85 b	12.20 a	1.22 a	91.00 a
T2: 25 kg ha-1 (AP + MN)	46.41 ab	12.20 a	1.33 a	94.00 a
T3: 50 kg ha-1 (AP + MN)	46.91 ab	12.60 a	1.35 a	96.00 a
T4: 25 kg ha-1 (AP + MN + AHF)	46.88 a	12.60 a	1.27 a	91.00 a
T5: 50 kg ha-1 (AP + MN + AHF)	49.79 a	12.80 a	1.33 a	93.00 a

Means with the same letter within each column do not differ statistically (Tukey, P ≤ 0.05). ns = not significant.

In terms of orange fruit size (Table 6), the greatest percentage was found for medium- to large-sized trees, exceeding 80% for the treated trees and approximately 20% or less for small-sized trees, whereas the control group reported the greatest number of small fruits.

During the flowering stage, no statistically significant differences in nitrogen concentration were detected, although the treated trees experienced slightly greater increases. For phosphorus, both treatments 2 and 5 were the best, with 0.58% and 0.60%, respectively, compared with 0.45% in the control, with highly significant differences. In treatment 4, the greatest difference in potassium concentration was detected, which was also significant. Calcium and magnesium were not significantly different. In terms of micronutrients, iron had the best effect, with almost 200 ppm in treatment 5 compared with 138 ppm in the control, representing an increase of more than 40%. Copper, zinc, and manganese had similar effects, with treatment 5 exhibiting the best results and highly significant increases of 23%, 24%, and 66%, respectively.

Table 6. Response of the application of agricultural gypsum with micronutrients and humic and fulvic acids on orange fruit size.

Treatments	Small-sized fruits (%)	Medium-sized fruits (%)	Large fruits (%)
T1: Control	16.68 ab	63.81 a	19.51 ab
T2: 25 kg ha^{-1} (AP + MN)	2.08 b	46.61 a	51.31 a
T3: 50 kg ha^{-1} (AP + MN)	11.79 ab	48.64 a	39.57 ab
T4: 25 kg ha^{-1} (AP + MN + AHF)	23.81 a	66.10 a	10.09 b
T5: 50 kg ha^{-1} (AP + MN + AHF)	3.31 b	58.63 a	38.06 ab

Means with the same letter within each column do not differ statistically (Tukey, $P \leq 0.05$). ns = not significant.

Table 7. Response of the nutrient concentration in orange leaves to the application of agricultural gypsum supplemented with micronutrients and humic and fulvic acids.

Crop stage	Treatments	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn
		---------- (%) ----------					----------(ppm) ----------			
Flowering	T1	2.60a	0.45b	1.30c	2.26a	0.37a	138ab	13c	25a	30c
	T2	2.64a	0.58a	1.46bc	2.28a	0.40a	128b	14bc	25a	28c
	T3	2.82a	0.56ab	1.66ab	2.54a	0.44a	176ab	17a	30a	35bc
	T4	2.84a	0.54ab	1.86a	2.41a	0.51a	167ab	14bc	31a	44ab
	T5	2.78a	0.60a	1.70ab	2.54a	0.53a	195a	16ab	31a	50a
Fruit filling	T1	2.44a	0.61a	1.01a	2.12bc	0.52a	121b	15a	41b	45b
	T2	2.49a	0.55a	1.10a	2.07c	0.56a	141ab	18a	56a	59ab
	T3	2.70a	0.61a	1.28a	2.60ab	0.56a	155ab	17a	56a	56ab
	T4	2.77a	0.65a	1.41a	2.42ab	0.56a	163a	18a	61a	69a

Crop stage	Treatments	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn
		---------- (%) ----------					----------(ppm) ----------			
	T5	2.79a	0.70a	1.05a	2.64a	0.58a	157a	18a	65a	62ab
Fruits at 50% of development	T1	2.50a	0.62a	1.07b	2.08a	0.56a	123c	15a	40b	38a
	T2	2.48a	0.56a	1.11ab	2.13a	0.59a	143bc	17a	54ab	48a
	T3	2.65a	0.64a	1.30a	2.53a	0.57a	157ab	18a	56a	57a
	T4	2.79a	0.64a	1.31a	2.25a	0.58a	163ab	20a	60a	63a
	T5	2.83a	0.68a	1.27ab	2.68a	0.62a	171a	20a	59a	65a
Refference*		2.20 – 3.50	0.12 – 0.50	1.20 – 3.00	1.10 – 4.00	0.30 – 0.50	60 – 250	6 –35	25 – 150	25 – 200

Means with the same letter within each column do not differ statistically (Tukey, $P \leq 0.05$). ns = not significant.

During the fruit set stage, the concentrations of none of the macronutrients (N, P, K, Ca, and Mg) significantly differed, although the treated trees presented relatively high concentrations. For micronutrients, the same trend continued, with higher concentrations reported in the treated trees than in the control. At the 50% fruit development stage, the concentrations of nitrogen, phosphorus, calcium, and magnesium did not significantly differ, whereas the concentration of potassium significantly differed. In terms of micronutrients, there were considerable increases in the treated trees compared with those in the control (Table 7).

CONCLUSION

The addition of micronutrients (Fe, Cu, Zn, and Mn) is highly important for citrus crops, as these trees have specific nutrient requirements. This integrated management ensures that nutrients are efficiently available to the plant, improving its physiological parameters, yield, and quality.

The application of agricultural gypsum with micronutrients, humic acids, and fulvic acids improves nutrient availability to the plant, efficiently meeting its nutritional needs.

A significant positive effect on the physiological variables, such as the chlorophyll index and nutrient concentration in the leaf tissue, was detected for the lemon, orange, and mandarin crops. Similarly, improvements in yield variables such as the number and weight of fruit and yield, as well as in quality parameters such as increased polar and equatorial diameters, juice percentage, total soluble solids (TSS), titratable acidity, fruit firmness, and shelf life, were observed.

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Chapter 13

Initial Agronomic Adaptation of *Stevia rebaudiana* Bertoni in the High Mountain Region of Veracruz and Sustainable Management of *Planococcus citri* Using a Neem-Based Bioinsecticide

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ABSTRACT

Assessment of seedling growth provides valuable information for understanding the early development and adaptation of plant species under specific cultivation conditions. In this study, *Stevia rebaudiana* Bertoni was evaluated as a plant model to analyze its early establishment in black soil under the environmental conditions of the High Mountain Region of Veracruz, Mexico. Additionally, the effectiveness of a potassium soap bioinsecticide enriched with *Azadirachta indica* (neem) extract, with potassium hydroxide as the active component, was evaluated for the control of the mealybug *Planococcus citri*. The study was conducted using a replicated 2^2 factorial experimental design, and the data were analyzed by ANOVA using Minitab 2023 software. The results showed that relative humidity had a positive and significant effect on stem growth, with a coefficient of determination of $R^2 = 94.51\%$. Under direct planting conditions, 30 seedlings showed better establishment and greater vigor, with an approximate average of 378 leaves per plant. In the phytosanitary trial, seven infested seedlings were sprayed every four days. Notably, in seedling 21, the visible insect population decreased from 53 to 7 individuals over a 13-day period, representing an 86.8% reduction. These results indicate that relative humidity is a determining variable for promoting the initial vegetative development of the soil-grown crop, whereas the evaluated bioinsecticide represents a sustainable alternative for the early management of this pest. Overall, this study provides an experimental basis for strengthening agronomic adaptation strategies and sustainable phytosanitary management for crops of regional interest.

Keywords: 2^2 factorial design, relative humidity, neem-based bioinsecticide, *Planococcus citri*, agronomic adaptation.

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INTRODUCTION

The growing interest in natural sweeteners has positioned *Stevia rebaudiana* Bertoni as a species of agronomic and industrial importance because of its content of steviol glycosides, mainly stevioside and rebaudioside A (Castillo-Vargas et al., 2025), compounds with high sweetening power and potential application in low-calorie products (Amaro-Arcos et al., 2025). The botanical nomenclature of this species was corroborated using the taxonomic record available in Plants of the World Online, Royal Botanic Gardens, Kew (n.d.).

The agronomic relevance of this crop is also associated with the need to strengthen leaf production systems, seedling management, and the generation of technical information useful for researchers, producers, and agricultural advisors, particularly in contexts where profitable and sustainable crops are sought under specific local conditions (Carneiro et al., 2024).

Although stevia is commonly associated with favorable development conditions at relatively low altitudes, edaphoclimatic gradients and soil properties can directly influence the physiological and metabolic responses of plants, thereby affecting their growth, adaptation, and stress tolerance (Ixmatlahua-Rodríguez et al., 2026). Therefore, its establishment under altitudinal conditions higher than those reported as optimal represents an aspect of agronomic interest, especially in regions where thermal variability and relative humidity may modify crop vegetative performance.

The early development of this species may also be affected by phytosanitary factors, including the presence of the mealybug *Planococcus citri*, which can reduce vegetative vigor and affect leaf formation. Mealybugs are pests of agricultural importance because they cause mechanical damage during feeding, reduce crop quality and productivity, and may act as vectors of plant viruses. Within this group, species of the genus *Planococcus* have been reported as relevant vectors; therefore, their monitoring and management are necessary within sustainable production schemes (Ahmed et al., 2023). In this context, the use of low-environmental-impact bioinputs, such as potassium soap and neem extract, represents an alternative compatible with sustainable agronomic management (Tamez et al., 2020).

The objective of this study was to evaluate, as a pilot trial, the initial establishment of *Stevia rebaudiana* Bertoni seedlings in black soil under the environmental conditions of Orizaba, Veracruz, as well as to assess the effectiveness of a neem-based bioinsecticide for the early management of this pest. This approach integrated the analysis of environmental variables with a sustainable phytosanitary management strategy aimed at strengthening stevia cultivation in the High Mountain Region of Veracruz.

Study site

The study was conducted in Orizaba, Veracruz, a municipality located in the central region of the state, at an approximate altitude of 1,230 m above sea level. This environment presents agroclimatic conditions of interest for evaluating the establishment and early development of *stevia rebaudiana* bertoni, since it is located above the altitudinal range commonly reported as optimal for this species.

The thermal variability characteristic of the region provides a relevant scenario for analyzing the adaptive capacity of the crop. According to Ramírez-Jaramillo and Lozano-Contreras (2016), altitudes above 1,200 m above sea level are considered unsuitable for the conventional establishment of *stevia rebaudiana*. Therefore, its evaluation in Orizaba represents a relevant setting for assessing its agronomic potential from a sustainable perspective.

Table 1. Agroecological requirements for the cultivation of *Stevia rebaudiana* Bertoni.

Parameter	Optimal	Suboptimal	Unsuitable
Climate	Tropical and subtropical	Temperate	Cold
Temperature (°C)	18–30	15–18; 30–43	Below 15; above 43
Precipitation (mm)	1000–1400	500–1000; 1400–2000	Below 500; above 2000
Altitude (m a.s.l.)	0–500	500–1200	Above 1200
Soil depth	Greater than 20 to 50 cm	Less than 20 cm	Deficient
Soil drainage	Good	Regular	Poor
Soil texture	Loam and sandy loam	Heavy	Heavy
Soil type	Luvisols, Nitisols, Regosols, and Fluvisols	Cambisols and Rendzinas	Solonchaks and Vertisols

Source: Adapted from Ramírez-Jaramillo and Lozano-Contreras (2016).

MATERIALS AND METHODS

Plant material and growing conditions

Stevia rebaudiana Bertoni seedlings obtained from shoots produced in a previous project were used as the plant material. These seedlings were gradually adapted to the conditions of the present study. An initial stock of 30 seedlings established in black soil was available. From this plant material, eight seedlings were randomly selected for the experimental evaluation associated with the factorial design.

The study period extended from October 2025 to February 2026. In addition to the results of the factorial analysis, the 30 seedlings established under direct planting conditions were monitored to assess their establishment and vegetative vigor. Complementarily, seven seedlings with visible *Planococcus citri* infestation were used in the phytosanitary trial to evaluate their response to the potassium soap bioinsecticide enriched with *Azadirachta indica* (neem) extract.

Irrigation was standardized by applying 800 mL of water per seedling every three days using a graduated cylinder to dose the supplied volume. From the third month onward, 17-17-17 NPK fertilizer was applied at a dosage of 10 g every 10 days as support for vegetative development during the experimental phase.

pH Measurement

The pH of the soil, the irrigation water, and the rainwater recorded during the experimental period was determined using the potentiometric method 4500-H^+ B from Standard Methods (American Public

Health Association et al., n.d.). The inclusion of this variable was relevant because pH influences nutrient availability, the chemical equilibrium of the growing medium, and the conditions of the root environment, all of which affect the establishment and early development of seedlings.

Under nursery conditions, the soil had a pH of 5.7, whereas the irrigation water had a pH of 6.5. These values made it possible to characterize the chemical conditions of the growing medium and provided a complementary criterion for interpreting the initial development of the seedlings. Additionally, from days 84 to 105 of the trial, an average daily rainwater volume of up to 317 mL was recorded. This volume was determined using four rain gauges placed inside the nursery and measured with a graduated cylinder.

Variables evaluated

The evaluated variables were stem growth, number of leaves per seedling, incidence of *Planococcus citri*, light intensity in the nursery, and environmental conditions of temperature and relative humidity. Stem growth was determined by direct measurement in centimeters using a millimeter ruler for general evaluations; when greater precision was needed, a Mitutoyo digital caliper with a nominal resolution of 0.01 mm was used. The number of leaves was recorded by manual counting for each seedling.

The incidence of this pest was evaluated through direct manual counting of visible insects on the infested seedlings before and after the application of the bioinsecticide. To facilitate the observation of small individuals, a 10× BelOMO/Trilomo-type triplet magnifying glass was used. This procedure made it possible to compare pest abundance during the experimental period. The selection of this method was supported by methodological precedents in which direct counting of mealybugs on defined plant structures has been used as a sampling procedure in field studies on *P. citri* (Rossati et al., 2025).

Temperature and relative humidity were monitored using an Elitech RCW-360 monitor, with wireless transmission and real-time data visualization through a Wi-Fi connection to a computer. As a complementary environmental reference source, the NASA POWER platform was used, which provides meteorological and solar data derived from satellite observations and models (NASA POWER, n.d.). During the monitoring period, the records obtained with the local equipment and the data consulted from NASA POWER showed an approximate variation of −1 to −2 °C in temperature and 0.2 to 0.3 units in relative humidity, which made it possible to corroborate a general trend of consistency between both sources.

In addition to morphological growth, environmental conditions may modify the physiological and biochemical responses of *Stevia rebaudiana*, including processes related to the biosynthesis of steviol glycosides. Therefore, the interpretation of early vegetative development should be linked to the cultivation environment and to the physiological response of the plant under specific local conditions (Sharma et al., 2026).

Light intensity in the nursery was determined using a Steren photocell lux meter equipped with a silicon photodiode sensor and optical filter, with a measurement capacity of up to 10,000 lux. All measurements were performed on the same experimental units, using the same instruments, under uniform observation criteria and at consistent intervals throughout the study period.

Statistical analysis

The study considered a replicated 2^2 factorial experimental design, in which the effects of temperature and relative humidity on the early growth of the seedlings were analyzed. Stem height was considered the main response variable, Y1, for the analysis of morphological performance.

The data generated during the experimental period were analyzed by analysis of variance (ANOVA) using Minitab 2023, with the purpose of determining the effects of temperature, relative humidity, and their interaction on the early growth of *Stevia rebaudiana* Bertoni seedlings. This analysis made it possible to identify the factors with a significant effect on the evaluated response and provided quantitative support for interpreting the agronomic behavior of the crop.

RESULTS AND DISCUSSION

Effects of Temperature and Relative Humidity on Stem Growth

The replicated 2^2 factorial experimental design made it possible to identify the influence of environmental conditions on the morphological performance of *Stevia rebaudiana* seedlings. An analysis of variance performed in Minitab 2023 revealed a positive and significant effect of relative humidity on stem growth, with a coefficient of determination of $R^2 = 94.51\%$. These results indicate that a high proportion of the variation observed in the response variable Y1 was explained by the factors included in the experimental model.

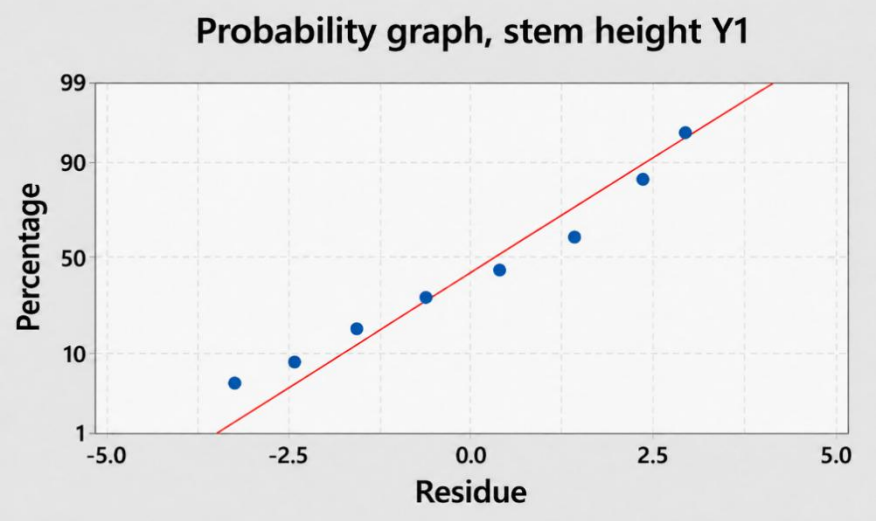


Figure 1. Probability graph (stem height).

Although temperature and relative humidity are closely related environmental variables, their interaction was not statistically significant in the evaluated factorial model. Nevertheless, the combined interpretation of these variables remains agronomically relevant, since previous studies have highlighted their influence on the adaptation and development of *Stevia rebaudiana* under specific cultivation conditions (Gutiérrez-González et al., 2025). Therefore, relative humidity should be considered a key factor in future crop management and adaptation strategies in high-altitude regions.

The critical reference line in the Pareto chart was located at 2.776, corresponding to the significance criterion established at $\alpha = 0.05$. Effects B and A exceeded this value and were therefore considered statistically significant for response Y1. In contrast, the AB interaction did not exceed the critical value, indicating that it was not statistically significant under the evaluated experimental conditions. Among the significant factors, relative humidity had the greatest effect, as shown in Figure 2.

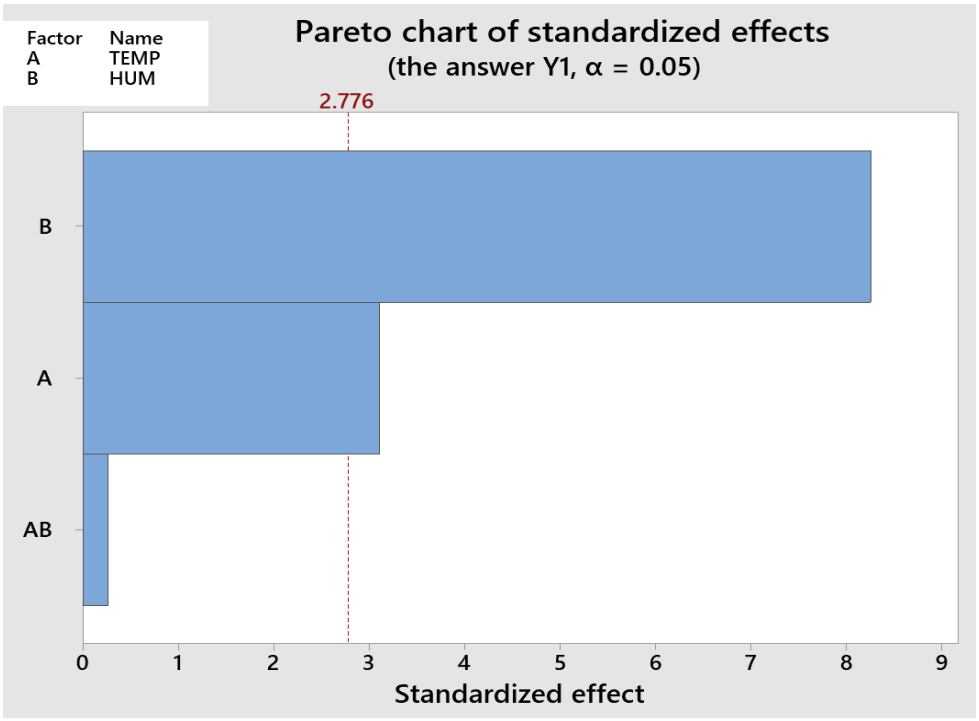


Figure 2. Pareto chart for temperature and humidity.

Relative humidity had the greatest effect, suggesting that atmospheric moisture availability was the most important component during the early stage of development. This behavior may be related to plant water balance, leaf expansion, and the maintenance of cell turgor. Although temperature also contributed to the crop response, its effect was lower than that observed for relative humidity.

Compared with seedlings previously managed in pots, those established in black soil showed better establishment and greater vegetative vigor. The average leaf development was approximately 378 leaves per seedling, with representative values ranging from 315 to 383 leaves in specimens 17 to 22. This variable is a relevant agronomic indicator in *Stevia rebaudiana*, since leaves are the main productive organ because of their relationship with the accumulation of steviol glycosides. In this context, the evaluation

of agromorphological, physiological, and biochemical traits allows a more accurate assessment of crop responses under specific production environments (Thakur et al., 2025).

From an agronomic perspective, these results suggest that soil cultivation favored a more stable vegetative response. Compared with previous trials, seedlings established in black soil developed approximately 11% more leaves, which may be attributed to better interactions between the root system and the substrate. Soil conditions and water management influenced not only the physical establishment of the seedlings but also their ability to sustain active leaf growth, a key aspect of a species whose agronomic value is closely associated with the accumulation of leaf biomass.

Complementarily, sustained seedling growth was observed throughout the monitoring period. The simultaneous increase in stem height and leaf development reinforces the interpretation that relative humidity plays a decisive role during the early stages of establishment by promoting the physiological processes associated with vegetative growth.



Figure 3. Adapted *Stevia rebaudiana* Bertoni seedlings grown in black soil under nursery conditions at TecNM, Orizaba Campus.



Figure 4. General view of *Stevia rebaudiana* Bertoni seedlings established under direct planting conditions at TecNM, Orizaba Campus.

Phytosanitary response of S. rebaudiana Bertoni seedlings to neem-based bioinsecticide treatment

Direct counting per seedling made it possible to quantify the response of *Planococcus citri* to the evaluated bioinsecticide treatment. In seedling 21, the visible insect population decreased from 53 individuals at the beginning of the application period to 7 individuals on day 13, corresponding to an 86.8% reduction. These results suggest that a potassium soap formulation enriched with neem may contribute to the early management of this pest under nursery conditions.

Table 2. Visible count of *Planococcus citri* on seedling 21 during bioinsecticide treatment.

Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Planococcus citri	53	48	41	45	43	38	34	31	28	25	23	11	7

Note: The percentage reduction was calculated on the basis of the initial count and the count recorded on day 13.

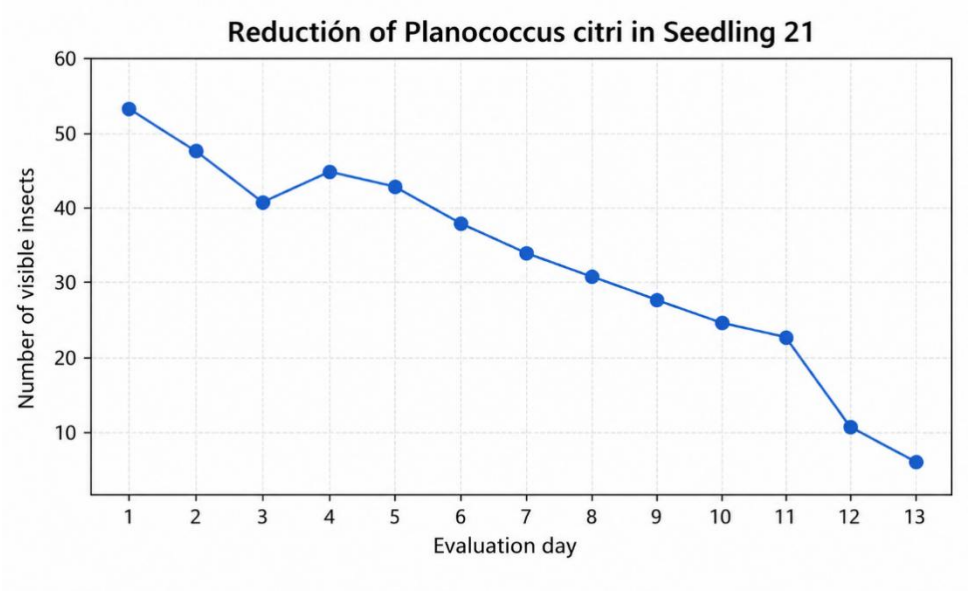


Figure 5. Reduction in the visible population of *Planococcus citri* in seedling 21 during the bioinsecticide treatment period.

From the perspective of sustainable agronomic management, these findings are relevant because they show that the use of bioinputs may represent a lower-environmental-impact alternative to conventional pesticides. In addition, its effectiveness during an early stage of infestation supports its potential use as both a preventive and corrective tool within integrated pest management schemes.

Overall, the results revealed that the early growth of *Stevia rebaudiana* under the conditions of the High Mountain Region of Veracruz was strongly influenced by relative humidity, whereas the use of bioinputs contributed to the development of more sustainable phytosanitary management strategies. From a sustainability perspective, the initial management of this crop can be strengthened through biocompatible strategies that promote plant growth and reduce dependence on chemical inputs. In this

context, beneficial plant–microorganism interactions have been identified as alternatives for improving the growth, yield, and sustainability of stevia cultivation (Abdelsattar et al., 2023). The integration of both dimensions, vegetative growth and ecological pest control, represents one of the main contributions of this pilot trial, as it allows the transition from a conceptual proposal for sustainable management to an experimental basis with regional agronomic relevance.

CONCLUSIONS

A pilot trial conducted in Orizaba, Veracruz, confirmed that the initial establishment of *Stevia rebaudiana* Bertoni in black soil was influenced mainly by relative humidity, which was identified as the variable with the greatest effect on seedling vegetative growth. These results show that crop success during its early stage depends largely on its response to local environmental conditions.

The factorial analysis revealed that both relative humidity and temperature significantly affected stem height. However, relative humidity presented the greatest magnitude of effect, while the interaction between the two factors was not statistically significant under the evaluated conditions. These findings support the relevance of environmental monitoring as a complementary tool for the initial management of the crop.

The potassium soap bioinsecticide enriched with neem showed potential for the early control of *Planococcus citri*, reducing the visible insect population in seedling 21 from 53 to 7 individuals over a 13-day period. These results support its use as a sustainable, low-environmental-impact alternative within early phytosanitary management strategies.

As a relevant contribution, this study suggests that *Stevia rebaudiana* may show promising initial adaptation to altitudinal and thermal conditions different from those commonly reported as optimal for its conventional establishment. Overall, this work provides an experimental and methodological basis for future agronomic evaluations of crops of regional interest exposed to variable environmental conditions.

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The coauthors dedicate this chapter to the memory of Dr. Eusebio Bolaños Reynoso, whose vision, guidance, and commitment gave rise to this project and strengthened its scientific and academic

development. His confidence in the potential of this work was essential to its consolidation, as was his support for the academic training of Mtro. Carlos Iván Gutiérrez González, to whom he provided the opportunity to pursue doctoral studies. This chapter is evidence of his commitment to research and his dedication to the training of new generations of professionals.

To the BioSecuraNet International Network—International Network for Biotechnology and Innovative Strategies for Global Food Security.

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Chapter 14

Evaluation of yield components of two F1 maize hybrids under controlled deficit irrigation in a moderately saline vertisol soil in Yaqui Valley, Sonora, Mexico

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ABSTRACT

Maize (*Zea mays*) is among the most important cereals worldwide because of its high consumer demand. Through genetic improvement, hybrids with high yields have been obtained compared with those of previous eras, as well as hybrids that are tolerant to damage from certain pests, diseases, salinity, and other biotic and abiotic factors. Currently, salinity is a global problem, particularly in arid and semiarid zones, such as the Yaqui Valley in Sonora, Mexico, where rainfall is quite scarce and the indiscriminate use of chemical fertilizers is prevalent. Therefore, implementing controlled deficit irrigation systems will be the best option or alternative for agricultural producers, not only in the Yaqui Valley but also in other regions with similar climate and soil salinity conditions. From this perspective, the present study was conducted to evaluate the grain yield components of two F1 single-crop hybrids using a split-plot experimental design in Vertisol soil with moderate salinity (3.8 ds/m) under drip irrigation. The results revealed variability in the response of the two hybrids to salinity, with a difference of one ton. The F1 hybrid from treatment 2 (L7 TNM-107-C x L5 TNM-72-P) exhibited the best response, achieving a yield of 14.4 t h^{-1}. The yield components ear length, number of rows, and number of grains per row were significantly greater in this hybrid, indicating greater salinity tolerance.

Keywords: *Zea mays* L., salinity, deficit irrigation, Vertisol soil, yield components

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INTRODUCTION

There is a wide variety of maize types, primarily white and yellow kernels, used for human and livestock consumption, respectively. This cereal is among the most important crops produced in Mexico because of its significant contribution to the Mexican diet (Franco; Galindo, 2023). Pigmented maize varieties also exist in Mexico in a considerable area (Preciado-Ortiz et al., 2025). In the Yaqui Valley, maize is considered a second crop option after wheat, which is cultivated on a larger number of hectares (Velázquez et al., 2025). Genetic improvement in maize has progressed considerably in recent years, primarily because of its great economic, social, and cultural importance, and second, because of the efficient development and implementation of various technological innovations that have allowed for physiological and molecular studies that were not possible in previous research (Valenzuela, 2024).

Drought, which is considered an abiotic factor, is currently an alarming problem in Mexico in all aspects of life (Ortega-Marín; González-Rosa, 2024). High temperatures and a lack of rainfall contribute to this problem, affecting almost 50% of Mexico's territory. Currently, 14 states are experiencing this climatic problem most acutely, with the northern states being the most critical. in the states of Chihuahua, Sonora, and Sinaloa (Noperi-Mosqueda et al., 2025).

The implementation of agricultural practices for sustainable agriculture has many benefits, primarily environmental and economic. Specifically, water conservation is highly important in regions with very low rainfall (Sperandio et al., 2026).

Therefore, opting for a drip irrigation system is an excellent alternative for the production of a crop that requires a high-water demand, in this case, maize (Monge-Freile et al., 2025).

Recently, in the Yaqui Valley, studies have been conducted to evaluate the tolerance of maize hybrids to conditions such as drought and salinity. Reduced water availability can affect the concentration of salts in the soil; thus, evaluating the tolerance of local hybrids to salinity in soils with limited water availability can be a sustainable option for increasing yields and contributing to regional food security (Pérez-López et al., 2025). Therefore, the objective of this study was to evaluate the yield components of two F1 single-crossed maize hybrids under controlled drip irrigation conditions in moderately saline Vertisol soil in the Yaqui Valley.

MATERIALS AND METHODS

Study site

The geographical location of the study area corresponds to the experimental field of the National Technological Institute of Mexico, Valle del Yaqui Campus (Figure 1). The ITVY is located on Calle 600, Block 611, Bácum, 85276 San Ignacio Río Muerto, Sonora, at an altitude of 26 meters above sea

level. The climate of the municipality is classified into two types: the area north of the Yaqui River has a very hot and hot dry climate, whereas half of the area south of the river has a temperate dry climate.



Figure 1. Image of the site where the trial was established at the National Technological Institute of Mexico, Valle del Yaqui campus, Bácum, Sonora, Mexico. Image taken from Google Earth on October 20, 2025.

Plant Material

In this study, two F1 single-cross hybrids that exhibited the best agronomic characteristics were used. These were obtained from 72 crosses in a diallelic design of 9 pure lines from the National Technological Institute of Mexico, Roque Campus, Celaya, Guanajuato, Mexico.

Table 1. shows the characteristics of the 4 pure lines that were crossed in the diallelic design using Griffing's Method I, which generated the 2 single-cross hybrids under study.

Line	Level of inbreeding	Origin of the lines	Source material
L3 TNM-111-DK	S_9	Pob. F_2 (DK2042)	Bayer
L5 TNM – 72-P	S_9	Pob. F_2 (P3030 W)	Corteva
L6 6-P	S_9	Pob. F_2 P3030 W)	Corteva
L7 TNM-107-C	S_9	Pob. F_2 (CAIMAN)	Bayer

The two F1 single hybrids evaluated in this study for treatments 1 and 2 were as follows: Treatment 1. L3 TNM-111-DK x L6-6-P; Treatment 2. L7 TNM-107-C x L5 TNM-72-P

Variability in Environmental Conditions at the Experimental Site

During crop development, climatic variables such as soil temperature were monitored using a TRUPER laser temperature meter. The maximum and minimum ambient temperatures and relative humidity were also monitored using a multiparameter temperature and relative humidity meter. Rainfall was recorded, with zero values for most months of the evaluation—only 0.5 mm in January. The soil moisture percentage was monitored with a tensiometer; the moisture content was maintained at 100% after irrigation and decreased to 75% at the time of the next irrigation. The relative humidity values ranged from 46% to 56%, indicating a dry climate in the region.

Experimental Design and Treatments

The experimental design used was a split-plot design with three replications and two F1 single hybrids that constituted the treatments; these consisted of the two F1 hybrids used. The experimental plot consisted of 4 rows per treatment, with a length of 12 m and 0.80 m spacing between rows and a spacing between plants of 10 cm, for a population of 1440 plants per treatment.

Evaluated variables

The agronomic variables of the grain yield components were evaluated. To evaluate these four variables, 10 ears of maize were randomly selected for each treatment. The number of rows per ear (NRE, unit), number of grains per ear (NGE, unit), ear length (EL in cm), and ear diameter (ED in cm) were determined. The latter two variables were measured using a TRUPER brand measuring tape and a TRUPER brand digital caliper, respectively.

Statistical analysis

For the statistical analysis of the data obtained from each treatment, the theoretical t distribution (Gosset, 1917) was used for the variables of the grain yield components at a significance level of 0.05%. The data were processed using the professional statistical package STATISTICA version 14.0 for Windows.

RESULTS AND DISCULTS

Variability in climatic conditions at the experimental site during crop development.

According to the monitoring of climatological variables during crop development, as shown in Figure 2, differences in occurrence between the evaluation months and a considerable correlation between these variables can be observed. The maximum temperature reached 38°C, and the minimum temperature was 10°C, which affected the relative humidity; the higher the temperature was, the lower the relative humidity.

It can also be observed that only rainfall during the crop cycle occurred in January, which coincided with the lowest temperature and highest relative humidity. From this perspective, the results of the present study allow us to corroborate that these arid and semiarid agricultural zones with very scarce rainfall encourage us to implement irrigation techniques for greater water use efficiency, such as controlled deficit irrigation (Franco-Luesma et al., 2025).

Similarly, the soil temperature increases according to the ambient temperature.

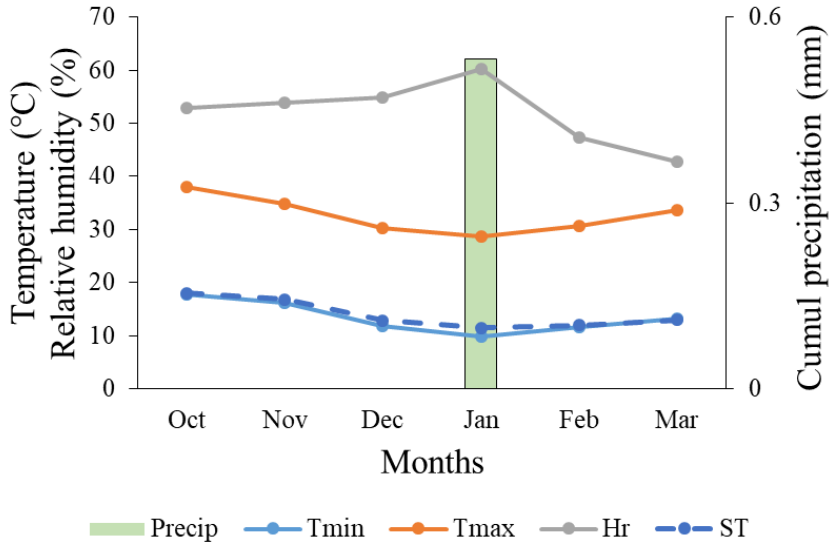


Figure 2. Variability in climatological conditions at the experimental site during the crop cycle from October to March 2025–2026.

Agronomic variables of grain yield components.

The overall average results for the grain yield components of the hybrids under study are shown in Figure 3. Ear length (EL) for both treatments was 19.95 and 21.25 cm, respectively, with significant differences between them. Ear diameter (ED), at 5.5 and 5.23 cm, did not significantly differ. The number of rows per ear (NRE), 15.6 and 14.6, did significantly differ, as did the number of kernels per row.

Table 2. Grain yield components in the treatments (EL: ear length; ED: ear diameter; NRE: number of rows per ear; NGE: number of grains per ear; t value: calculated Student's t value; p probability).

Treatments	Yield components variables			
	EL (cm)	**ED (cm)**	**NRE (unit)**	**NGE (unit)**
T1	19.95	5.5	15.6*	31.20
T2	21.25*	5.23	14.6	33.60*
t-value	1.23421	-1.27621	-0.97435	1.1832
p	0.023299	0.54096	0.0072027	0.0251940

* represents a significant difference according to Student's t test.

It is essential for maize breeding programs to select homogeneous materials with high yields and stable morphological and agronomic attributes. These attributes contribute to good genetic adaptability and high grain yield (Ponce-Encinas et al., 2022).

Furthermore, considering that these hybrids have not yet been genetically improved, they present very satisfactory values compared with others already on the market as a production alternative for farmers.

With respect to grain yield (Figure 3), significant differences were found between the two hybrids evaluated under this controlled deficit irrigation scheme, which contributed to grain yield. The significant differences in yield and most of the components between these two genetic materials indicate differences in their tolerance to soil salinity. The F1 hybrid of treatment 2 (L7 TNM-107-C X L5 TNM-72-P), which achieved a yield of 14.4 t h^{-1}, was superior to that of treatment 1 (L3 TNM-111-DK X L6-6-P), whose yield was 13.4 t h^{-1}.

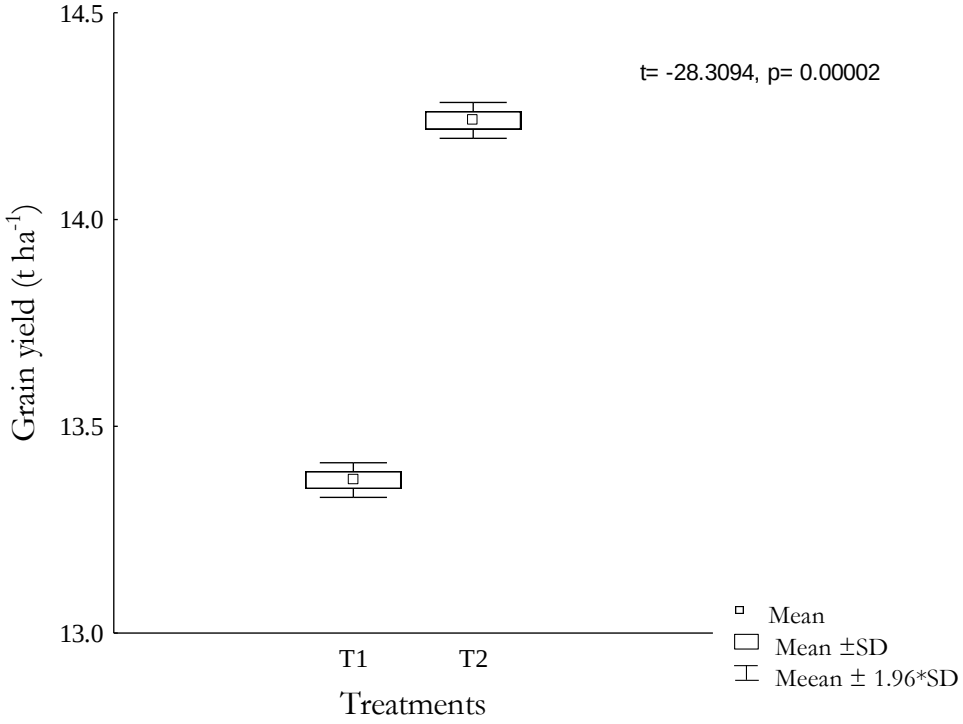


Figure 3. Grain yield in t ha^{-1} of the two hybrids evaluated.

The tolerance of maize to salinity is becoming a vital element of study because salt damage affects plant development (Li et al., 2023). This is due to the importance of maize cultivation for human and animal consumption and the need to increase production on the basis of the increasing population density in Mexico (Sobrino, 2024).

The salinity in the Yaqui Valley is known to increase significantly each year (Dévora-Isiordia, 2016). Therefore, obtaining local genetic materials that can tolerate these conditions is highly economically important.

The use of drip irrigation systems improve water availability (Solano et al., 2021), making it an economically viable option for increasing water use efficiency in semiarid regions (Díaz et al., 2008; Ortiz-Diaz et al., 2025). This study provides evidence of the work being performed at the local level in northwestern Mexico, particularly in the Yaqui Valley, to help mitigate the effects of salinity on the development and productivity of crops such as maize and to contribute to regional and national food security.

CONCLUSIONS

The results demonstrated variability in the response of the two hybrids to salinity conditions, with a difference of one ton. The F1 hybrid from treatment 2 (L7 TNM-107-C x L5 TNM-72-P) showed the best response, achieving a yield of 14.4 t ha^{-1}.

The yield components ear length (EL), number of rows per ear (NRE), and number of grains per row (NGE) were significantly greater in this hybrid, indicating greater salinity tolerance.

A key conclusion of this study is that when water resources are used efficiently—in this specific case, with controlled drip irrigation—and when the level of fertilization is much lower than that typically recommended in technological packages, the grain yield is 1.0 t^{-1} greater than that achieved with flood irrigation.

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Aquaculture

Chapter 15

Effects of partially replacing fish meal with mesquite pod meal (*Prosopis juliflora)* on the growth of nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Food represents one of the main costs in aquaculture; therefore, the search for alternative protein sources to fish meal is a priority for the sustainable development of the sector. In this study the effects of partially replacing fish meal with mesquite (*Prosopis juliflora*) pod and seed meal on the growth, survival, and proximate composition of juvenile Nile tilapia (*Oreochromis niloticus*) were evaluated. Mature pods were harvested, dried, ground, and sieved to obtain meals, which were used to formulate experimental diets using the Pearson square method. Three treatments were established: a pod meal diet, a seed meal diet, and a control diet. The fish were fed during a 165-day experimental period, with weight and length recorded every 15 days, in addition to monitoring physicochemical water parameters. Proximate analysis revealed that the formulations containing pod meal had adequate protein and carbohydrate contents. At the end of the experiment, the fish fed pod meal achieved the highest growth values, with an average weight of 175.7 g and a length of 248.49 mm, outperforming the treatments with seed meal and commercial feed. Furthermore, no mortality was recorded in the groups fed plant-based meals, whereas the cumulative mortality rate of the control group was 10%. The results indicate that mesquite pod meal represents a viable and sustainable alternative as a partial substitute for fish meal in tilapia diets, contributing to improved growth and reduced dependence on conventional.

Keywords: Nile tilapia, Fish meal, Alternative protein sources.

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INTRODUCTION

In recent decades, aquaculture has increased significantly, becoming a key source of fish for human consumption (FAO, 2024). Its role in global food security is crucial, as it provides protein, essential fatty acids, and micronutrients, especially in developing countries (Bogard et al., 2017). Furthermore, it has driven economic and agricultural development in various regions (Kariuki et al., 2024). According to the FAO, by 2050, fish and aquaculture production will need to increase by more than 60% to meet global demand (Subasinghe, 2014).

Tilapia is among the most widely farmed species because of its rapid growth, disease resistance, and adaptability to various environmental conditions (Méndez-Martínez et al., 2018). Nile tilapia (*Oreochromis niloticus*) stands out for its ease of rearing and market acceptance, making it a profitable alternative for large-scale aquaculture (Rodríguez-Hernández et al., 2025).

One of the greatest challenges in aquaculture is the use of fish feed, as it accounts for between 30% and 70% of production costs (Munguti et al., 2021). Fish meal is the predominant protein source because of its high nutritional value and digestibility. However, its growing demand has led to economic and environmental problems, increasing costs and reducing its availability. This has prompted the search for sustainable alternatives (Annamalai et al., 2021).

Various plant- and animal-based protein sources have been explored to reduce the dependence on fish meal. A promising resource is mesquite (*Prosopis spp.*), a fast-growing perennial shrub found in semiarid regions worldwide (Armijo-Nájera et al., 2019; Chaturvedi et al., 2013). In Mexico, it is estimated that there are more than four million hectares of mesquite, notably *Prosopis leavigata*, *Prosopis glandulosa*, and *Prosopis juliflora* (Andrade-Montemayor et al., 2011). This species is ecologically significant because of its ability to fix nitrogen in the soil, improve fertility, reduce erosion, and provide shelter for wildlife (Villegas-Espinoza et al., 2014).

Mesquite has been evaluated as a forage alternative in arid regions, where feed availability for livestock is limited (Armijo-Nájera et al., 2019). Its pods have been incorporated into the diets of cattle, sheep, camels, buffalo, rabbits, and poultry in South America, Africa, and India. Its protein and soluble sugar contents improve the quality of animal diets and provide a valuable nutritional resource during times of scarcity (Armijo-Nájera et al., 2019).

The use of mesquite pod meal in fish feed is an area of study that is still underdeveloped but has high potential because of its nutritional characteristics. As a legume, mesquite shares properties with other protein sources widely used in aquaculture, such as soybean meal. The latter has been incorporated into various aquaculture diets because of its availability and nutritional profile, although its complete replacement with fish meal depends on the specific needs of each species (Ahmad et al., 2020). However, the presence of antinutritional factors, such as lectins and protease inhibitors, in soybean meal can negatively impact fish growth and health (Veer et al., 2021). In this context, mesquite pod meal emerges

as a viable alternative, as its protein composition positions it as a potential substitute for soybean meal in the formulation of sustainable aquaculture diets.

Given the high cost of fish meal and the decline in the number of fish populations used in its production, finding sustainable solutions is crucial. The development balanced diets that combine more accessible protein sources without compromising nutritional quality is a viable strategy (McGoogan et al., 1997). Studies have shown that the use of plant proteins and animal byproducts can reduce costs without affecting fish growth or health. However, completely replacing fish meal remains a challenge because of the need to maintain an adequate balance of essential amino acids and minimize the effects of antinutritional factors (Ahmad et al., 2020). Aquaculture faces significant challenges in terms of the sustainability of inputs, particularly in the area of fish feed. Exploring alternative protein sources, such as mesquite pod meal, presents an opportunity to reduce the dependence on fishmeal and mitigate environmental impacts. To achieve an effective transition, it is essential to continue investigating its impact on fish performance, feed conversion, and the quality of the final product (Kariuki et al., 2024). The objective of this study was to evaluate the effects of partially replacing fish meal with mesquite pod meal (*Prosopis juliflora*) on the growth, health, and body composition of juvenile Nile tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

Selection and Preparation of Plant Material

The experiments were conducted at the National Technological Institute of Mexico/Yaqui Valley Technological Institute which is located in Bacum, Sonora, Mexico. Mature pods were collected from the mesquite tree (*Prosopis juliflora*) and manually selected in a wild area of southern Sonora, Mexico. The selection process involved careful visual inspection to remove pods showing visible signs of physical damage, insect infestation, or fungal growth, with the aim of ensuring the microbiological quality and safety of the final product. This stage is critical, as the presence of contaminants can affect both the nutritional profile and the safety of the feed intended for animal consumption.

Production of Mesquite Pod Flour (Prosopis juliflora)

Once selected, the pods underwent a natural drying process for 15 days. The average temperature in the shade reached 37 °C; measurements were taken daily, which promoted a rapid reduction in surface moisture. This combined strategy of solar and shade drying prevents overheating of the plant material, which could lead to the degradation of nutritional and phytochemical compounds, especially polyphenols and other natural antioxidants present in mesquite (Moreno-Limón et al., 2011; Ochoa-Meza et al., 2023).

Grinding and Sifting

After drying, the completely dehydrated pods were subjected to mechanical grinding using a food-grade hammer mill. The objective was to reduce the material to a homogeneous powder. The resulting flour was sieved using a stainless-steel sieve with 80 mesh, thus ensuring the production of fine, uniform particles suitable for incorporation into feed mixtures and to facilitate digestibility in juvenile organisms. This degree of fine grinding is also important for maximizing the bioavailability of the nutrients contained in the plant product (Geankoplis, 1998).

Proximate Chemical Analysis

Quantitative analyses were performed using standardized procedures of the Association of Official Analytical Chemists AOAC (2000).

Moisture Content

The total moisture content of the food was determined using the AOAC method (950.46). To do this, the sample was dried in a forced-air oven at 105 °C for 4 to 5 hours to completely remove the moisture present. The total moisture content was subsequently calculated using the following equation:

$$Moisture\ Content\ (\%) = \frac{\text{Weight loss}}{\text{Starting weight}}\ X100$$

Total ash

Three grams of powdered sample was placed in each crucible and calcined at 550 °C for 6 hours. Once the process was complete, the crucibles were allowed to cool in a desiccator and were then weighed to determine the ash content.

$$\text{Total ashes}\ (\%) = \frac{W - Z}{N} X\ 100$$

where W is the weight of the crucible and the ash; Z is the weight of the empty crucible; and N is the weight of the sample.

Determination of Lipids

The determination of crude fat in the powdered sample was performed using the Soxhlet method, in accordance with AOAC method 920.39 (2000), employing petroleum ether as the solvent. Three zero-point grams of sample were placed in prelabeled thimbles, which were subjected to an extraction process

for 24 hours in a Soxhlet apparatus. Upon completion of the extraction, the ether was evaporated, the flask was cooled, and it was weighed to calculate the amount of fat extracted.

$$\text{Lipids (\%)} = \frac{\text{Weight of lipids}}{\text{Sample weight}} X\ 100$$

Crude protein analysis

The crude protein content was determined using the micro-Kjeldahl method, in accordance with the methods of the AOAC (990.04). To do this, 0.15 g of flour was weighed and placed in a 250-mL Kjeldahl flask, along with 2.5 mL of 98% sulfuric acid and a catalyst. The mixture was heated until complete clarification was achieved. It was then allowed to cool, and 7 mL of distilled water was added.

The digested solution was transferred to a micro-Kjeldahl distillation apparatus, where 30% (w/v) sodium hydroxide was added to initiate distillation. The released ammonia was captured in a 2% boric acid solution, with methyl red as an indicator. The color change from red to green indicated complete capture of the ammonia. Finally, the solution was titrated with 0.1 N hydrochloric acid until a color change indicating the endpoint was observed. A blank sample was included in the analysis, and the nitrogen percentage was calculated using the following equation.

$$\text{Proteín (\%)} = \frac{(\text{N of HCl})(Volume\ of\ HCl\ used)(1.4\ \text{g/mol})(6.5)}{Sample\ grams}$$

Nutritional Design of the Experimental Diet

Crude protein analysis

The diet was designed using Pearson's square method, a tool widely used in animal nutrition to balance ingredients on the basis of their nutrient content and the needs of the target organism. For this study, a four-variable model was used, allowing for precise adjustment of the proportion of each feed ingredient, and ensuring that protein, energy, lipid, and fiber requirements were met, in addition to essential vitamins and minerals.

Sequential mixing

The ingredients were mixed in a horizontal-shaft mixer, starting with those present in the highest proportions, such as vegetable and protein flours. Subsequently, the ingredients with lower volumes, such as vitamin-mineral premixes, were incorporated, and finally, the oils were added. This sequential procedure ensures a homogeneous mixture and an even distribution of nutrients throughout the feed matrix.

Addition of Water and Homogenization

Following dry mixing, potable water was added; it was incorporated slowly while continuous mixing was maintained. Fifty milliliters of water was added to every 100 grams of dry mixture, allowing certain compounds to be activated and facilitating the formation of a cohesive mass suitable for the pelletizing process. Hydrating the mixture also improved the texture of the final pellet and reduced dust production during handling and storage.

Manual pelleting

Once a moist, homogeneous mass was obtained, it was left to rest and aerate at room temperature for approximately two hours. This resting period allows the ingredients to properly absorb moisture, improving the structural integrity of the pellet. The mixture was subsequently subjected to a pelletizing process using a manual meat grinder, fitted with a nozzle to form compact feed cylinders. This artisanal method is efficient for pilot or small-scale studies, where detailed control over the texture and size of the pellets is needed.

Final drying of the pellets

The obtained pellets were placed on clean trays and subjected to a drying process for two days. This extended drying reduces the final moisture content of the product to levels safe for storage, minimizing the risk of microbial growth. Once dry, the pellets were ground into particles suitable for feeding to juvenile organisms

Measurement of feeding and parameter

During the bioassay, the fish were fed three times a day, and biometric assessments were conducted every 15 days over a 90-day period to adjust the feed ratio of the basis of biomass. The assessments involved measuring the weight and length of each individual. To calculate the biomass of each experimental unit, ten organisms per tank were weighed. The length of each fish was determined using an ichthyometer, measuring from the head to the tip of the caudal fin (Afram et al., 2021; Cadena-Cadena et al., 2023).

To maintain optimal water conditions, parameters such as temperature, pH (using an Ohaus ST10 meter), and dissolved oxygen (measured with a YSI oximeter) were recorded twice daily, at 8:30 a.m. and 1:00 p.m. (Cadena-Cadena et al., 2023). Water was siphoned twice a week to remove settleable particles, and 25% of the total water volume was replaced daily. Additionally, 50% of the water was replaced every ten days by washing the aeration stones (Cadena-Cadena et al., 2023; Claudett et al., 2022). Mortality was determined by counting all the specimens that died during the three replicates of the experiment and was expressed as the ratio of the number of dead fish to the total number of organisms (Afram et al., 2021).

$$Mortality = \left[\frac{Number\ of\ dead\ animals}{Total\ number\ of\ animals}\right] x\ 100$$

Statistical Analysis

Means and their respective standard deviations were calculated for the data obtained. Prior to the statistical analysis, the assumptions of normality and homogeneity of variance were verified. To evaluate possible differences between treatments, one-way analysis of variance (ANOVA) was performed. Differences for which the p-value was less than 0.05 were considered indicate statistical significance. All analyses were performed using SPSS statistical software, version 23.

RESULTS AND DISCUSSION

Response of Growth Performance and Proximate Composition to Pod and Seed Meal Diets

In this study the growth of tilapia fed mesquite pod and seed meal was compared to that of control group that received commercial feed. The results revealed differences in growth patterns among the different treatments, highlighting the potential of mesquite pod meal (*Prosopis juliflora*) as an effective nutritional source for aquaculture species.

During the 165-day experimental period, both the proximate composition of the feed formulations and the growth in weight and length of the organisms fed pod meal, seed meal, and a control treatment based on commercial feed were evaluated. Overall, all the groups showed a progressive increase in growth parameters, although differences in growth efficiency and rate were observed among the treatments.

From the outset, weight (6.20 g) and height (66.6 mm) of the organisms feed seed meals were greater than those of those fed pod meal (5.5 g; 63.7 mm) and the control group (3.2 g; 47.25 mm). Concurrently, proximate composition analysis indicated that the 50/50 formulation—an equal mixture of pod meal and seed meal—contained 17.69% protein, 6.39% fat, 63.17% carbohydrates, 3.46% ash, and 9.30% moisture, with values intermediate to those of its individual components.

At 60 days, the control group weighed more (38.9 g) than the pod (35.1 g) and seed (30.8 g) treatments did. However, in the later stages, the group fed pod flour exhibited more rapid growth. At 90 days, this treatment resulted in a weight of 62.3 g and a length of 166.76 mm, while the seed group maintained the same weight (62.3 g) but with a shorter length (139.2 mm), and the control reached 72.8 g with a length of 130.51 mm. In terms of nutritional content, the pod/commercial feed and seed/commercial feed formulations resulted in the highest protein levels (29.94% and 26.40%, respectively), whereas the control group had 23.80% protein, along with the highest levels of fat (8.97%) and ash (8.97%).

On day 165, highest values of weight and length were recorded for the fish feed pod meal in the study: 175.7 g and 248.49 mm, respectively. In comparison, the group fed seed meal reached 121.2 g and 196.7 mm, and the control group reached 138.7 g and 162.44 mm. These results, supplemented by proximate analysis, suggest that vegetable meals, particularly pod meal, not only offer a balanced nutritional profile but also promote superior growth in the medium and long term under experimental conditions.

Table 1. Proximate analysis of the 2 treatments based on flour and seeds of the mesquite pod (*Prosopis juliflora*) and the control treatment.

formulation 50/50	%Protein	%Lipids	%Carbohydrates	%Ash	%Moisture
Pod Flour	16.91	7.21	62.9	3.76	9.22
Seed Meal	18.47	5.57	63.43	3.16	9.37
Commercial Pod/Food	29.94	6.59	45.94	8.32	9.21
Seed/Commercial Feed	26.4	4.94	50.55	8.77	9.34
Control	23.8	8.97	48.01	8.97	10.25

Table 2. Mean weight (g) and length (mm) of fish fed pod meal, seed meal, and control diet during the 165-day experimental period.

	Sheath		Seed		Control	
Time (days)	weight (g)	size (mm)	weight (g)	size (mm)	weight (g)	size (mm)
0	5.5	63.70	6.20	66.6	3.20	47.25
15	10.2	88.1	9.70	79.2	12.10	80.37
30	17.3	102.8	13.90	95.8	23.40	86.22
45	22.6	115.2	22.50	101.7	19.80	99.63
60	35.1	130.5	30.80	116.9	38.90	109.89
75	55.9	145.9	54.80	133.3	50.40	122.22
90	62.3	166.76	62.30	139.2	72.80	130.51
105	85.7	199.54	73.80	159.5	66.70	137.65
120	106.7	207.46	80.90	165.8	91.30	143.18
135	135.8	218.79	99.80	173.7	105.00	146.80
150	145.6	243.43	104.80	182.2	112.50	160.01
165	175.7	248.49	121.20	196.7	138.70	162.44

In the initial phase of the study, during the first 30 days, superior growth was observed in the organisms fed mesquite pod meal (*Prosopis juliflora*) compared with those fed mesquite seed meal (*Prosopis juliflora*) and commercial feed. This initial difference could be explained by the high energy density and high bioavailability of nutrients present in the formulated feed, even more so than the commercial feed that was designed specifically for tilapia (Cabrera-B. et al., 2001; Guimarães et al., 2012). Starting on day 75 of the experiment, the fish fed the diet containing mesquite pod flour began to show a more rapid growth pattern compared than those in others treatments did, both in terms of weight gain and increase in length. This behavior suggests a possible progressive physiological adaptation to the feed formulated

with plant-based components, which could be associated with improved feed conversion efficiency or greater bioavailability of the nutrients present in the pod meal during later stages of growth (Armijo-Nájera et al., 2019). On the other hand, regarding survival, none of the fish fed plant-based feeds (pods and seeds) died throughout the experimental period, indicating good tolerance to these alternative diets. However, the control group fed commercial feed exhibited a cumulative mortality rate of 10%, which could be related to factors such as the immune response to specific ingredients in the commercial feed or differences in water quality resulting from the type of diet (Abdel-Tawwab et al., 2007; Li et al., 2018).

These finding suggest that mesquite pod flour not only has an adequate nutritional profile but also may induce positive physiological adaptations in the medium and long term. The progressive increase in size observed in this group could be linked to the presence of slowly digestible carbohydrates, soluble fibers, and secondary compounds that, far from acting as antinutrients, could improve digestive efficiency and energy metabolism (de Melo Cavalcante et al., 2022).

In contrast, the performance of the group fed seed meal was more variable. Although the animals initially grew rapidly, the weight gain rate subsequently stabilized, resulting in a final yield lower than that of the pod meal group. This effect could be related to the presence of antinutritional compounds typical of raw seeds, such as trypsin inhibitors, saponins, or tannins, which interfere with protein digestibility and the utilization of essential amino acids (de Melo Cavalcante et al., 2022). Additionally, the slightly lower lipid and protein contents in the seed meal, than in the pod meal, may have limited tissue synthesis in advanced stages of development (de Souza et al., 2019).

Notably, although the commercial feed showed a continuous and stable growth pattern, the height results were lower than those observed in the groups fed plant-based diets. This could suggest that plant-based meals induce greater somatic elongation, possibly because of a different macronutrient ratio or a fatty acid and fiber profile that stimulates body expansion without necessarily increasing total mass (Figueiredo-Silva et al., 2013).

Several studies have reported that the use of ingredients from nonconventional sources in aquaculture can be a viable strategy provided that the availability of limiting amino acids is ensured and the presence of antinutritional factors is controlled (Cadena-Cadena et al., 2023; de Souza et al., 2019; Tibbetts, 2018). The results of the present study are consistent with those of previous research highlighting the use of nonconventional legumes such as *Canavalia ensiformis* and *Vigna unguiculata*, whose starches present nutritional properties comparable to those of corn in tilapia diets (Olivera-Novoa et al., 1997).

Furthermore, it is important to consider the influence of other environmental factors such as salinity, temperature, and stocking density, which can significantly alter the performance of the culture. Studies conducted on tilapia hybrids have shown that they can grow efficiently in environments with salinities of up to 37%, and that the stocking density and protein formulation of the diet directly impact

the feed conversion and growth rate (Watanabe, 1991; Watanabe et al., 1997). Although salinity was not controlled as an experimental variable in this study, the sustained increased in the use of vegetable flours suggests good physiological adaptation of the organisms to the farming system. Another study conducted in Brazil using mesquite pods indicated that the nutritional profile is comparable to that of corn in terms of dry matter digestibility, gross energy, and protein, as well as essential amino acids such as lysine and methionine. These findings suggest the high potential for digestive utilization by *Oreochromis niloticus*, which supports its use as an alternative ingredient in extruded diets. However, although high levels of starch and sucrose were observed in the diets, a 20% substitution resulted in lower protein utilization, as evidenced by reduced plasma amino acids and increased activity of transaminase and deaminase enzymes, suggesting increased catabolism of endogenous proteins without signs of physiological stress or adverse effects on overall health or survival of the fishs (de Souza et al., 2019). Furthermore, in the São Francisco Valley in Brazil and Benue, Nigeria, it was demonstrated that mesquite pod meal can be incorporated into extruded diets for Nile tilapia fingerlings at levels up to 38.67% without compromising production performance or fish health. Although significant differences were observed in parameters such as the feed conversion ratio and blood glucose levels, digestive enzyme activity and transaminases, as well as hematological variables, no relevant alterations were detected. These results suggest a viable alternative for reducing the cost of tilapia feed, which contributes to the sustainability of aquaculture without negatively affecting the growth or physiological condition of the fish (Aroyehun et al., 2019; Nascimento et al., 2019). The use of plant-based flours in aquaculture diets represents a sustainable and economically viable alternative, especially in regions where commercial feed costs are high. The use of mesquite pods as a feed ingredient could significantly reduce the dependence on animal-based or imported ingredients, contributing to the development of sustainable production systems.

CONCLUSIONS

Mesquite pod flour represents a promising plant-based alternative for the formulation of extruded diets for Nile tilapia (*Oreochromis niloticus*), and shows performance comparable to or better than that of commercial feeds at certain growth stages. Its inclusion at levels of up to 50% did not negatively affect growth parameters, supporting its potential as a partial substitute that can improve economic and environmental sustainability in aquaculture. However, further studies are needed to optimize inclusion levels and evaluate the environmental factors that influence mesquite growth, as well as to promote management and conservation strategies that enhance its ecological, cultural, and economic value.

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Chapter 16

Consumer preferences and value-added opportunities in the commercialization of *Oreochromis niloticus* in Tenosique, Tabasco, México

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ABSTRACT

Tilapia, *Oreochromis niloticus,* is among the most important aquaculture species worldwide because of its high adaptability, rapid growth, and broad market acceptance. In this context, the present study aimed to analyze consumer preferences and value-added opportunities in the commercialization of tilapia in Tenosique, Tabasco, Mexico. A quantitative, descriptive, and cross-sectional study was conducted through the application of 303 surveys to fish consumers. The data were analyzed using descriptive statistics. The results revealed that tilapia is a frequently consumed product, with a predominant preference for fresh (32%) and whole (54%) presentations, as well as fried preparations (38%). The main factors influencing purchasing decisions were taste (28%), place of purchase (28%), and health benefits (23%). Additionally, a high willingness to purchase value-added products (88%) and tilapia from certified production systems (94%) was identified, highlighting opportunities to strengthen commercialization through product differentiation. In conclusion, tilapia consumption preferences in Tenosique are determined by sensory attributes, accessibility, and perceived quality, allowing the identification of strategies aimed at value generation and strengthening the aquaculture value chain. These findings provide relevant evidence for decision-making in the sector and open avenues for future research incorporating inferential approaches to better understand consumer behavior.

Keywords: tilapia; consumer behavior; fish consumption; value-added products; aquaculture marketing.

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INTRODUCTION

Aquaculture has become among the fastest-growing food production sectors worldwide, contributing significantly to food security, nutrition, and economic development. According to the Food and Agriculture Organization (FAO), global fish consumption has steadily increased in recent decades, driven by the recognition of its nutritional benefits and its role in healthy diets (FAO, 2022).

Among aquaculture species, tilapia, *Oreochromis niloticus,* stands out because of its high adaptability to diverse production systems, rapid growth, and wide market acceptance, positioning it as one of the most widely cultivated species globally. Its economic and productive relevance has increased significantly in recent years, driven by the growing demand for affordable protein and the expansion of both local and global markets (El-Sayed, 2019). In this context, consumer preference for fish products, including tilapia, has been shown to be influenced by attributes such as price, product presentation, origin, and production methods, which directly affect purchasing decisions (Andoh-Odoom et al., 2024; Owusu et al., 2025).

In southeastern Mexico, particularly in the state of Tabasco, aquaculture represents a relevant activity with significant potential for economic development, especially in areas with abundant water resources. The municipality of Tenosique, located along the Usumacinta River, is characterized by the presence of aquaculture production units, cooperatives, and commercial farms dedicated to fish production. However, there is limited scientific evidence addressing local consumer preferences for tilapia, which restricts the ability of producers to make informed decisions regarding product diversification and market positioning, as commercialization is predominantly based on fresh products sold directly at the farm level (Rusdian et al., 2023; Rusdian et al., 2024). Therefore, the objective of this study was to analyze consumer preferences for tilapia in Tenosique, Tabasco, to identify the factors influencing consumption, propose more efficient commercialization strategies, and contribute to strengthening the aquaculture value chain.

MATERIALS AND METHODS

Study design

A quantitative, descriptive, and cross-sectional study was conducted to analyze consumer preferences for tilapia and the factors associated with its commercialization in the municipality of Tenosique, Tabasco, Mexico. This type of design is widely used in consumer behavior research, as it allows the characterization of consumption patterns within a specific context without manipulation of variables (Malhotra, 2019).

Study area

The study was carried out in the municipality of Tenosique, Tabasco, Mexico, which is located in the southeastern region of the country. This area is characterized by its proximity to the Usumacinta

River and by the presence of aquaculture production units, including commercial farms and cooperative societies dedicated to tilapia farming, making it a relevant setting for analyzing local consumption patterns.

Population and sample

The target population consisted of fish consumers residing in the municipality of Tenosique. A nonprobabilistic convenience sampling approach was employed, with participants selected on the basis of accessibility and availability. This sampling method is typically used in exploratory and consumer preference studies aimed at obtaining direct information from local markets (Hair et al., 2019). A total of 303 valid surveys were collected, providing a sufficient dataset for analyzing consumer preferences. Participation was voluntary and anonymous, and the confidentiality of the information was guaranteed. The data collected were used exclusively for academic and research purposes.

Instrument design and data collection

Data were collected using a structured questionnaire designed on the basis of approaches commonly used in consumer preference studies for food and fish products (Verbeke et al., 2007; Claret et al., 2012). The instrument included qualitative categorical variables organized into three analytical dimensions: a) sociodemographic characteristics, including variables such as age, sex, and occupation, to characterize the consumer profile; b) consumption habits, including frequency of fish consumption, types of products consumed, and general consumption patterns (Claret et al., 2012); and c) consumption preferences and decision-making criteria, including variables related to product presentation, place of purchase, and selection criteria such as price, quality, freshness, and origin. These attributes have been identified as key determinants in fish product acceptance and purchasing decisions (Verbeke et al., 2007; Honkanen et al., 2005). The questionnaire was administered in person at strategic locations within the municipality, including commercial areas, markets, and high-traffic zones, to capture a diverse range of consumer profiles.

Study limitations

This study employed a nonprobabilistic convenience sampling approach, which has certain limitations in terms of population representativeness. Although this type of sampling is widely used in consumer behavior research because of its operational feasibility, it does not ensure that all individuals in the population have an equal probability of being selected, which may introduce bias into the results (Hair et al., 2019; Malhotra, 2019).

Therefore, the findings reflect the preferences of the surveyed consumers within the specific context of Tenosique, Tabasco, and should be generalized to other regions with caution. Nevertheless,

this sampling approach is appropriate for obtaining preliminary and descriptive insights into consumption patterns in local markets, providing valuable inputs for decision-making in specific contexts (Etikan et al., 2016).

Data analysis

The collected data were organized and analyzed using descriptive statistics. Absolute frequencies and relative frequencies (percentages) were calculated for each variable, allowing the identification of consumption patterns, consumer preferences, and commercialization trends. This type of analysis is widely used in food preference studies for market characterization (Hair et al., 2019).

RESULTS AND DISCUSSION

Sociodemographic distribution of fish consumers

The results indicate that tilapia consumption in Tenosique, Tabasco, is influenced by a combination of sociodemographic factors, consumption habits, and product attributes that directly affect consumer decision-making. A total of 303 valid responses were obtained from fish consumers. The ages of the respondents ranged from 18 to 50 years, with the highest proportion concentrated in the 26–33 age group (Table 1).

Table 1. Sociodemographic distribution of fish consumers (n=303).

Variable	Category	Percentage (%)
Gender	Female	54
	Male	46
Age (years)	18 - 25	19
	26 - 33	28
	34 - 41	27
	42 - 50	26
Occupation	Employer	14
	Employee	48
	Homemaker	38

Source: Own elaboration.

This distribution reflects a diverse sociodemographic profile, which is essential for understanding the consumption patterns of fish and seafood products. Additionally, 54% of the respondents were female, and 46% were male. With respect to occupation, most participants were classified as employees (48%), followed by homemakers (38%) and employers (14%). These findings suggest that tilapia consumption is associated with economically active population groups, which is consistent with the findings of Claret et al. (2012), who reported that age and occupational profile significantly influence fish consumption patterns. The slight predominance of female participants also aligns with previous studies

indicating that food purchasing decisions are often influenced by women, particularly in household contexts.

Consumption habits and product preferences

Table 2 presents the consumption preferences for tilapia. The most relevant findings indicate that respondents reported a weekly consumption frequency (38%), suggesting that tilapia is part of the regular diet in the study area. With respect to purchasing preferences, fresh (32%) and prepared products (31%) tended to have similar preferences, whereas whole fish was the most preferred presentation (54%).

Table 2. Consumer preferences for tilapia fish consumption (n=303).

Variable	Category	Percentage (%)
Consumption frequency	Weekly	38
	Biweekly	32
	Monthly	30
Purchase preference	Frozen	25
	Fresh	32
	Prepared	31
	Canned	13
Product presentation preference	Whole	54
	Fillet	28
	Steak (cut)	18
Preparation preference	Grilled	28
	Fried	38
	Baked	18
	Wrapped (foil-cooked)	16
Purchase quantity preference	0.5 - 1 kg	46
	400 - 450 g	36
	300 - 350 g	18

Source: Own elaboration.

In terms of preparation methods, fried tilapia was the most preferred material (38%), and the most common purchase quantity ranged between 0.5 and 1 kg (46%). These results reinforce the role of tilapia as an accessible source of animal protein, which is consistent with the findings of Andoh-Odoom et al. (2024), who reported that fish consumption in developing countries is strongly influenced by availability, accessibility, and cultural habits. The preference for whole and fresh presentations reflects the importance of freshness as a quality indicator, in agreement with Del Río-Zaragoza et al. (2022), who identified freshness as a key factor in fish selection in Mexico. Additionally, the preference for fried preparation suggests the influence of local culinary traditions.

Factors influencing purchasing decisions

With respect to the factors influencing purchasing decisions, consumers identified taste (28%) and place of purchase (28%) as the most important attributes, followed by health benefits (23%) and price (21%) (Figure 1). The preferred purchasing locations were restaurants or small food establishments (30%), followed by local markets (29%), street vendors (21%), and supermarkets (20%). This distribution highlights the relevance of traditional and short marketing channels in fish commercialization. These findings reinforce the role of local markets in shaping fish consumption behavior in developing regions. These findings are consistent with those of Owusu et al. (2025), who reported that fish consumption decisions are influenced by a combination of intrinsic (such as taste) and extrinsic (such as accessibility and price) factors. From a consumer behavior perspective, these results can be interpreted as part of the product evaluation process prior to purchase (Rusdian et al., 2023).

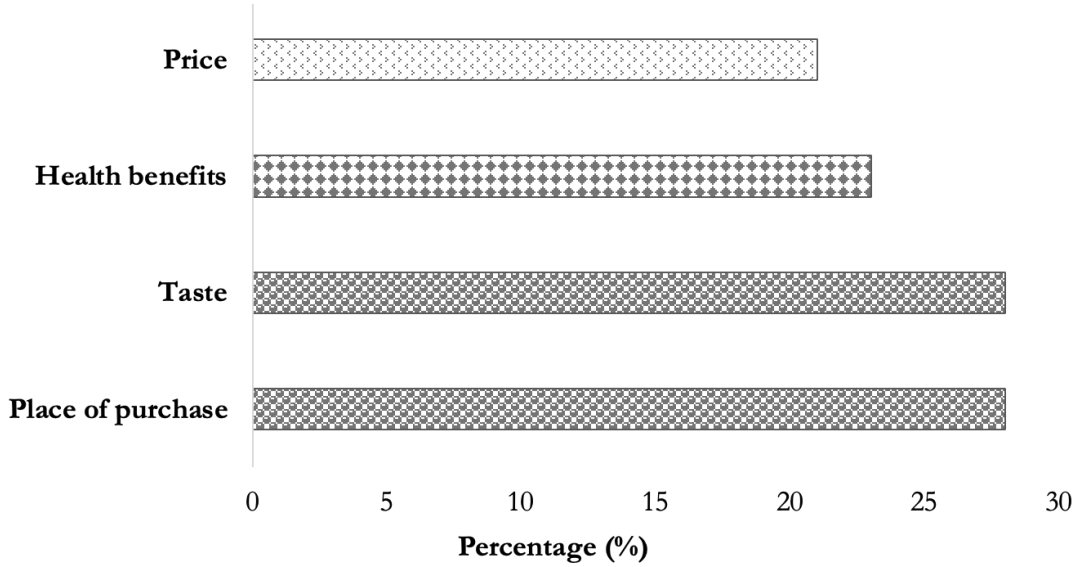


Figure 1. Distribution of the main factors influencing consumer purchasing decisions for tilapia in Tenosique, Tabasco.

Commercialization trends and value-added opportunities

Table 3 shows consumer perceptions regarding commercialization trends. A high willingness to purchase value-added tilapia products was observed (88%), particularly those offering convenience in preparation (57%). Additionally, there was a strong preference for tilapia products from certified farms (94%), indicating a growing interest in quality assurance and production standards. With respect to product presentation, 54% of the consumers preferred vacuum-packed, marinated, and frozen whole tilapia, followed by smoked products (30%) and canned products (16%). Moreover, consumers expressed interest in alternative value-added products such as nuggets (35%) and sausages (32%). These findings reveal significant opportunities for market diversification through the development of value-added products and certification strategies. The preference for traditional purchasing channels further supports

the importance of local distribution systems, as reported by Verbeke et al. (2007). Furthermore, the incorporation of value-added processes has been identified as a key strategy for improving product acceptance and competitiveness in emerging markets (Del Río-Zaragoza et al., 2022).

Table 3. Commercialization trends and value-added opportunities for tilapia consumption (n=303).

Variable	Category	Percentage (%)
Willingness to purchase value-added products	Yes	88
	No	12
Preferred type of value-added product	Easy-to-prepare products	57
	Ready-to-eat products	20
	Lack of knowledge regarding fish preparation	23
Preference for certified production	Yes	94
	No	6
Preferred presentation of value-added products	Vacuum-packed, marinated, frozen whole tilapia	54
	Smoked products	30
	Canned products	16
Interest in alternative products	Sausages	32
	Nuggets	35
	Snacks	13
	Products with souces	20

Source: Own elaboration.

CONCLUSIONS

This study demonstrates that tilapia consumption preferences in Tenosique, Tabasco, are determined by the interaction of sociodemographic factors, consumption habits, and product attributes, with freshness, taste, and place of purchase identified as key drivers of consumer decision-making. Additionally, the high willingness to purchase value-added and certified products highlights strategic opportunities for product differentiation and strengthening the aquaculture value chain, contributing to regional economic development. Overall, these findings provide relevant evidence for designing more efficient commercialization strategies; however, future research should incorporate probabilistic sampling and inferential approaches to enhance generalizability and deepen the understanding of consumer behavior.

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Chapter 17

Partial substitution of pechita (*Prosopis glandulosa*) and alfalfa (*Medicago sativa*) flour with commercial flour in diets for tilapia (*Oreochromis niloticus*) farming

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ABSTRACT

Feed represents between 50% and 80% of operating costs in aquaculture production, with protein ingredients constituting more than two-thirds of diet costs (FAO, 2024). Therefore, the search for alternative, economical, and locally available plant-based ingredients has become a priority for the sustainability of tilapia (*Oreochromis niloticus*) farming. In this study, the effects of partial substitution of commercial fish meal with mesquite (*Prosopis glandulosa*) and alfalfa (*Medicago sativa*) meal in the diets of juvenile tilapia were evaluated. Three treatments were formulated: a diet including mesquite meal, a diet including alfalfa meal, and a control diet (commercial feed without substitution). A total of 135 juveniles with an initial weight of 5 ± 2 g were used, distributed in nine tanks with 15 organisms each, and fed for 41 days at 4% of their total biomass three times daily. Water quality parameters (dissolved oxygen, temperature, and pH) were monitored daily, and biometrics were performed every 15 days to evaluate growth. The results revealed that although plants-maintained survival above 77% in the plants-based diet treatment group, their growth was significantly lower than that in the control treatment group. The control diet reached a final weight of 20.0 ± 0.9 g, with a feed conversion ratio (FCR) of 4.1, while the weights of the alfalfa and mesquite diets reached 12.0 ± 0.9 g (FCR=10) and 8.0 ± 0.9 g (FCR=9.5), respectively. Water quality parameters remained mostly within tolerable ranges for the species, although extreme values of dissolved oxygen (1.5 mg/L) and temperature (33°C) were recorded, which may have

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affected fish metabolism. Mesquite and alfalfa meals can be used as supplements in tilapia feed, but formulation optimization is needed to improve the digestibility and essential amino acid profile.

Keywords: *Oreochromis niloticus*, feeding, plant ingredients, mesquite, alfalfa, aquaculture.

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is the predominant farmed species worldwide, accounting for more than half of global tilapia production since 1992 (FAO, 2024). This species is particularly suitable for aquaculture: it is omnivorous with the ability to filter phytoplankton, tolerates a wide range of environmental conditions, and grows rapidly at optimal temperatures between 31°C and 36°C (FAO, 2024). However, the success of intensive farming critically depends on the availability of low-cost formulated feeds.

In aquaculture, feed can represent between 50% and 80% of operating costs, and protein ingredients constitute more than two-thirds of diet costs (Padhan, 2022). Traditionally, fish meal has been the main protein source in fish feeds because of its excellent amino acid profile and high digestibility (Watanabe, 2002). However, global fish meal production has declined, while its price has increased, making it necessary to identify and produce sustainable alternatives to replace this feed source (FAO, 2024).

In this context, plant-based ingredients have emerged as promising alternatives (FAO, 2024). Alfalfa (*Medicago sativa*) is a legume with high protein (15–22%), vitamin, and mineral contents and is widely available and low in cost. Mesquite (*Prosopis glandulosa*), known as sweet mesquite, is a tree native to the southwestern United States and northern Mexico whose pods have been traditionally used for human and animal feed. Mesquite pods contain sugars, proteins, and fibers, representing an underutilized local resource with potential for use in aquaculture feed (Botanic Gardens Conservation International, 2020; Becker; Grosjean, 1980).

When new ingredients in tilapia diets are being evaluated, it is essential to maintain constant protein and energy levels (isoproteic and isoenergetic diets) so that any observed differences can be attributed to the ingredient being evaluated and not to nutritional imbalance (García Meilán, 2016). In addition to protein levels, the balance of essential amino acids such as lysine, methionine, and threonine is decisive, since when one of them falls below the ideal level, protein synthesis is reduced, even if the total protein content appears adequate (Furuya et al., 2023).

Previous research has shown that the inclusion of plant ingredients in tilapia diets can be successful, when necessary, adjustments are made. Studies with soybean meal, sunflower meal, and pea concentrate have shown that it is possible to partially substitute fish meal without significantly affecting growth, as long as a balanced amino acid profile is maintained (Refaey et al., 2025). Similarly, the use of chaya leaves (*Cnidoscolus chayamansa*) as a partial substitute for formulated feed has been evaluated, and

substitution levels of 25–50% can maintain weight gains similar to those of the control (Poot-López et al., 2012).

The present study aimed to evaluate the effects of partial substitution of commercial fish meal with mesquite (*Prosopis glandulosa*) and alfalfa (*Medicago sativa*) meals on the growth, survival, and feed conversion of juvenile tilapia (*Oreochromis niloticus*) and to monitor water quality parameters during bioassays.

MATERIALS AND METHODS

Study area

This work was carried out in the aquaculture laboratories of the Technological Institute of the Yaqui Valley, located in the municipality of Bácum, Block 611, using its facilities and tools for the bioassay and the production of the evaluated feeds.

Feed preparation

For the preparation of experimental feeds, nutritious, low-cost, and easily available ingredients, namely, mesquite (*Prosopis glandulosa*) and alfalfa (*Medicago sativa*), which provide vitamins, protein, and other nutrients (Becker & Grosjean, 1980), were selected. The fish meal (commercial feed) used as a base and control was obtained from the aquaculture laboratory.

To obtain mesquite and alfalfa meals, the ingredients were placed in industrial blenders for grinding, yielding 1 kg of meal from each raw material. The same grinding process was applied to commercial fish meal to homogenize all the ingredients, following standard plant ingredient processing methods (Hernández, 2019).

Feed formulation

Using alfalfa, mesquite, and fish meal (commercial feed), the feeds to be evaluated were prepared. Each meal was placed in clean buckets, enriching each formulation as shown in Table 1 so that each feed matched the profile of the commercial feed used as a control. Afterward, 1.5 L of hot water was added to bind and mix all the ingredients until a uniform dough was obtained. The dough was introduced into a pelletizer, and each formulation was processed separately. The obtained pellets were left to dry and cut to a size that allowed the juveniles to ingest them easily. The finished feed was kept refrigerated to avoid contamination. Throughout the preparation process, good personal hygiene and clean instruments were maintained.

Table 1. Composition of experimental feeds (per kg of diet).

Ingredients	Mesquite Diet	Alfalfa Diet
Mesquite meal	700 g	-
Alfalfa meal	-	700 g
Corn meal	140 g	140 g
Fish meal	50 g	50 g
Vitamins (Vitamix)	2 g	2 g
Vegetable oil	52 g	52 g
Gelatin	6 g	6 g
Starch	20 g	20 g
Lard	30 g	30 g

Source: Own elaboration.

Experimental organisms

Juvenile black tilapia (*Oreochromis niloticus*), a species characterized by its ease of handling and high tolerance to adverse environmental conditions (FAO, 2024), were used. The organisms came from the fishing cooperative "La Cochi de Marcela," located on the Esperanza to Hornos highway, from which they were transferred to the aquaculture laboratory of the Technological Institute of the Yaqui Valley.

Juvenile fish with body weights between 4 and 8 g were selected, and those with poor physical appearance, bruises, or obvious signs of disease were discarded, following the standard selection criteria for bioassays (Martínez, 2003).

To avoid high mortality rates and stress during transport, the organisms were placed in plastic bags with oxygen at a density of 15 organisms per bag, yielding a total of 135 transported organisms, following recommended protocols for aquatic organism handling (Martínez, 2003).

Once in the laboratory, the bags with the organisms were placed in the experimental tanks to equalize the temperature and avoid thermal stress. The fish were subsequently fed according to the stipulated feed during a 7-day adaptation period.

Bioassay

Distribution of organisms

After the acclimatization period, the organisms were placed in nine experimental tanks with a density of 15 organisms per tank. Through random assignment, the type of feed each tank received was

determined, with three replicates per treatment (control, alfalfa, and mesquite), following a completely randomized design (Poot-López et al., 2012).

The fish were fed the three treatments (control diet, alfalfa meal diet, and mesquite meal diet). The daily ratio was calculated on the basis of the total biomass of the organisms in each tank, which accounted for 4% of the total body weight and was divided into three daily rations administered every four hours, in accordance with the recommendations for juvenile tilapia (El-Sayed, 2004).

To maintain water quality and inhibit contaminants, temperature and dissolved oxygen were monitored daily with the help of an oximeter. The pH was measured periodically. Dissolved oxygen was maintained using a constant aeration system. Water exchanges of 50–60% were performed every three days and 90% every week, supplemented with siphoning to remove feces and feed residues, following recommended practices for static systems (Fagbenro; Jauncey, 1995).

Biometrics of all the organisms were performed every 15 days from the start of the bioassay, and individual weight and size were recorded. These data allowed the evaluation of growth and adjustment of the feed ratio on the basis of increased biomass (Olvera-Novoa, 1994).

Statistical analysis

The growth, survival, and physicochemical parameter data were analyzed using descriptive statistics, and the results are presented as the means and standard deviations and were identified at the 5% level of significance using Tukey's one-way ANOVA.

RESULTS AND DISCULTS

Water quality parameters

Water quality parameters during the bioassay varied across treatments and over time. Dissolved oxygen (DO) fluctuated because of the increase in organic matter (feces and uneaten feed) and water exchange. The highest value was recorded in the 15.73 mg/L alfalfa treatment on day 34, whereas the lowest value was observed in the 1.5 mg/L mesquite treatment on day 22.

These extreme values deserve special attention. The concentration of dissolved oxygen (1.5 mg/L) recorded in the mesquite treatment was below the recommended range for tilapia, which should be maintained above 3 mg/L to avoid severe stress and hypoxia, with optimal concentrations above 4–5 mg/L to maximize feed conversion (Matter et al., 2025). Such low values can cause reduced feed intake and directly affect growth, which could partially explain the poor performance of the fish in this treatment. Studies in recirculating systems have shown that dissolved oxygen is among the most critical parameters for tilapia welfare and growth (Refaey et al., 2025).

The temperature progressively increased from the beginning of the bioassay and stabilized after day 15. The highest value was recorded in the alfalfa treatment on day 41 at 33°C, whereas the lowest value was observed in the male treatment on day 1 at 27.8°C. Tilapia is a tropical species whose optimal

temperature range is between 26°C and 30°C, although it can tolerate temperatures up to 36°C (Nivelle et al., 2019). The recorded temperatures (27.8–33°C) are within the tolerable range, although values close to 33°C increase oxygen consumption by fish (Leonard; Skov, 2022), which, combined with low DO concentrations, could generate metabolic stress. Research with tilapia has shown that temperature directly affects the feeding rate and conversion efficiency (Verdegem et al., 2016; Leonard; Skov, 2022).

The pH in the three treatments remained similar throughout the experiment, ranging between 5.0 and 5.5, indicating acidic conditions. The optimal pH range for tilapia is between 6.5 and 8.5, although they can tolerate values from 5 to 11 (Matter et al., 2025). pH values less than 6.5 can reduce growth and cause damage to the gills and skin (Boyd, 1998). The acidic pH recorded (5.0–5.5) could have negatively affected the metabolism and feed efficiency of the organisms in all the treatments. Studies in recirculating systems have reported that the pH tends to decrease because of nitrification and respiration, which require constant monitoring (Refaey et al., 2025).

Overall, water quality parameters, although mostly within tolerable ranges, presented suboptimal conditions (low DO and acidic pH) that likely contributed to the low growth observed in all the treatments, including the control. Previous studies have shown that when ammonia begins to accumulate or when water quality conditions deteriorate, fish respond by reducing their feeding activity, which directly affects growth and feed conversion (Zhang et al., 2016).

Growth, survival, and feed conversion ratio

The results of the productive parameters are presented in Table 2. Survival was greater than 70% in all the treatments, with 93.33% in the alfalfa treatment, 77.77% in the mesquite treatment and 71.11% in the control. These values are acceptable considering the experimental conditions and management and are similar to those reported in other studies with plant ingredients (González-González, 2023; Lan et al., 2023).

Table 2. Productive parameters of juvenile tilapia.

Treatment	Initial weight (g)	Survival (%)	Final weight (g)	FCR
Mesquite	5 ± 0.28	77.77	8.0 ± 0.76	9.5
Alfalfa	5 ± 0.34	93.33	12.0 ± 0.85	10.0
Control	5 ± 0.31	71.11	20.0 ± 0.91	4.1

The final weight differed notably between the treatments. In the control treatment, the yield reached 20.0 ± 0.91 g, which was significantly greater than that in the alfalfa (12.0 ± 0.85 g) and mesquite (8.0 ± 0.76 g) treatments. This difference suggests that partial substitution of fish meal with plant meals,

in terms of proportions used, did not maintain the same growth level as the commercial diet did. Similar results were reported by Barragán et al. (2017), who, when evaluating different inclusion levels of plant meals in tilapia diets, reported that a diet with higher fish meal content (54.9%) resulted in better growth than a diet with a higher proportion of plant ingredients, although the FCRs obtained in their study (2.53–2.60) were notably better than those of the present work (Mohammadi et al., 2020).

The feed conversion ratio (FCR), which indicates the amount of feed needed to produce a unit of live weight, was notably more efficient in the control treatment (4.1) than in the alfalfa (10.0) and mesquite (9.5) treatments were. These values indicate that fish fed plant-based diets required more than twice the amount of feed to gain the same weight as those on the control diet did. These FCRs are higher than those reported in the literature for tilapia under optimal conditions, which usually range between 1.5 and 2.5 (El-Sayed, 1998), suggesting that the general experimental conditions (water quality, diet formulation) were not adequate.

The results obtained can be explained by multiple factors. First, the nutritional quality of the plant meals used likely differed significantly from that of the fish meal in terms of the amino acid profile and digestibility. Studies with protein hydrolysates in tilapia have shown that the peptide and free amino acid profile of the ingredient influences acceptance and digestibility (Afuang et al., 2003). Alfalfa and mesquite meals, although they provide protein, may be deficient in essential amino acids such as lysine, methionine, and threonine, which are frequently limiting plant ingredients (Yousif et al., 1994).

Second, the presence of antinutritional factors in plant meals may reduce protein digestibility and nutrient availability. Like other legumes, alfalfa may contain saponins, tannins, and protease inhibitors that affect digestion (Francis et al., 2001). Studies in rainbow trout have shown that phytic acid, which is present in many vegetables, can reduce feed intake and growth when it exceeds certain threshold levels (Kaiser et al., 2021). When new ingredients are evaluated in the literature, ensuring that digestible protein, essential amino acids, and energy remain balanced is essential (García Meilán, 2016).

Similar results have been reported in studies with moringa meal included in tilapia diets, where González-González (2023) reported that the inclusion levels of 25–100% moringa meal did not significantly differ between treatments, although survival was 100% for the lowest inclusion levels. In contrast, Rivas-Vega et al. (2010) evaluated the inclusion of moringa leaf meal in tilapia cultured in seawater and reported that up to 20% substitution of sardine meal protein did not affect growth, with a protein digestibility of 89.1%.

Studies with other plant ingredients, such as chaya, have shown promising results. Poot-López et al. (2012) evaluated the partial substitution of formulated feed with raw chaya leaves in tilapia and reported that compared with the control treatment, treatments with 50–75% formulated feed resulted in weight gains similar to those of the control, and the feed conversion ratio of formulated feed decreased between 5.38 and 40.23% depending on the climatic season.

The high survival in the alfalfa treatment (93.33%) suggests that, although growth was limited, plant-based diets were not toxic and allowed the maintenance of organisms. This finding is consistent with reports indicating that moderate inclusion of alternative ingredients can maintain survival even if growth is compromised (Fasakin et al., 2005). The lower survival in the control treatment (71.11%) is atypical and could be related to uncontrolled factors, such as water quality in those specific experimental units.

Notably, the FCR values obtained in this study (4.1–10.0) are high compared with those reported in the literature. Fontainhas-Fernandes et al. (1999) reported FCRs between 1.5 and 2.5 for tilapia fed plant-protein-based diets. Köprücü and Özdemir (2005) reported apparent digestibility coefficients for various plant ingredients in tilapia ranging from 70–90%, suggesting that good efficiency can be achieved with adequately processed plant ingredients.

The processing of plant ingredients is crucial for improving their digestibility. Studies on snook have shown that extrusion can significantly improve the digestibility coefficients of plant meals, which can reach protein digestibility values between 73.4 and 96.9% (Hernández, 2019). Microbial fermentation has also been consolidated as an effective strategy to reduce antinutritional factors and improve nutrient bioavailability in plant ingredients for use in aquaculture (Ahmed et al., 2025).

CONCLUSIONS

Compared with the control diet, partial substitution of commercial fish meal with mesquite (Prosopis glandulosa) and alfalfa (*Medicago sativa*) meals in the diet for juvenile tilapia (Oreochromis niloticus) maintained acceptable survival (above 77%) but resulted in significantly lower growth and higher feed conversion ratios.

In terms of final weight (12.0 g vs 8.0 g) and survival (93.33% vs 77.77%), the alfalfa meal treatment resulted in better performance than the mesquite treatment did, suggesting that alfalfa could be a more promising alternative for future formulations.

Water quality parameters (temperature, dissolved oxygen, and pH) remained mostly within tolerable ranges for the species, although suboptimal conditions (mainly low oxygen and acidic pH) were recorded that may have affected the metabolism and feed efficiency of the organisms.

Mesquite and alfalfa meals represent local resources with potential for use in aquaculture, but further research is needed to optimize their use and maximize their productive efficiency, considering aspects such as processing, amino acid supplementation, and optimal inclusion levels.

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Chapter 18

Hydrolysate of the alga *Spirulina* influences the proliferation, growth, and development of *Spathiphyllum uxpanapense in vitro*

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ABSTRAC

Spathiphyllum uxpanapense is an ornamental species of commercial interest, but its propagation is limited by slow growth, low shoot production, and a lack of suitable seed-based propagation material. In this study, the effects of Spirulina hydrolysate on the in vitro proliferation and growth of axillary shoots of *S. uxpanapense cv.* were evaluated. Spirulina hydrolysate was obtained through acid and alkaline hydrolysis, and the growth variables included total plant length, number of roots, root thickness, number of shoots, number of leaves, survival rate, and photosynthetic pigments. Although acid and alkaline hydrolysis did not differ significantly, alkaline hydrolysis at a concentration of 1 M was found to be the most favorable mineral profile for subsequent biological evaluation. In the establishment trials, low hydrolysate concentrations, especially 5% and 10%, improved root development and thickness, plant vigor, and overall plant quality; conversely, high concentrations reduced survival and growth. The contents of chlorophyll a, chlorophyll b, and carotenoids did not differ significantly among the treatments but were higher in most cases. Overall, the spirulina hydrolysate acted more clearly as an additive that promoted growth and development and promoted plant rooting and as a direct stimulator of shoot proliferation. These results support the use of low concentrations of spirulina-derived hydrolysate as a complementary component in the in vitro culture of *S. uxpanapense*. This can be used as a biostimulant in agricultural practices after field trials.

Keywords: *Spathiphyllum uxpanapense*; *Spirulina sp.*; axillary buds; biostimulant; in vitro culture.

INTRODUCTION

The genus *Spathiphyllum* comprises 47 species, 94% of which are found in the Neotropics, from Mexico to northern Bolivia and Brazil (Cardona, 2016). It is the second best-selling houseplant in the world and of commercial interest because of its attractive foliage and white spathe-shaped inflorescences (Cardona, 2016). The use of synthetic growth promoters and mineral fertilizers has been practiced for many decades. Nevertheless, their continued use has led to negative effects, including soil chemical alteration and reduced plant growth (Srijaya et al., 2010). Seaweed extracts have gained prominence and

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are used as nutritional supplements, biostimulants, or biofertilizers in agriculture and horticulture (Hernández et al., 2013). Consequently, new natural materials are being developed and can be used as fertilizers or plant growth biostimulants. Plant biostimulants, regardless of their nutrient content, contain substance(s), compound(s), and/or microorganism(s) whose functional use, when applied to plants or the rhizosphere, improves crop development, vigor, yield, and/or quality by stimulating natural processes that promote growth and responses to biotic stress (Palazon, 2013). The presence of at least six types of cytokinins and their precursors has been scientifically demonstrated in cyanobacteria, microalgae, and three types of marine macroalgae, such as cis- and trans-zeatin riboside, trans-zeatin, dihydro-Zetina N6 (isopentenyl) adenine and its 9-β-ribosides, as well as in microalgae and cyanobacteria (Blunden; Wildgoose, 1977; Meeting et al., 1988; Mooney; Van Staden, 1987; Zhang et al., 1991; Crouch; Van Staden, 1993).

Various studies have demonstrated the potential of these bioactive substances as alternatives to synthetic hormones used in plant micropropagation. Among them, the use of Pectimorf®, obtained from a mixture of oligosaccharides of peptidic origin (Cabrera, 1999), has been employed to promote various processes in plants; hormonal effects have been reported both in vitro and *ex vitro*, in somatic embryogenesis in coffee, sugarcane, and potato, and as a rooting agent for rootstocks in *Ficus sp.* and guava cuttings, among others (Ramírez et al., 2003). The addition of seaweed extract (SWE) with and without growth hormones significantly increased total biomass production, total plant length, number of shoots, root length, and number of roots in *Picrorhiza kurroa* plants; furthermore, this is the first report demonstrating the potential of SWE as an inducer to increase the growth and yield of *P. kurroa* (Sharma et al., 2014). Currently, various extraction processes are employed to obtain stimulants from seaweed.

Methods for producing seaweed extracts can generally be divided into three groups: biological methods (enzymatic degradation), physical extraction methods (high-pressure and cold processes and supercritical fluid extraction), and chemical hydrolysis methods (organic and inorganic solvents) (Kim; Chojnacka, 2015). Enzymatic extraction has emerged as a promising alternative for isolating bioactive compounds from algae. This method uses enzymes; however, its large-scale application is limited by the high cost of these enzymes (Barzana et al., 2002). The disadvantages include the high volumes of solvent required and the prolonged extraction time (Ramnluckan et al., 2014). Acid hydrolysis: The purified material is mixed with deionized water at a specific acid concentration. Sulfuric acid is generally used, as it produces a negative surface charge on the particles and stabilizes the suspension (Habibi et al., 2010).

Alkaline hydrolysis: In this process, the algal biomass is first dried and then mixed with alkaline solutions. The most commonly used compounds are sodium hydroxide or potassium hydroxide, either alone or in combination (Harun; Danquah, 2011). Marine algae products can be classified as extracts or suspensions depending on the production process. The term "extract" is used to refer to products obtained by using chemical agents to hydrolyze the algae, while the term "suspension" refers to those obtained without chemical agents (Chojnacka et al., 2012). Toxicity tests are used to study the

relationships between dose or concentration and effect, and between dose or concentration and the dose–response effect: a biological change that can be evaluated on a scale of intensity or severity; response: the proportion of the exposed population that exhibits a defined effect (Cárdenas, 2009).

Spirulina platensis, also known as *Arthrospira*, is a filamentous, microscopic cyanobacterium (blue–green algae) with a long history of use as a food source and as a biofertilizer. An evaluation of *Spirulina platensis* as a biostimulant for organic farming systems revealed that it contains 6.7% N, 2.47% P, and 2.14% K, up to 60% protein, and adequate amounts of the trace elements necessary for plant nutrition. This biostimulant was inoculated under *ex vitro* conditions, resulting in higher yields (Aly; Esawy, 2008; Spínola et al., 2024).

Spathiphyllum sp. is an important tropical ornamental plant. Other species are distinguished within their genus; one of them is *S. uxpanapense*. It is of commercial interest, but its production requires large quantities of plants. In vitro culture enables the production of these compounds in relatively short periods, underscoring the need to standardize a micropropagation protocol for this species. On the other hand, *Spirulina platensis* is a potential biostimulant microalga. The use of extracts in crops has positive effects; therefore, it is essential to verify whether these extracts can increase the proliferation of *S. uxpanapense* in vitro. Consequently, new biostimulant alternatives based on the development of natural products that mitigate the harmful effects of chemical substances are needed.

MATERIALS AND METHODS

Collection of plant material and induction of in vitro culture

Explants (axillary buds) from *Spathiphyllum uxpanapense*, cultivar Callisto, were used to induce shoots. The explants were subsequently sown in MS media (Murashige; Skoog, 1962) supplemented with 30 $g \cdot L^{-1}$ sucrose and 7.5 $g \cdot L^{-1}$ agar, and the pH was adjusted to 5.8 with concentrations of 2 $mg \cdot L^{-1}$ benzyl aminopurine (BA) and 2 $g \cdot L^{-1}$ naphthaleneacetic acid (NAA). The cultures were incubated under a 16-hour light/8-hour dark cycle; shoot numbers were evaluated after 30 days, and treatment means were compared using analysis of variance and Tukey's HSD test.

Preparation of a hydrolyzed extract of Spirulina sp.

The hydrolyzed extract was obtained by varying the acid and alkaline hydrolysis conditions from *Spirulina sp.* biomass. The samples underwent heat treatment, centrifugation, and pH neutralization, as described by Setyaningsih (2012). Subsequently, with minor modifications, the optimal hydrolysis was adjusted. The yields from acid and alkaline hydrolysis were analyzed to determine the most effective hydrolysis process, and the CIATEJ Analytical and Metrological Services Unit determined the essential mineral contents.

Establishment of Cultures with Hydrolyzed (Extracts of Spirulina sp.)

The effect of the hydrolyzed extract of *Spirulina sp.* (5%, 10%, 20%, 30%, 40%, and up to 100%) was evaluated both alone and in combination with MS media with and without BA. Treatments without the hydrolyzed extract were used as controls (Table 1). Each treatment consisted of 5 replicates; the media were adjusted to a pH of 5.8; 25 ml was poured into each test tube and sterilized for 15 min at 121 °C. The cultures were maintained at 25 ± 2 °C under a 16 h light/8 h dark photoperiod. The effects of hydrolyzed spirulina extract (HE) on the plants were evaluated every 30 days. The variables evaluated were the number of shoots per explant, survival rate, height, root thickness, number of shoots, number of leaves, chlorophyll content, and concentration of the hydrolyzed extract.

Table 1. Composition of media containing different percentages of hydrolyzed extract (HE): Medium 1 (M1), (water + HE); Medium 2 (M2), (MS + HE); and Medium 3 (M3), (MS + BA + HE).

Treatments	Medium 1	Medium 2	Medium 3
Control	WATER + EH (0)	MS + EH (0)	MS + BA + EH (0)
T1	WATER + EH (5)	MS + EH (5)	MS + BA + EH (5)
T2	WATER + EH (10)	MS + EH (10)	MS+BA + EH (10)
T3	WATER + EH (20)	MS + EH (20)	MS + BA + EH (20)
T4	WATER + EH (30)	MS + EH (30)	MS + BA + EH (30)
T5	WATER + EH (40)	MS + EH (40)	MS + BA + EH (40

Measurement of chlorophyll a, b, and carotene

A modified method by Wellburn (1994) was used to measure chlorophyll a, b, and carotene levels. Three replicates of each treatment were used. A leaf sample of approximately 20 mg was taken from the explant, cut into small pieces, and placed in test tubes. Two milliliters of absolute methanol were added, and the samples were incubated in the dark at 4 °C for 24 hours until all the tissue became white. The optical density at 666, 653, and 470 nm was subsequently measured using a spectrophotometer. The concentrations of chlorophyll a and b and the carotenoid content were calculated for each sample using the formula of Wellburn (1994).

Statistical analysis

The evaluation results were analyzed using ANOVA, and the treatment means were compared using Tukey's HSD test at the 5% significance level in the STATGRAPHICS program.

RESULTS

Analysis and Selection of the Best Hydrolyzed Extract

An analysis of the yields from acid and alkaline hydrolysis revealed that compared with the other hydrolysis methods, 1 M alkaline hydrolysis yielded the greatest amount of minerals of interest (Table 2). Compared with the other alkaline and acid hydrolysis methods, the 1 M and 0.4 M alkaline hydrolysis

methods result in a large amount of phosphorus (P); for calcium (Ca), potassium (K), and iron (Fe), 1 M alkaline hydrolysis is superior to the other hydrolysis methods, except for the contents of two minerals: magnesium (Mg) and sulfur (S). The 0.4 M acid hydrolysis resulted in a higher magnesium content, and the 1 M acid hydrolysis resulted in a high amount of sulfur.

Table 2. Performance analysis of hydrolysis methods for the determination of major and minor elements.

Minerals mg/kg	Acid Hydrolysis 0.4 M	Acid Hydrolysis 0.6 M	Acid Hydrolysis 1 M	Alkaline Hydrolysis 0.4 M	Alkaline Hydrolysis 0.6 M	Alkaline Hydrolysis 1 M
Phosphorus	971.1	336	874,3	1851	576.9	1798
Calcium	53.39	37.71	42.47	38.59	48.92	59.68
Magnesium	133.2	108.9	107	40.96	34.69	45.02
Sulfur	13410	14370	23160	244.5	258	309.2
Potassium	1721	979.8	1610	12570	9989	38520
Iron	7.4	9.82	8.38	10.05	7.3	10.96

Hydrolyzed Extract Toxicity Test

A toxicity bioassay was performed 15 days after sowing, and chlorosis was observed only in the leaves of *S. uxpanapense* at extract concentrations greater than 40%. After 30 days, the previously chlorotic plants exhibited necrosis and complete plant death (Figure 1B). A suitable concentration without any toxicity was observed at concentrations of nearly 10% or less than 20% (Figure 1C).

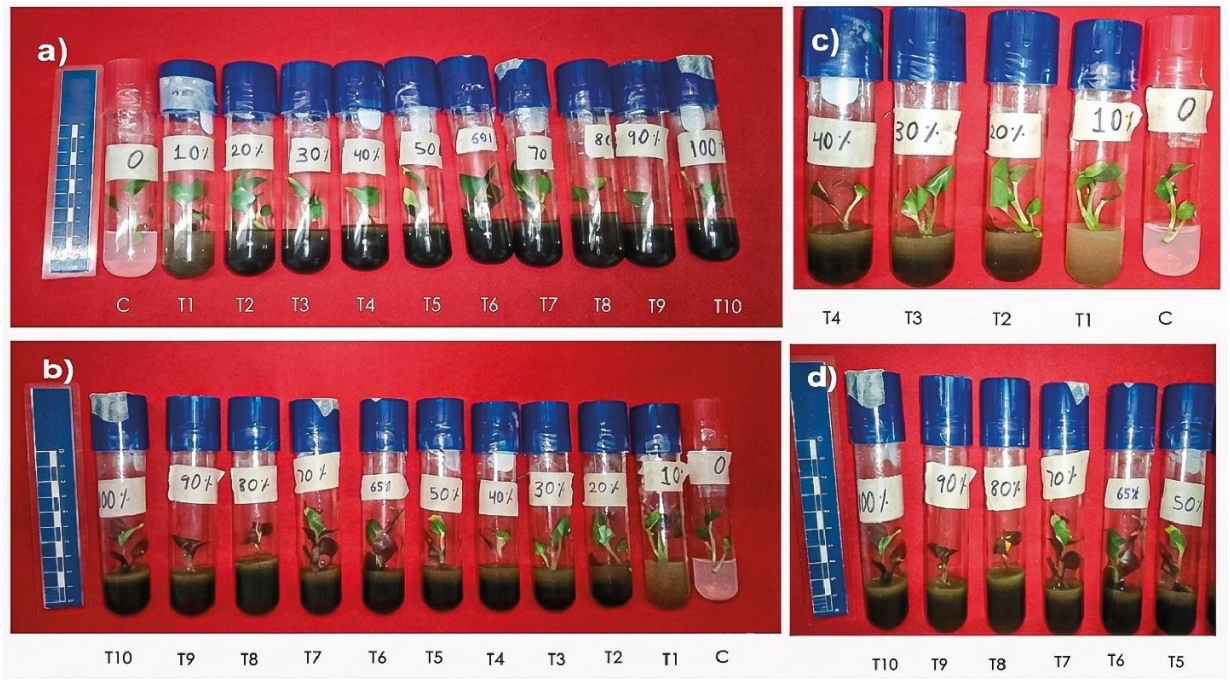


Figure 1. Effects of different concentrations of hydrolyzed extract on the axillary buds of *S. uxpanapense*. (a) Day 1 after sowing: control (C 0%) and treatments with 10, 20, 40, 50, 60, 70, 80, 90, and 100% hydrolyzed extract (T1–T10); (b) evaluation 15 days after sowing under the same concentrations; (c) evaluation at 30 days for treatments of 0–40% (C–T4); (d) evaluation at 30 days for treatments of 50–100% (T5–T10).

Effect of the hydrolyzed extract on the establishment of in vitro *cultures*

The effects of the extract on the in vitro culture of *S. uxpanapense* revealed that the type of medium and the treatment percentage significantly affected ($p \leq 0.05$) plant length. Moreover, their interaction was not significant ($p \geq 0.05$), indicating that both factors independently influence plant length. With respect to root number and thickness, the type of medium did not significantly differ ($p \geq 0.05$). In contrast, the treatment percentage and its interaction did ($p \leq 0.05$), indicating that these variables depend primarily on the treatment level and its interaction with the medium (Fig 2). In contrast, the number of shoots, the number of leaves, and the survival rate were significantly affected by the type of medium, the treatment percentage, and their interaction ($p \leq 0.05$), indicating that the plant's response depends on the combination of both factors (Figure 2).

In continuation, the analysis of means using Tukey's test ($\alpha = 0.05$) showed that the plant length was highest in media M3 (3.81 cm) and M1 (3.69 cm), with no statistically significant difference between them, and both were greater than M2 (3.21 cm). No significant differences were observed among the three media evaluated for root number and root thickness. For the number of shoots, medium M3 produced a significant number of shoots (3.0), while M1 and M2 produced no shoots. Regarding the number of leaves, M3 also showed the highest value (6.17), statistically higher than that of the other treatments. Finally, in terms of survival rate, medium M3 had the highest value (71.36%), followed by M1 (64.70%) and M2 (58.41%), with significant differences among them (Figure 2).

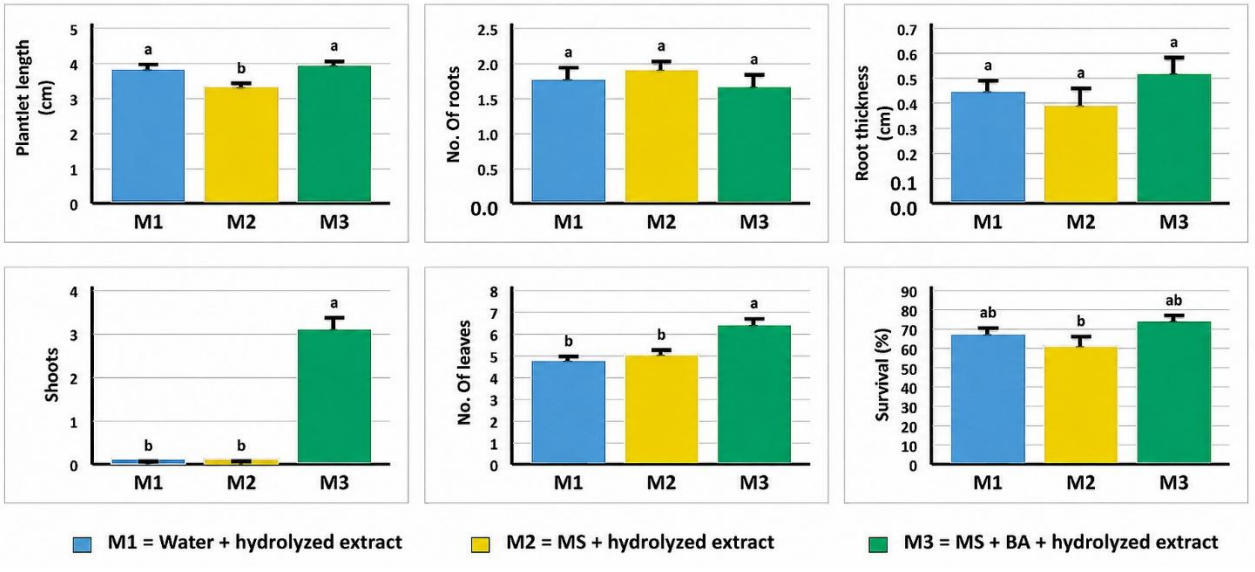


Figure 2. Multiple comparison of means for medium type and extract concentrations regarding total plant length, number of roots, root thickness, shoots, number of leaves, and (%) survival of axillary buds of *S. uxpanapense.* Medium 1 (M1) Water + Hydrolyzed extract, Medium 2 (M2) MS + Hydrolyzed extract, Medium 3 (M3) MS + BA + Hydrolyzed extract. Using the Tukey HSD test. Groups with the same letter do not differ significantly from one another. p=0.05.

The effect of different extracts of hydrolyzed extract on in vitro cultures

On the other hand, the extract concentration had varying effects depending on the variable evaluated, as seen in Table 3. For plant length, the 20% concentration (3.97 cm) yielded the highest value, though it was not statistically different from most treatments, whereas the 40% concentration (3.16 cm) yielded the lowest value. For root numbers, the 0%, 5%, and 10% concentrations showed the highest values, with no significant differences, whereas a significant decrease was observed at 20%.

Table 3. The effect of different hydrolyses of Spirulina extract (from 5 to 40%, 0 is without extract) on total plant length, number of roots, root thickness, shoots, number of leaves, and (%) survival of axillary buds of *S. uxpanapense.* Using the Tukey HSD test. Groups with the same letter do not differ significantly from one another. p=0.05.

% of Extract	Length of Plantlets	No. of Roots	Thickness of Roots	Shoots	No of Leaves	Survival %
0	3.44 ab	3.87 a	0.69 b	4.8 a	6.9 a	92.21 a
5	3.66 ab	3.4 a	0.7 ab	1 b	5.83 ab	77.96 a
10	3.66 ab	2.67 a	0.8 a	0.2 b	5.2 bc	80.94 a
20	3.97 a	0.13 b	0.0 c	0 b	5.13 bc	76.47 a
30	3.55 ab	0.2 b	0.03 c	0 b	4.17 cd	43.93 b
40	3.16 b	0.0 b	0.0 c	0 b	3.8 d	17.44 c

Regarding root thickness, 10% concentration had the highest value (0.8), whereas higher concentrations significantly reduced it. As for shoots, the greatest formation was observed at the 0% concentration; it also had the highest value (6.9), with a decreasing trend as the extract concentration increased. Finally, the survival rate was highest at the 0%, 5%, and 10% concentrations, while the 30% and 40% concentrations significantly reduced survival, with 40% showing the lowest rate (17.44%) (Table 3).

The effect of the combination of Medium with hydrolyzed extracts

The results of the effect of different concentrations of hydrolyzed extract on the growth of axillary buds of *S. uxpanapense* in combination with three different culture media show very interesting results (Figure 3). The treatment of extract without nutrients (M1: Water + hydrolyzed extract), like MS salts, showed better growth compared to nutrients (M2: MS medium + hydrolyzed extract). The medium containing a growth regulator offers the best of the three (M3: MS medium + BA + hydrolyzed extract) (Figure 3).



Figure 3. Effect of different concentrations of hydrolyzed extract on the growth of axillary buds of *S. uxpanapense* in three culture media. (a) Medium 1 (M1): Water + hydrolyzed extract, (b) Medium 2 (M2): MS medium + hydrolyzed extract, (C) Medium 3 (M3): MS medium + BA + hydrolyzed extract. Six treatments were evaluated in each medium: control without extract (c) and concentrations of 5, 10, 20, 30, and 40% hydrolyzed extract (T1–T5), respectively.

Effect of Hydrolysate of Spirulina on Chlorophyll a, b, and Carotene

The results of pigmentation on the influence of different concentrations of the hydrolysate of spirulina are presented in Figure 4. The Chlorophyll concentration in the three media was not statistically significant (Figure 4); however, in medium 1 (water + hydrolyzed extract), the 20% treatment showed a higher level of chlorophyll content than the other media and their treatments. For chlorophyll b, higher amounts were found in media 1 and 2 with a 40% treatment, and in medium 3 with a 10% treatment. Regarding carotene content, higher levels were observed in medium 1 with treatment percentages of 20% and 40%; in medium 2, carotene content did not differ significantly; and in medium 3, with treatment percentages of 30% and 40%, higher carotene levels were observed.

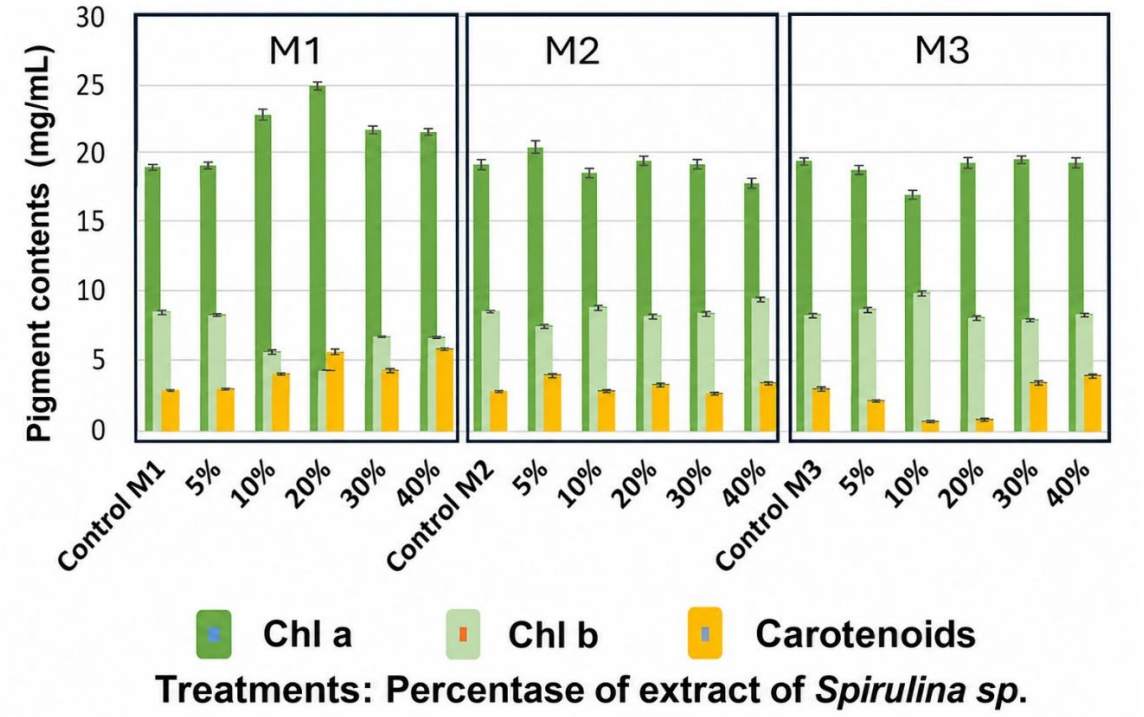


Figure 4. Chlorophyll (Chl) a, b, and carotenoid content of *S. uxpanapense* cultured in different media (M): M1, water control (water + EH), M2, MS control (MS + EH), M3, MS + BA (MS + BA + EH). Where hydrolyzed extract is represented as EH, Murashige Skoog as MS, and Benzylaminopurine as BA.

DISCUSSION

Estimation of hydrolyzed extract yield

The results of the statistical analysis indicated that the type of hydrolysis and concentration did not have a significant effect; however, mineral content differed, with higher concentrations of Ca, K, Fe, and Mg in the alkaline hydrolysis. These results are consistent with those reported by Kadam et al. (2013), who noted that alkaline conditions and high temperatures promote cell wall disruption and the release of bioactive compounds compared to other extraction methods.

In this study, alkaline hydrolysis at 80 °C and pH 12 (1 M) yielded an extract with a higher mineral content, supporting its use as a supplement in in vitro culture media. However, other authors have noted that extreme pH conditions can degrade certain sensitive compounds (Michalak and Chojnacka, 2015), suggesting the need to optimize extraction conditions.

Toxicity levels of the hydrolyzed extract

The toxicity assessment showed that concentrations of the hydrolyzed *Spirulina sp.* extract exceeding 40% were harmful, causing chlorosis in the explants. This behavior is consistent with the findings of Khan et al. (2009), who reported that algal-derived biostimulants exhibit dose-dependent effects, with high concentrations capable of causing phytotoxicity. Likewise, Calvo et al. (2014) note that excessive accumulation of bioactive compounds can alter the physiological balance of plants, which explains the negative effects observed at high concentrations. Therefore, the range of 10% to 40% was considered appropriate for evaluation.

Effect of the hydrolyzed extract of Spirulina sp. on the in vitro cultures of S. uxpanapense

The results showed that the hydrolyzed Spirulina extract had positive effects on in vitro cultures of *S. uxpanapense*, as demonstrated by increased *S. uxpanapense* growth and higher values for plant height, leaf number, and root development, especially at low concentrations of hormensis. These results are consistent with those reported by Zhang and Ervin (2008), who observed that algal extracts promote plant growth by stimulating physiological and metabolic processes.

The very promising effect on the root system is consistent with Craigie's (2011) findings, which noted that algal extracts contain auxins and cytokinins that promote root formation and elongation. However, the extract in this study did not show a significant effect on shoot formation, unlike the findings of Kavipriya et al. (2011), who observed increased shoot formation in other species. This difference may be due to the species evaluated, the concentration used, or interaction with the growth medium. The superior performance at low concentrations and negative effects at high concentrations reinforce Du Jardin's (2015) findings that biostimulants act within specific concentration ranges, where inappropriate doses can inhibit growth. Taken together, these results confirm the potential of the hydrolyzed extract of

Spirulina sp. as a biostimulant in vitro culture, constituting a viable alternative to synthetic growth regulators, as reported in the literature.

CONCLUSIONS

Among the hydrolysis methods of acid and alkaline processes, the alkaline hydrolysate extracts at 0.6 and 1 M were found to have higher mineral content than those from acid hydrolysis. The hydrolysate of *Spirulina sp.* was tested in an *in vitro* culture of *S. uxpanapense*, with 5% and 10% hydrolyzed microalgae extracts, alone or combined with MS or MS plus BA, which influenced root proliferation, producing greater root mass and improving plant development by stimulating vigor, bud growth, and root thickness. Importantly, the hydrolysate of *Spirulina sp.* without nutrients has been effective for establishing and in vitro culturing of *S. uxpanapense.*

Modifying this hydrolysis process can improve the yield of the Spirulina extraction process. Further investigation is needed to evaluate the hydrolyzed extract across different plant species, both in vitro and in the field, to determine its potential as a bioestimulant.

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Chapter 19

Growth of the wild juvenile common snook *Centropomus undecimalis* in captivity

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ABSTRACT

Aquaculture can be a high-impact alternative for this species since wild populations are overexploited because of fishing. This study contributes to the incorporation of *Centropomus undecimalis* (common snook) into aquaculture, providing information on the growth of wild juveniles and the formation of a stock of breeders. The study was conducted in the Tropical Aquaculture Laboratory at the Biological Sciences Academic Division in Universidad Juárez Autónoma de Tabasco. Wild fish were captured at different sites in Mecoacán Lagoon and maintained in marine water for one week; after this, they were slowly transferred to fresh water. Samplings were conducted monthly to determine growth in weight and length. At the end of this experiment, we obtained a batch of 126 young adults to be used as broodstock measuring, on average, 26.92 cm in length and 136.81 g in weight.

Keywords: common snook, grow-out, culture.

INTRODDUCCIÓN

The overexploitation of fishery resources has led to the decline of important fish populations that hold high ecological, cultural, and economic value for a large number of residents in southeastern Mexico. An example of this is the snooks of the genus *Centropomus*, which inhabit the basins of numerous rivers flowing into the Gulf of Mexico. This genus, represented mainly by *C. undecimalis*, constitutes a group of predatory fish (primarily ichthyophagous) with distinctive characteristics; they are protandric hermaphrodites that change sex, although the mechanism underlying this change is unknown. They are diadromous fish, as they are found in both marine and freshwater environments (Rivas, 1986). They spawn in the sea and spend extended periods in freshwater, brackish, or marine waters, depending on their feeding or reproductive needs (Greenwood, 1976). The described breeding season for *C. undecimalis* runs from May to October (Chávez, 1963; Rojas, 1975). As part of one of the highest links in the food

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chain, it acts as a regulator for a large number of fish. Its role in maintaining food webs is fundamental for both inland and marine waters.

From a social perspective, common snook are a resource of great cultural and commercial value; they have been part of the local population's diet since pre-Hispanic times and are currently the species of greatest commercial value in the national market, supporting a large number of families that depend on coastal fishing (Tucker; Campbell, 1985). This high demand, driven by the exquisite flavor, texture, and color of its flesh, has led to increased catch volumes and made the pursuit of larger specimens a priority, thereby reducing resource availability and necessitating the establishment of a fisheries management plan (DOF, 2014).

Considering its reproductive biology, this results in the capture of large females of incalculable population value, as they represent the guarantee of fishery stock renewal. Owing to fishing pressure, its wild populations have come to be considered at potential risk. Perera-García et al. (2009) evaluated indicators of the fishery for this species and concluded that the overfishing of adult organisms could alter the minimum size at sexual maturity. To address this situation, aquaculture can be used as a strategy to develop farming techniques and, where appropriate, produce juveniles that can be used to restore overexploited populations. In this context, common snook have been widely considered for inclusion in aquaculture since they can easily adapt to various types of culture (Álvarez-Lajonchere; Tsuzuki, 2008) as well as to artificial feed and variations in physicochemical parameters (Chapman et al., 1982).

Studies on the growth of juvenile fish in captivity are highly important, as they can generate essential information for evaluating the potential for captive management of a species, as well as the quality of a fishery's stock (Aliaume et al., 2000). These studies also allow for the establishment of culture conditions for a batch of organisms that can be used as broodstock, making the evaluation of an appropriate diet very important (Watanabe et al., 2003). It is also feasible to plan the timing, quantity, and type of feed to obtain high-quality spawn. In this study, the growth in captivity of a group of wild juvenile common snook was determined with the aim of assessing its suitability for establishing a broodstock of the species.

MATERIALS AND METHODS

Obtaining Juveniles

C. undecimalis juveniles were captured in Mecoacán Lagoon, Paraíso, Tabasco, using a trawl net made of 10 m long and 2 m high mosquito netting. Collection was carried out at shallow sites near mangrove areas, which are devoid of vegetation and mangrove roots. A total of 125 juveniles were collected on the basis of their size; they were considered to have been from the same reproductive season and were only a few months old (age 0). They were initially transferred to the Marine Aquaculture Station of the Universidad Juárez Autónoma de Tabasco (UJAT), located in Jalapita, municipality of Centla. The fish were kept for one week in 1,500-liter tanks with filtered seawater and constant aeration for a 24-h

period and subsequently transferred to the Tropical Aquaculture Laboratory of the Academic Division of Biological Sciences at UJAT, where they were acclimated to freshwater through gradual changes in salinity over the course of a week. The final arrangement of the fish in the laboratory was organized by size to prevent aggression by larger fish toward smaller ones, with the fish assigned to four 1,500-liter tanks as follows: 40 fish measuring 19.73 ± 3.14 cm (large); 25 fish measuring 16.09 ± 0.96 cm (medium); 35 fish measuring 12.08 ± 1.48 cm (small); and 25 fish measuring 8.66 ± 0.66 cm (very small). The initial biomass in each tank was 1.34 kg/m^3 (large), 0.47 kg/m^3 (medium), 0.30 kg/m^3 (small), and 0.09 kg/m^3 (very small).

Feeding

The common snook were subjected to a feeding regimen interspersed with fasting periods at each change in feed type: During the first 14 days, they were fed live feed consisting of juvenile Castarrica cichlids and tilapia (*Mayaheros urophthalmus* and *Oreochromis niloticus*) ad libitum; on days 15 and 16, the fish were fasted, and on days 17 and 18, they were fed ad libitum with whole fresh fish; from days 19 to 20, they were fasted again; and from days 21 to 22, they were fed ad libitum with pieces of fresh fish. From the 23rd to the 24th, they were fasted, and finally, from the 25th onward, they were fed a semimoist diet provided three times a day until apparent satiety. The diet was formulated using fish meal, ground fish fillet, shrimp meal, fish oil, soy lecithin, a vitamin and mineral premix, vitamin C, unflavored gelatin, soy milk, and sorghum flour. These ingredients have been used in similar studies with striped bass (Cerqueira and Tsuzuki, 2009). The fish were maintained in a recirculating system for 12 months, with monthly sampling of weight and length to determine growth, followed by prior anesthesia with tricaine methanesulfonate (MS-222).

Water Quality

The temperature and dissolved oxygen concentration were recorded daily using a YSI® Model 55 oximeter (Yellow Springs, OH, USA), and the pH was measured using an UltraBasic® UB-10 potentiometer (Denver Instruments, Denver, CO, USA). All the tanks were connected to a recirculation system equipped with a mechanical and biological filter. Water changes were performed monthly, and approximately 10% of the water lost to evaporation was replenished daily. The water quality throughout the study for the measured parameters was 6.83 (± 0.98) mg/L of dissolved oxygen, 28.09 (± 2.38) °C, and 7.04 (± 0.62) pH units.

Statistical analysis

One-way analysis of variance (ANOVA) was performed to determine significant differences in growth between the sampling months. In addition, a simple linear regression analysis was conducted. All

the statistical analyses were performed using STATGRAPHICS CENTURION® software, version 15.0 (Statgraphics Technologies, Inc., The Plains, VA, USA). Statistical significance was set at $p < 0.05$. Graphs were generated using SIGMAPLOT® software version 11.0 (Systat Software, Richmond, CA, USA).

RESULTS AND DISCUSSION

Wild juveniles of *C. undecimalis* adapted rapidly to captive conditions. Most of these organisms did not present significant problems in accepting the different foods provided, consuming live food, whole fish, fish pieces, or, finally, the semimoist food provided after short periods of forced fasting.

Statistical analysis revealed statistically significant differences between sampling months for both weight and length (ANOVA; $P<0.01$) in the four grow-out tanks. In the large fish tank, the average final weight and length were 258 ± 65.64 g and 34.08 ± 3.14 cm, respectively; in the medium fish tank, 139.05 ± 29.93 g and 28.38 ± 1.79 cm, respectively; in the small fish tank, 112.43 ± 34.21 g and 26.13 ± 1.48 cm, respectively; and in the very small fish tank, 68.11 ± 21.79 g and 22.18 ± 2.33 cm, respectively (Figure 1). The weight gain was 207.91 g at the end of the sampling year (0.57 g/day) for large fish; the gain for medium-sized fish was 110.67 g at the end of the year (0.30 g/day); for small fish, the value was 99.58 g per year (0.27 g/day); and for very small fish, the gain was 62.95 g per year (0.17 g/day).

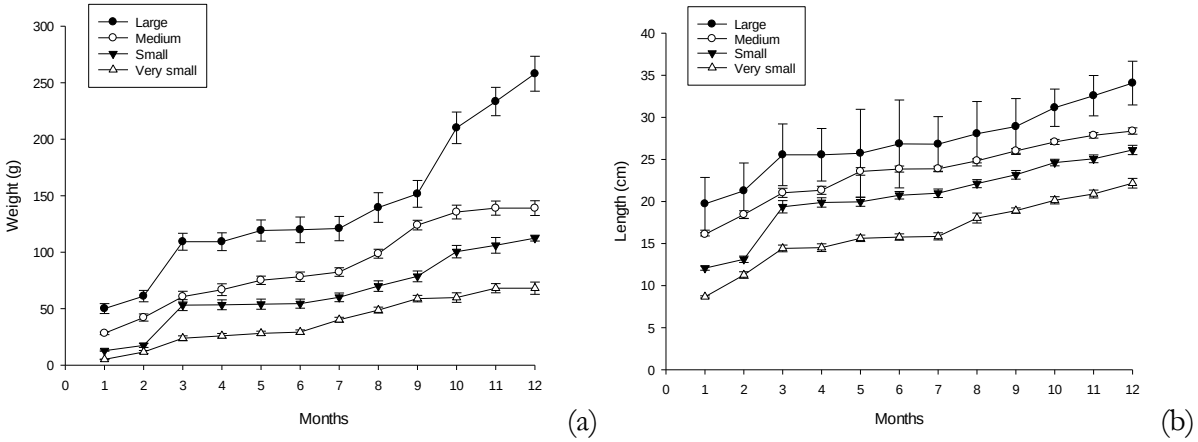


Figure 1. Average values (± SE) for weight gain (a) and total length (b) for the four different sizes of captive juvenile common snook.

When the data were evaluated as a whole, the results also revealed statistically significant differences between the sampling months for both weight and length (ANOVA; $P<0.01$). The average initial weight of all the organisms evaluated was 26.30 ± 24.05 g. The final weight was 145.37 ± 79.43 g (Figure 2), representing a gain of 119.07 g over the 12-month sampling period (0.33 g/day). The initial

average total length was 14.65 ± 4.66 cm, and the final average was 27.82 ± 4.72 cm (Figure 2).

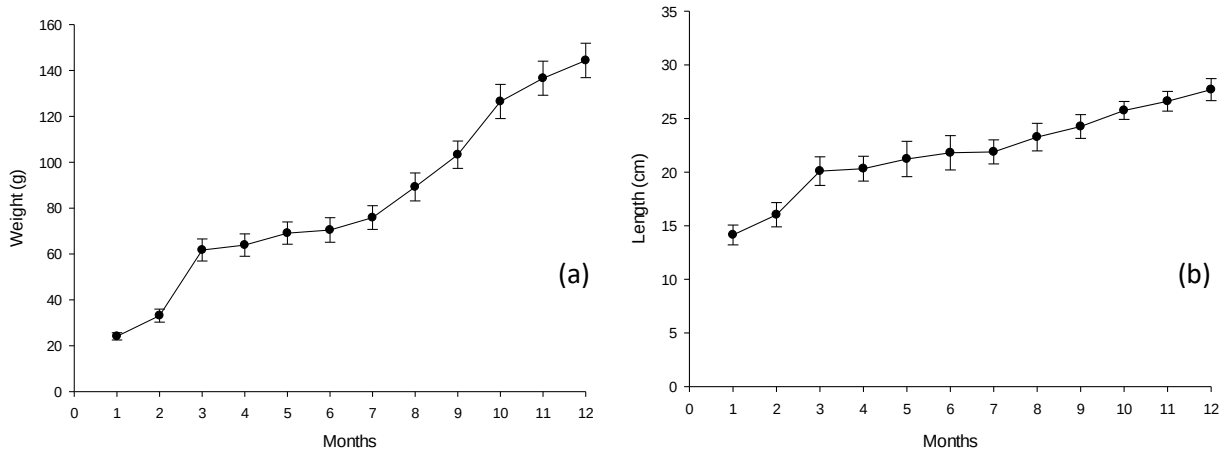


Figure 2. Average values (± SE) for weight gain (a) and total length (b) for all the organisms included in the study.

The feed conversion ratios were 1.24 for large fish, 1.11 for medium-sized fish, 1.29 for small fish, and 6.1 for very small fish. Survival varied considerably among the batches, at 45%, 84%, 57%, and 64%, respectively.

The study of captive growth of a batch of wild juveniles of *C. undecimalis* to establish a broodstock suggests that this species adapts easily to captive conditions. Although mortality was recorded throughout the experimental phase, the overall survival rate exceeded 60%. Acceptance of artificial feed was achieved by applying a protocol that begins with live fish, moves to fish pieces, and culminates with wet balanced feed. Compared with that in other studies, the growth obtained in this study is slow, which may be due to the stocking densities in this study, resulting from the limited space available in the tanks used. Sánchez-Zamora et al. (2003) reported in a study with the same species that the daily growth rate of the fish was 2.7 g, which is considerably higher than that obtained in our study, with growth rates ranging from 0.17 to 0.57 g/day. It is important to note the significant differences between the two studies, as they used seawater, whereas this study used freshwater.

In the aforementioned study, the fish were kept in 20 m^3 tanks, whereas in our study, the fish were housed in plastic tanks with a capacity of 1.5 m^3 at a density of between 25 and 40 fish/m^2, with space being a limiting factor for good growth performance. With respect to 20-g juveniles, Tucker (1987) reported that this species of common snook can reach 450 g in just 8 months (1.8 g/day) and 2 kg in two years. Additionally, Reyes et al. (2006) reported that 280.1-g samples in earthen ponds can reach a weight of up to 761.9 g (5.0 g/day) in just 150 days of culture. These same authors compared the previous results with the growth of juveniles in fiberglass tanks in a marine environment, with an average initial weight of 371.5 ± 82.4 g, and over 162 days, they reached a final average weight of 716.7 ± 56.1 g (4.42 g/day). Polonía et al. (2017) reported a specific growth rate of 0.6 g/day, a condition factor of 0.8, and a feed

conversion ratio of 3.8 in juveniles. However, the survival rate was 50%. This variability could be related to differences in the salinity of the water used, the feed provided, and/or the stocking densities used in the studies. Recent studies have indicated that the combination of appropriate temperature and salinity improves growth and feed conversion in juvenile common snook (Mello, 2024).

In other common snook species, such as chucumite (C. *parallelus*), which are reared in an extensive system, good results have been observed when freshwater forage fish were used, with the fish reaching 500 g in two years (0.69 g/day; Cerqueira 2005), and in terms of growth length, capture–recapture studies by Aliaume et al. (2000) reported a daily growth rate of 0.06 cm in fish collected during the rainy season and 0.04 cm in those collected during the dry season. Studies by Zarza-Mesa et al. (2006a) report similar results, with daily growth rates of 0.04 cm in freshwater concrete ponds for both white common snook and chucumite common snook. Absolute daily growth was also determined for white common snook and chucumite common snook in earthen ponds, with growth rates of 0.06 and 0.02 cm, respectively (Zarza et al., 2006b). These results are consistent with those obtained in our study, which revealed 0.03 cm of daily growth.

It is possible to adapt wild juvenile white common snook to captive conditions using a balanced diet. Adaptation to artificial feed is relatively rapid, as the fish accept it after two days of fasting, with all the fish consuming it by the fifth day. However, more information is needed regarding the growth of this species under brackish and marine conditions, in addition to evaluating different stocking densities and feed types. The aquaculture and commercial potential of this species justifies these studies and requires research aimed at developing a technological package for its commercial cultivation.

CONCLUSIONS

The adaptation of the common snook to captive conditions is relatively easy and accessible, allowing for the establishment of a broodstock with reproductive potential in captivity using practical diets, which is highly beneficial for contributing to the development of marine aquaculture, as well as for strengthening wild populations by exerting less pressure on them by relying on this resource rather than on what is produced by aquaculture. Furthermore, obtaining a broodstock from wild conditions supports the release of larvae or juveniles to facilitate the reestablishment of populations through the reintroduction of organisms capable of natural reproduction *in situ*.

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Coastal Sciences

Chapter 20

Notes on the recreational fishing of snook (*Centropomus* spp.) in the coastal zone of Centla, Tabasco, Mexico

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ABSTRACT

Snook sport fishing (*Centropomus* spp.) is a socioeconomic and recreational pillar in southeastern Mexico. This research characterized the recreational fishery in Centla, Tabasco, using biological, operational, and regulatory frameworks. Through access-point intercept surveys and biological sampling during a tournament, 18 specimens were identified across two species: common snook (*C. undecimalis*, n = 14) and Mexican snook (*C. poeyi*, n = 4). Morphometric analysis revealed that compared with C. undecimalis, *C. poeyi* was significantly larger in terms of size (86.25 ±3.7 cm) and biomass (8,251±861 g). Both species exhibited negative allometric growth (b<3), a phenomenon linked to high tournament selectivity for large adults. From an operational perspective, the total cumulative effort reached 193.00 angler hours, yielding an overall catch per unit effort (CPUE of 0.0933 snook/h. Socioeconomically, the activity generated $337,000.00 MXN in direct revenue, with an average expenditure per angler of $18,722.22 MXN. Regulatory analysis confirmed strict compliance with NOM-017-PESC-1994, with sizes exceeding 70 cm, ensuring the harvest of sexually mature organisms. In conclusion, sport fishing in Centla is a high-yield sustainable development driver and a strategic biological refuge for the genus *Centropomus*.

Keywords: Recreational fishing, *Centropomus*, Catch unit effort, economic impact, Centla.

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INTRODUCTION

Snooks (family Centropomidae) comprise 13 species of the genus Centropomus Lacépède, 1802, which are distributed along both the Atlantic and Pacific coasts of the Americas (Nelson et al. 2016; Carvalho-Filho et al., 2019; Ferreira et al. 2026). Given their abundance and high commercial value, these species represent a socioeconomic mainstay for western Atlantic fisheries, contributing significantly to regional development across Central and South America (Taylor et al. 2000; FAO 2005).

These species are characterized by their frequent occurrence in mangrove ecosystems and their remarkable euryhaline capacity, which allows them to adapt to extreme salinity fluctuations and even establish themselves in inland waters (Allen; Robertson, 1998; Bussing, 1998). Furthermore, owing to the excellent quality of their flesh, they represent a key resource with high socioeconomic value for both recreational and commercial fisheries (Allen; Robertson, 1998; Bussing, 1998; Ortíz et al., 2015).

The primary objective of sport fishing is the capture of organisms for competitive or recreational purposes. It is distinguished from commercial fishing by the explicit prohibition of sale, export, or use for subsistence. In Mexico, this sector is governed by the General Law of Sustainable Fisheries and Aquaculture and, specifically, by NOM-017-PESC-1994, which prohibits the trade of any resulting catch (DOF 1994, 2013).

Geographically, this activity is distributed across more than 159 inland bodies of water. In the northern and central regions, the exploitation of the largemouth bass (*Micropterus salmoides*) is most prominent. Conversely, in the south southeast, specifically in the states of Campeche, Tabasco, and Chiapas, the focus shifts to species such as common snook (*Centropomus* spp.), macabíl (*Brycon guatemalensis*), tenguayaca (*Petenia splendida*), and Atlantic horse mackerel (*Trachurus trachurus*). Under the National Development Plan for this sector, fishing effort is restricted to a single rod per angler, with a maximum daily bag limit of five specimens (Gaspar-Dillanes., 2023).

There has been a marked surge in interest in recreational angling within Mexico's coastal waters. This trend is particularly evident in the southern region, where the activity's growing popularity has spurred the organization of annual tournaments targeting local species, such as snook in southern Veracruz, Tabasco, and Campeche. The objective of the present study was to characterize the recreational snook fishery in the coastal zone of Centla, Tabasco.

MATERIALS AND METHODS

Study site

This study was conducted in the coastal region of the municipality of Centla, Tabasco, within the influence zone of the Pantanos de Centla Biosphere Reserve (CONANP, 2000) and the estuaries of the Grijalva and Usumacinta Rivers. This ecosystem is considered essential for the life cycle of the genus

Centropomus spp. because of the presence of salinity gradients and extensive mangrove forests that support their development.

Study design

We conducted a descriptive and cross-sectional study in the coastal region of Centla, Tabasco. Access-point intercept surveys (Creel Surveys) were employed according to the methods of Pollock et al. (1994). This methodology is the recognized benchmark for assessing fisheries where fishers return to discrete access points, including marinas, boat ramps, and docks.

Socioeconomic and operational data

Structured collection questionnaires were administered to recreational anglers throughout the duration of the tournament. The variables measured included fishing effort, effective fishing time (hours/day) and the number of fishing gear used. Angler Profile: Origin, level of experience, and direct trip-related expenses (fuel, bait, and guide services).

***Catch and effort*:** Catch per unit effort (CPUE) was calculated and defined as the number of snook caught per angler per hour (CPUE = N/angler-hour).

Biological sampling: For the biological characterization of species within the genus *Centropomus,* data were recorded from specimens caught during the tournament:

Identification: Dichotomous keys by Rivas (1986) were used to differentiate sympatric species, such as *Centropomus*.

Morphometrics: Measurements of total length (TL) and total weight (TW).

Size structure: Data were grouped into size-class intervals to analyze the distribution of the exploited population.

***Regulatory analysis*:** Compliance with NOM-017-PESC-1994, which regulates recreational fishing in Mexico, was evaluated by verifying daily bag limits (5 specimens per angler in inland/coastal waters) and the minimum size limits suggested in local management plans.

Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated with Levene's test. To compare the mean total length (TL) between *C. undecimalis* and *C. poeyi*, an independent samples Welch's t test was performed, with the significance level set at $\propto = 0.05$.

RESULTS AND DISCUSSION

Socioeconomic and operational results

The analysis of angler profiles and economic impact revealed significant regional dynamics within the municipality of Centla. According to the intercept surveys, the total direct expenditure generated by participants was $337,000.00 MXN, with an average expenditure per angler of $18,722.22 MXN (Table 1).

Table 1. Distribution of economic impact and participation by the origin of recreational anglers.

Origin	n	Total expenditure (MXN)	Average expenditure (MXN)	Participation (%)
Villahermosa, Tabasco	8	$105,000	$13,125	44.4
Campeche, Campeche	4	$91,000	$22,750	22.2
Palenque, Chiapas	2	$40,000	$20,000	11.1
Tampico, Tamaulipas	1	$38,000	$38,000	5.6
Mérida, Yucatán	1	$25,000	$25,000	5.6
Emiliano Zapata, Tabasco	1	$20,000	$20,000	5.6
Tenosique, Tabasco	1	$18,000	$18,000	5.6
Total/Average	18	$337,000	$18,722	100%

Note: *n* = number of anglers; MXN = Mexican Pesos. Expenditures include fuel, guide services, registration fees, and travel logistics.

Although the highest turnout of anglers originated from Villahermosa (44.4%), out-of-state participants from regions such as Tamaulipas and Yucatán recorded the highest average expenditures (> $25,000 MXN). This highlights the capacity of the Grijalva–Usumacinta system to attract long-range trophy fishing tourism. The economic impact per unit of biomass is significantly greater than that generated by local commercial fisheries, which is consistent with the findings of Espinoza-Tenorio et al. (2011) regarding the potential of recreational fishing as a driver of sustainable economic development in the riparian communities of Tabasco.

From an operational perspective, the total cumulative effort reached 193.00 angler hours, yielding an overall catch per unit effort (CPUE) of 0.0933 snook/h. When the data were disaggregated, the species-specific CPUE values were virtually identical: 0.0931 for *C. undecimalis* and 0.0937 for *C. poeyi*. This index reflects a requirement of nearly 11 effective hours per catch and highlights that high-end centropomid fishing demands significant technical proficiency. Furthermore, these results support the hypothesis that *C. poeyi* is less susceptible to lures than to congener within these estuarine environments.

Biological sampling results

A total of 18 specimens of the genus *Centropomus* were analyzed, all of which were harvested during a recreational fishing tournament in the coastal region of Centla, Tabasco. Taxonomic identification using the keys of Rivas (1986) confirmed the sympatric occurrence of two species: common snook (*C. undecimalis*, n = 14) and Mexican snook (*C. poeyi*, n = 4). The predominance of *C. undecimalis* in the landings suggests either a greater relative abundance or a heightened susceptibility of this species to sportfishing efforts at the Grijalva and Usumacinta River mouths during February. These observations are consistent with the distribution patterns reported by (Arévalo-Frías; Mendoza-Carranza., 2014; Mendoza-Carranza et al. 2010) for this deltaic system.

Morphometric analysis revealed significant dimensional differences between the two species (Table 2). Compared with *C. undecimalis* (77.12± 4.9 cm), *C. poeyi* was significantly longer (86.25± 3.7 cm), as validated by Welch's t test (Welch, 1938) (t = -3.96, p = 0.0067). This disparity in size suggests that, within the context of the tournament, the Mexican snook serves as the primary "trophy" target because of its substantially higher mean biomass (8,251± 861 g).

Table 2. Biometric parameters and length-weight relationship of the captured species.

Species	n	Mean TL (cm)	Mean BW (g)	b	R^2	K
C. undecimalis	14	77.12 ±4.9	5,483±990	2.37	0.65	1.19±0.13
C. poeyi	4	86.25±3.7*	8,251±861	2.29	0.96	1.28±0.04

Note. Asterisk (*) indicates a significant difference (p< 0.01). TL = Total Length; BW = Body Weight; b = allometry coefficient; K =condition factor.

The obtained allometry coefficients (2.37 for *C. undecimalis* and 2.29 for *C. poeyi*) indicate negative allometric growth (b< 3). These values are lower than those reported by Perera-García et al. (2008), who reported growth near isometry (b = 2.98) in wild populations within the Pantanos de Centla Biosphere Reserve. However, this divergence can be attributed to the selectivity of the landing site intercept surveys (Creel Surveys); by focusing on large individuals, these surveys introduce a statistical bias that flattens the slope of the length-weight regression. Despite the low allometry, the condition factor (K) remained above 1.1 for both species, indicating a robust physiological state and adequate energy availability within the estuarine ecosystem during the sampling period.

Regulatory compliance analysis

The analysis confirmed strict adherence to NOM-017-PESC-1994, as no recorded catches exceeded the daily limit of five specimens per angler. Furthermore, all individual lengths exceeded 70 cm,

ensuring that the harvested organisms were above the size at first sexual maturity reported for these species in the Gulf of Mexico.

CONCLUSIONS

Biological and operational assessments of the 2025 Frontera Sport Fishing Tournament in Centla, Tabasco, demonstrate the coexistence of two sympatric species of high ecological and recreational value: *C. undecimalis* and *C. poeyi*. The statistically validated morphometric superiority of *C. poeyi* establishes it as the quintessential trophic species of the region, despite the need for significantly greater fishing effort (CPUE). While both taxa exhibited negative allometric growth ($b < 3$), this phenomenon is identified as an artifact of the tournament's high selectivity for large adults; this, coupled with an optimal condition factor (K), confirms the robust population health of centropomids within the Grijalva–Usumacinta Delta.

From a socioeconomic perspective, the results underscore that recreational fishing within the Pantanos de Centla Biosphere Reserve is a high-yield development driver, generating a per capita economic impact that far exceeds that of traditional artisanal fishing. Strict compliance with NOM-017-PESC-1994, as evidenced by the exclusive landing of mature organisms (>70 cm), validates this activity as a model for sustainable use. In conclusion, the coastal zone of Centla is reaffirmed as both a strategic biological refuge and a premium recreational sanctuary; its proper management is not only an environmental necessity but also an indispensable economic investment for the sovereignty of aquatic resources in southeastern Mexico.

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Chapter 21

Linkage among climatic variation and artisanal fisheries behavior of common snook (*Centropomus undecimalis*) in tropical Atlantic coast

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ABSTRACT

The artisanal fishery of common snook *Centropomus undecimalis*, is of high socio-economic importance along the coasts of Tabasco, Mexico. Understanding its temporal dynamics is essential for sustainable management. This study aimed to analyze the temporal variability of catch per unit effort (CPUE) and its relationship with fishing effort, environmental factors (precipitation and temperature), and El Niño–Southern Oscillation (ENSO) anomalies. A directed sampling design was applied based on fishing cooperatives with the highest landings. Data from logbooks (September 1999 to June 2005) and direct vessel records were analyzed. CPUE was calculated as total catch (kg) per boat. Monthly variation was evaluated over a 19-month period, and correlation analyses were performed to examine relationships between CPUE, environmental variables, and ENSO anomalies. Marked seasonal fluctuations in CPUE and total catch were observed, associated with reproductive migration and life cycle patterns. The highest CPUE peaks occurred in January–February and July–October 2000 (86.43 kg and 37 kg, respectively), coinciding with increased fishing effort. Although no statistically significant correlations were found between CPUE and environmental variables, higher catches were associated with periods of increased precipitation and optimal temperatures (25–29°C). Negative ENSO anomalies corresponded with higher rainfall, which in turn was linked to increased fishing effort and CPUE. A decreasing trend in CPUE over time was observed, although it was not statistically significant ($r^2 = 0.14$). The common snook fishery in Barra San Pedro is influenced by the interaction between fishing effort, hydrological conditions, and climatic variability. These findings highlight the need for long-term monitoring and further research to support sustainable fisheries management.

Key words: common snook, Gulf of Mexico, CPUE, artisanal fisheries, ENSO

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INTRODUCCIÓN

The common snook (*Centropomus undecimalis*) is the most important commercial species of snook in Gulf of Mexico coast. This species represents an important incoming for artisanal fishermen of Tabasco, Veracruz and Campeche states, representing the 68% (Perera-García et al., 2012, Contreras-García et al., 2024). Common snook is an accessible resource for the most of fishermen; the reasons are related with its migratory habits along rivers of Tabasco and for the easiness to catch it. High technifications is not necessary, only are necessaries small boats with or without outboard motor, seines named "robaleras" or special tarpons known locally as "fizga" (Caballero, 1995). Almost for Tabasco the capture per unit effort (CPUE) and total catch along year present a marked decline. This diminution in total catches and CPUE could be related with an accelerate increase in effort and probably to change in pluvial regimes, river curse modifications and water pollution (Quiroga, et al., 1996; Muhlia et al., 1994; Caballero, 2003, Perera-García, 2011).

On the other hand, total catch and CPUE of common snook in Tabasco present a noticeable seasonal fluctuation. These fluctuations are related with its life cycle and principally with its reproductive migration. However, from Tabasco aquatic ecosystems the life and reproductive cycle are not well known and it is probably that the size of reproductive school and migratory season could be related with change in environmental conditions. In certain form this variation could be related with local variation of environmental conditions but influenced strongly by global climatic anomalies. ENSO phenomena originating in the southeastern Pacific could directly influence fish assemblages and fisheries in rivers and coastal waters over a large portion of the earth (Fiedler et al., 1986, Swales et al., 1999, Sanchez-Velaso et al., 2000, Hernández-Vidal et al., 2014). For these reasons is very important to consider the possible relation among total catch, CPUE of common snook in Tabasco coast and local environmental variations as rain and sea surface temperature and its relation with global climatic anomalies.

MATERIALES Y MÉTODOS

Study site

The study was carried out at the fishing port of Barra de San Pedro, located in the municipality of Centla, in the state of Tabasco, Mexico. This municipality is situated in the hydrological region of the Grijalva and Usumacinta rivers, within the Pantanos subregion, with its municipal seat being the city and port of Frontera. Barra de San Pedro is located on the Gulf of Mexico coast, east of Frontera, approximately 22.3 km to the southeast. Its geographic coordinates are 18.6422° north latitude and -92.47124° west longitude, and it lies at an approximate altitude of one meter above sea level, characterizing it as a low-lying coastal zone influenced by hydrological and marine processes (INEGI, 2022).

Data collection and analysis

Common snook fishery data were collected directly in a particular storage center located in San Pedro Port, Centla, Tabasco. A directed sampling design was implemented based on the cooperatives where the highest catches were recorded. At the landing site, the common snook (*Centropomus undecimalis*) is distinguished from other snook species under the local name 'common snook' (robalo blanco).

The monthly average sea surface temperature along the fishing area was estimated from weekly sea surface temperature images based on OI.v2 analysis (Reynols et al., 2002). Weekly sea surface temperature images were obtained from the web site of the International Research Institute for Climate Prediction (2003). Monthly average precipitation of Grijalva-Usumacinta hydrological basin, which influences the hydrology and salinity of the fishing area, was estimated from monthly images (Adler et al., 2003). Images were acquired from the web site of the Global Precipitation Climatology Centre (2004).

For the purposes of this study, statistical information recorded (logbooks) on arrivals by cooperative from September 1999 to June 2005 was available, as well as data collected directly from the boats that arrived at the cooperative. Assuming that this value is proportional to the abundance of a resource, the following equation was applied:

$$CPUE = C/E$$

where: (C) is the total catch and (E) is the fishing effort.

In general terms, and due to the absence of more precise data, CPUE was expressed based on kilograms of snook caught per boat. The temporal variation (monthly variation over 19 months) was analyzed. The latter defined the variation in the abundance of these species over time, allowing inferences about the relationships between this abundance and local climatic variations (Sparre; Venema, 1995). A correlation analysis was estimated (Gulland, 1983; Sparre; Venema, 1995).

RESULTS AND DISCUSSION

It was observed that the artisanal white snook fishery on the coasts of Tabasco exhibits seasonal fluctuations in total catch and catch per unit of effort (CPUE), associated with its life cycle and reproductive migratory habits. CPUE showed variations over the last seven years, with peaks in January–February and July–October 2000 (86.43 kg and 37 kg), related to higher fishing effort. Subsequently, in November–December, catch decreased to 7.9 kg per vessel, coinciding with a reduction in effort. In later years, catch increased to 67.80 kg in May–June 2001 and September–December 2002, then declined along with effort. Overall, CPUE shows a downward trend per vessel (Figure 1), although without a significant correlation with time ($r^2 = 0.14$).

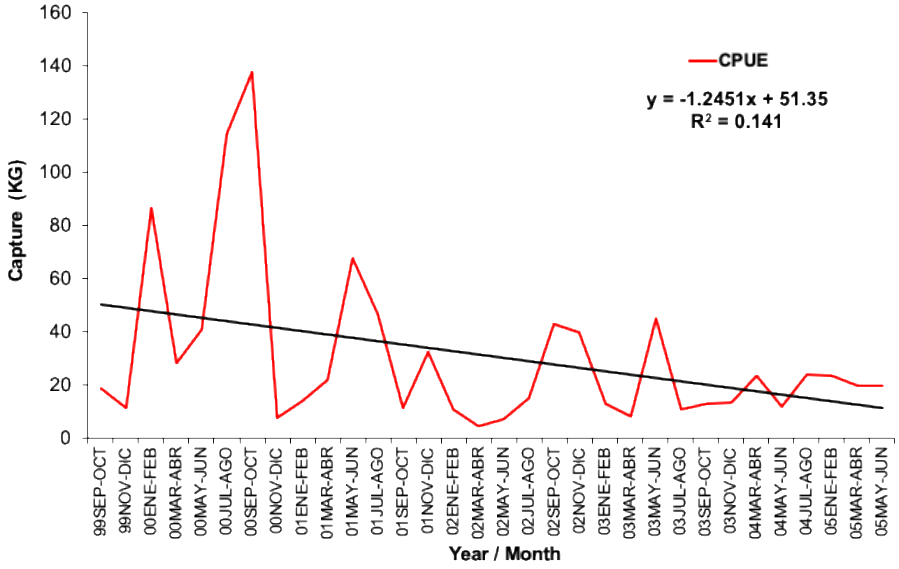


Figure 1. Bimonthly variation of catch per unit of effort (CPUE) for *C. undecimalis* observed from September 1999 to July 2005, at San Pedro Port, Centla, Tabasco.

These results coincide with the spawning and reproductive migration periods, particularly in the months of May, June, and July, suggesting a greater availability of the resource during these seasons. Considering CPUE as an indirect indicator of abundance (Yañez-Arancibia; Sánchez, 1988; França; Olavo, 2015), it is inferred that there was a greater availability of the resource in certain periods; however, catches did not follow a defined trend in subsequent years. This variability coincides with reports from fishermen, who indicate an increase in fishing pressure, as well as impacts derived from human activities such as pollution, sediment input, and alteration of coastal ecosystems—factors that have contributed to the decline in the biomass of *C. undecimalis* (Gilmore et al., 1983, Chávez, 1989, Quiroga et al., 1996, Farías-Tafolla et al., 2022).

Regarding the results relating ENSO (El Niño–Southern Oscillation) anomalies to rainfall peaks in Tabasco, it was observed that when ENSO anomalies are negative, higher rainfall peaks are recorded. In contrast, during positive anomalies, precipitation decreases or is distributed more evenly throughout the year, with even more than two rainy periods occurring, as observed between 1993 and 2003. Likewise, fishing effort shows a close relationship with these changes in the hydrological regime, as the highest effort peaks coincide with maximum precipitation. This behavior was reflected in both total catch and catch per unit of effort (CPUE), and a general decreasing trend in both parameters over time was also observed (Figure 2).

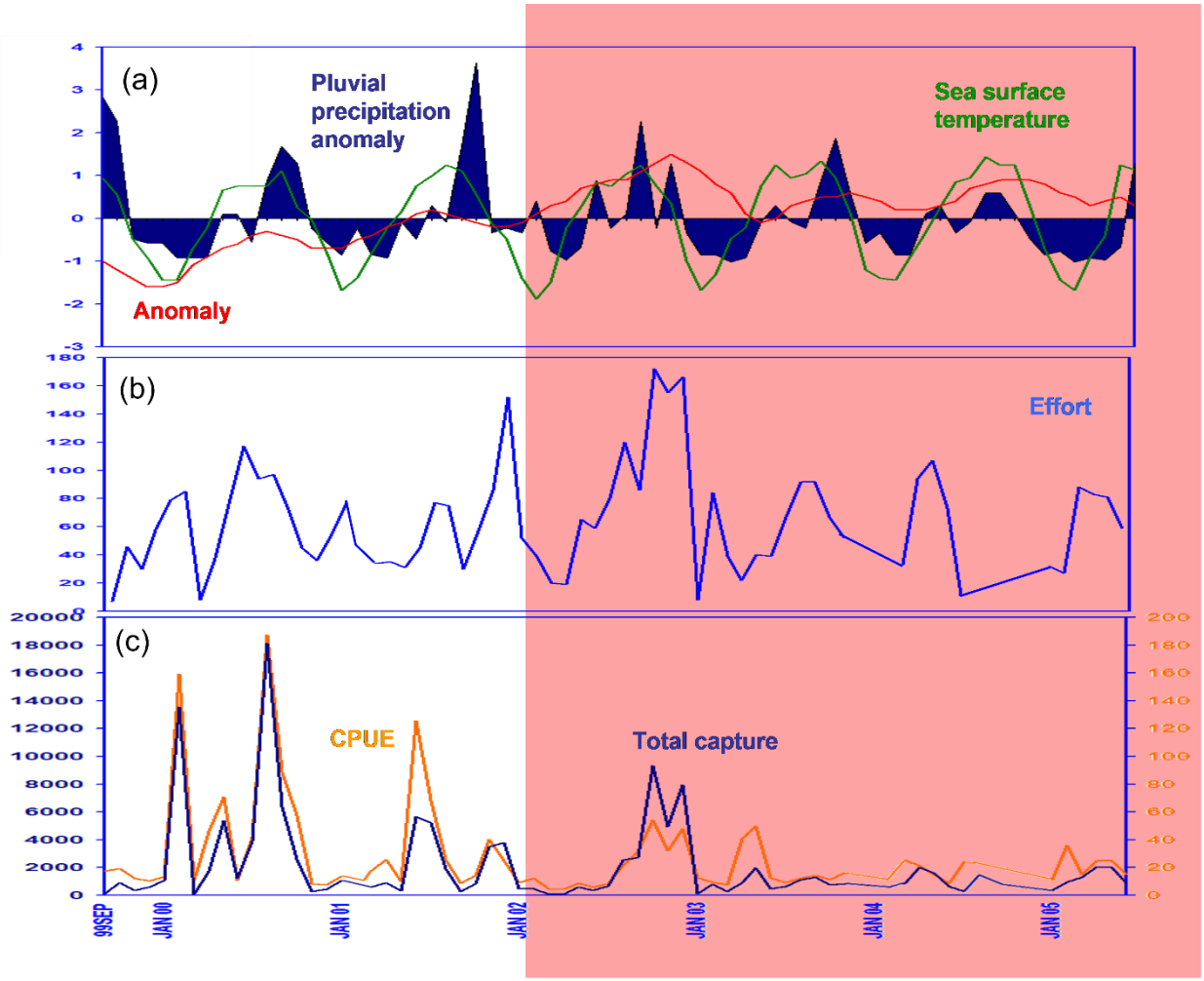


Figure 2. Annual values for (a) anomalous rainfall at the Barra San Pedro port, Centla, Tabasco, (b) fishing effort, and (c) catch per unit of effort (CPUE). The shaded areas show the relationship between fishing effort, rainfall regime, and CPUE from 1999 to 2005, respectively.

A relationship was established between effort and CPUE over three different time periods (Figure 3). Prior to the El Niño event, there is an exponential increase in CPUE in response to increased effort. During the El Niño event, effort increases twice but no significant increase in CPUE is observed. After the El Niño event, the effort-CPUE relationship becomes negative. It is important to note that in two particular cases before the El Niño event, effort is very high but CPUE is too low, breaking the mathematical relationship; these points correspond to the beginning and end of the fishing season. This is related to fisher behavior and seasonal variation in the abundance of snook

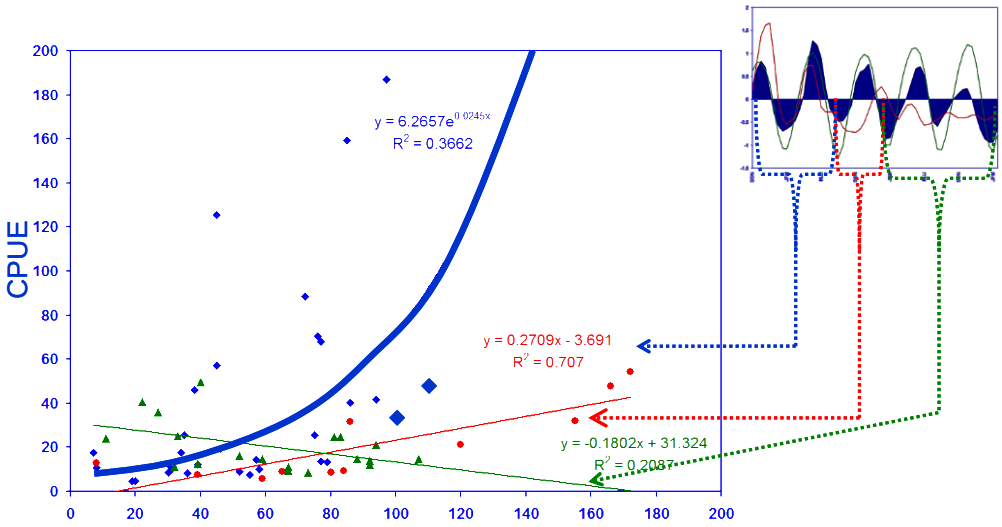


Figure 3. Relationship between fishing effort and CPUE of *C. undecimalis* before, during, and after the El Niño event at Barra San Pedro, Centla, Tabasco.

Although no statistically significant correlations were found between CPUE and environmental variables, a biological relationship with precipitation and temperature is possible (similar result in Matsunaga, 2017). The highest catches occur under heavy rainfall and temperatures of 25–29 °C, which favor recruitment and growth. Nutrient-rich riverine inputs have positive effects on recruitment, growth, and mortality, enhancing the productivity of *C. undecimalis*, while temperature directly affects its recruitment and spawning (Gilmore et al., 1983; Ramírez-Rodríguez et al., 2006). Likewise, enrichment from the river mouth, micro-upwellings (Díaz et al., 1999), nutrients from the upper basin, and coastal upwelling influence growth, mortality, and recruitment, thereby reinforcing fishery production (Grimes, 2001; Gamboa-García; Galván-Duque, 2023). These factors partially explain the spatiotemporal differences in catch and CPUE. Together, the findings reveal the complexity of the fishery system at Barra San Pedro, where fishing effort, environmental conditions, and ecological processes interact. As indicated by Weng and Sibert (2000), areas with high CPUE are relevant when they contribute significantly to total production, whereas areas with high effort and low CPUE may indicate excessive pressure or resource degradation, underscoring the need for science-based sustainable management strategies.

CONCLUSIONS

Given the social and economic importance of this resource, it is necessary to continue research on its biological and fishery aspects in order to achieve sustainable use. Selectivity studies of the fishing gear used to capture these species should be carried out, using appropriate mesh sizes, with special attention to those that cause the least environmental damage and, above all, retain organisms with average sizes close to the size at first capture. In Tabasco, the magnitude of the fishing effect on *C. undecimalis*

populations is unknown. It is necessary to establish permanent monitoring programs in order to obtain reliable data for decision making based on the biology and fishery dynamics of the resource.

It is advisable to expand knowledge about the population structure of this species through age and growth studies using indirect techniques (reading of bony structures and modal progression of sizes), systematically collecting data on this fishery. Studies on the magnitude of mortality through mark recapture experiments could constitute a fruitful field of collaboration with research institutions and higher education institutions along the entire Gulf of Mexico coastline.

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Environmental Sciences

Chapter 22

Fungal-based arsenic bioremediation: linking enzymatic detoxification mechanisms with bioreactor engineering

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ABSTRACT

Arsenic contamination represents a major environmental challenge due to its toxicity, persistence, and impact on water resources used for human consumption and agriculture. Fungal-based bioremediation has emerged as a promising alternative to conventional physicochemical treatments, owing to the metabolic versatility and adaptive capacity of fungi to tolerate and transform arsenic species. This work presents an integrative approach that links enzymatic detoxification mechanisms in fungi with the design of a controlled bioreactor system aimed at improving experimental reproducibility and analytical precision. A systematic literature analysis was conducted to identify key biochemical pathways involved in arsenic detoxification, including arsenate uptake, enzymatic reduction to arsenite, thiol-mediated complexation, efflux through Ars systems, methylation, and antioxidant responses. Based on these biological requirements, a modular bioreactor was designed using computer-aided engineering tools, incorporating GL45-based cultivation units, integrated sensors for temperature, pH, and dissolved oxygen, and mechanical agitation. The system also includes an electronic architecture for real-time monitoring and feedback control, as well as an IoT-based platform for data acquisition and remote supervision. The proposed design enables precise control of environmental variables and continuous data collection, facilitating the study of fungal responses under defined conditions. By reducing experimental variability, this platform supports the establishment of robust correlations between operational parameters and enzymatic activity. Overall, this work provides a scalable and accessible framework that strengthens the integration of biotechnology and engineering, contributing to the advancement of fungal-based strategies for arsenic bioremediation.

Key words: arsenic bioremediation, fungal detoxification, bioreactor engineering.

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INTRODUCTION

Arsenic contamination is currently a serious and concerning environmental problem worldwide, as it often affects groundwater intended for human consumption (Monteiro De Oliveira et al., 2021) and/or agricultural activities (Patel et al., 2023). This heavy metal can exist in two chemical forms: arsenate [As(V)] or arsenite [As(III)] (Bjørklund et al., 2020). Both forms are toxic to living organisms, causing oxidative stress, cellular damage, and metabolic alterations (Ganie et al., 2024). Conventional physicochemical treatments for remediating this contaminant, such as chemical precipitation, ion exchange, or adsorption, typically involve high operating costs, high energy consumption, and the generation of secondary contaminating residues (Priya et al., 2023), which limits their long-term sustainability. Therefore, research and development of alternatives based on biological processes have become a priority in impactful environmental engineering.

In the previously mentioned context, an alternative that arises is the use of organisms such as fungi, which have high potential for the bioremediation of heavy metals such as arsenic, chromium, or lead (Sher; Rehman, 2019; Ayele; Godeto, 2021; Kumar; Singh, 2024). Their morphology generates a high surface-to-volume ratio (Alsharari et al., 2018), and their metabolic diversity allows significant interaction with contaminants (Deshmukh et al., 2016), making them useful in biosorption, bioaccumulation, and biotransformation processes (Bibbins-Martínez et al., 2023). Previous studies have shown that fungal species tolerate high concentrations of arsenic and are even capable of modifying its chemical state through mechanisms involving enzymatic processes dependent on arsenate reductases, glutathione, or metallothioneins (Singh et al., 2018; Gupta et al., 2022; Mohsin et al., 2023). However, there are still gaps in the understanding of the mechanisms involved due to the physiological complexity of fungi and the high metabolic variability influenced by culture conditions.

The study of detoxification mechanisms presents several challenges, notably those associated with cultivation in flasks or open batch-type systems (Bahera et al., 20219, where variations in temperature, oxygen availability, and pH occur (Xiao et al., 2022; Karayannis et al., 2023; Rocha et al., 2025). These fluctuations can significantly alter the metabolic and proteomic responses of the fungus, making reproducibility difficult and hindering the identification of correlations between culture conditions and enzymatic expression (Bakar et al., 2020; Rämä; Quandt, 2021). This may lead to discrepancies between studies and limitations in interpreting the fungus–arsenic interaction.

For this reason, the use of controlled cultivation platforms may be a valuable strategy for advancing the understanding of fungal mechanisms involved in arsenic bioremediation. Bioreactors enable regulation of critical process variables, reducing experimental noise and facilitating the collection of homogeneous biological samples for subsequent analysis (Lin et al., 2025). Automated control (Palladino et al., 2024) and remote monitoring (Hausmann et al., 2017), features of mechatronic

engineering, allow continuous recording of operating parameters, thereby establishing reproducible conditions useful for proteomic and molecular studies.

This chapter first reviews the key factors in arsenic bioremediation research and then proposes a conceptual design for an automated bioreactor with remote monitoring. In this way, it establishes a link between the biological requirements of fungi and the engineering operating variables necessary to generate standard experimental conditions in environmental bioremediation research.

MATERIALS AND METHODS

Literature Analysis of Enzymatic Detoxification Mechanisms

A systematic literature review was conducted to identify and analyze the main enzymatic detoxification mechanisms involved in fungal arsenic bioremediation. The search strategy was based on the use of structured Boolean queries designed to retrieve relevant scientific articles focusing specifically on fungal systems, while minimizing the inclusion of studies related to plants or other organisms.

Scientific databases including Google Scholar, Scopus, and Web of Science were consulted to ensure comprehensive coverage of peer-reviewed literature. The search process employed combinations of keywords related to arsenic speciation, transport mechanisms, enzymatic detoxification pathways, and fungal biology. Boolean operators (AND, OR, NOT) were used to refine the search, allowing the inclusion of synonymous terms and the exclusion of unrelated research areas.

For example, search strings included combinations such as: ("arsenate uptake" OR "As(V) transport") AND ("arsenite transport" OR aquaglyceroporin) AND (fungi OR fungal OR yeast) NOT (plant OR Arabidopsis OR rice). Additional queries were designed to target specific mechanisms, including arsenate reduction, glutathione-mediated detoxification, Ars systems, and oxidative stress responses. These queries were iteratively refined to improve specificity and relevance of the results.

Inclusion criteria considered peer-reviewed research articles that explicitly addressed arsenic detoxification mechanisms in fungi at the molecular, biochemical, or physiological level. Studies involving gene expression, protein characterization, enzymatic activity, and transport systems were prioritized. Review articles were also considered when they provided integrative insights or summarized experimental evidence relevant to the mechanisms of interest.

Exclusion criteria included studies focused primarily on plants, bacteria (unless used for comparative purposes), or environmental assessments lacking mechanistic detail. Articles without clear experimental or molecular evidence related to arsenic detoxification pathways were also excluded. This methodological approach ensured a structured and reproducible selection of literature, providing a robust foundation for the analysis of enzymatic detoxification mechanisms discussed.

Bioreactor Engineering Design and Development

The design of the bioreactor system for fungal-based arsenic bioremediation was carried out through a computer-aided engineering (CAE) approach, integrating mechanical, electronic, and system-level components into a unified platform. The entire design process was developed using SolidWorks, which enabled precise modeling, visualization, and iterative optimization of the proposed system.

The conceptual design phase involved the three-dimensional modeling of the bioreactor structure, including the support frame, control housing, and integration of GL45 glass vessels as cultivation units. Mechanical components such as the agitation system (motor and impeller), adjustable shaft, and sensor ports were designed to ensure proper alignment, stability, and compatibility with standard laboratory materials. Special attention was given to the modification of the GL45 cap, incorporating multiple inlets for sensor integration (pH, temperature, dissolved oxygen), aeration, and mechanical stirring, ensuring a functional and compact configuration.

For the electronic architecture, schematic-level planning was performed in parallel with the mechanical design to ensure proper spatial distribution and compatibility of components within the control unit. This included the arrangement of the power supply, microcontroller, signal conditioning modules, and actuator drivers. Although the electronic circuits were not simulated within the CAD environment, their physical integration and housing were designed in SolidWorks to ensure adequate heat dissipation, accessibility, and protection of components.

Additionally, the IoT-based monitoring system was conceptualized through a systems integration approach, where data flow between sensors, control units, and user interfaces was defined. The Human-Machine Interface (HMI) and remote monitoring capabilities were incorporated into the design as functional modules, allowing visualization of process variables and interaction with the system.

The design process followed an iterative methodology, where multiple versions of the system were evaluated in terms of spatial arrangement, ergonomics, modularity, and scalability. Virtual assemblies and renderings generated in SolidWorks were used to validate component compatibility, optimize layout, and communicate the system architecture. This approach ensured that the final design integrates mechanical robustness, electronic functionality, and digital connectivity, providing a comprehensive platform for controlled fungal bioremediation studies.

RESULTS AND DISCUSSION

Enzymatic Detoxification Mechanisms

Arsenic detoxification in fungal systems is a complex and highly regulated process involving coordinated biochemical and molecular responses. These mechanisms are strongly influenced by arsenic speciation, primarily As(V) and As(III), as well as by the intracellular redox state.

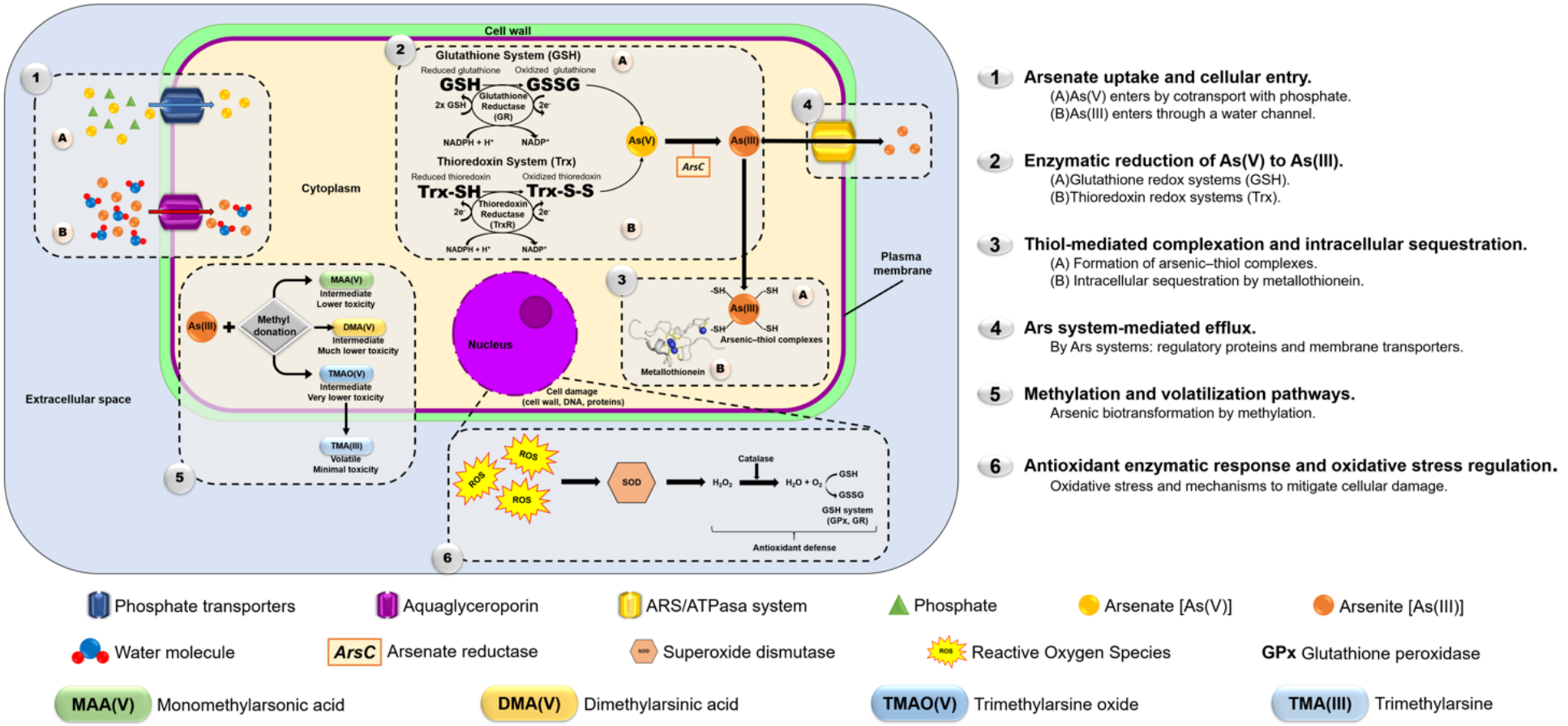


Figure 1. Integrated overview of fungal arsenic detoxification mechanisms, including cellular uptake pathways, enzymatic reduction of arsenate to arsenite, thiol-mediated complexation and intracellular sequestration, efflux via Ars transport systems, methylation and volatilization processes, and activation of antioxidant defenses under oxidative stress conditions.

In fungi, detoxification is achieved through a series of enzymatic and metabolic strategies that allow tolerance, transformation and removal of arsenic from the cellular environment (Figure 1). The main enzymatic detoxification mechanisms can be described as follows:

Arsenate uptake and cellular entry. As(V) enters fungal cells primarily through phosphate transporters due to its structural similarity to phosphate (PO_4^{3-}). This molecular similarity allows As(V) to be recognized by high- and low-affinity phosphate permeases in the plasma membrane. Consequently, arsenate uptake is closely linked to phosphate availability; under phosphate-limiting conditions, transporter expression is upregulated, inadvertently increasing arsenate influx and cellular toxicity. In contrast, As(III) predominates under reducing conditions and is uncharged at physiological pH. This enables its transport through aquaglyceroporins, a class of membrane channels that facilitate the passive diffusion of small neutral solutes such as glycerol. Unlike arsenate, arsenite uptake is less dependent on nutrient-regulated systems and more influenced by concentration gradients and membrane permeability. These distinct transport pathways have important implications for arsenic bioavailability and intracellular fate. Environmental factors such as pH, redox potential, and competing ions can modulate transporter activity and arsenic speciation. Therefore, cellular entry represents a critical control point that determines intracellular arsenic levels and directly influences the activation of downstream detoxification mechanisms (Liang et al., 2021; De Francisco et al., 2021; Sanaa-Qasem, 2025).

Enzymatic reduction of As(V) to As(III). One of the most critical steps in arsenic detoxification is the enzymatic reduction of As(V) to As(III), catalyzed by arsenate reductases. This reaction is essential because As(V), once inside the cell, can interfere with phosphate-dependent metabolic processes, whereas its reduction to As(III) allows further processing through detoxification pathways such as efflux and thiol complexation. Arsenate reductases are typically linked to cellular redox systems, primarily involving glutathione (GSH) or thioredoxin, which function as electron donors. These systems provide the reducing equivalents necessary for the conversion of As(V) to As(III), thereby coupling arsenic detoxification to the intracellular redox balance. As a result, the efficiency of this process is strongly influenced by the oxidative state of the cell and the availability of reducing molecules. Although As(III) is generally more toxic and reactive due to its high affinity for thiol groups, its formation is a strategic step in detoxification. This is because As(III) can be more readily sequestered through binding to thiol-containing compounds or actively exported out of the cell via specific transport systems. Therefore, arsenate reduction represents a central biochemical hub that connects arsenic transformation with redox homeostasis and downstream detoxification mechanisms (Mohsin et al., 2023; William; Magpantay, 2023; Georgiou-Siafis; Tsiftsoglou, 2023; Khan et al., 2024).

Thiol-mediated complexation and intracellular sequestration. Once reduced, As(III) exhibits a high affinity for thiol groups (-SH) present in intracellular molecules such as GSH and cysteine-rich peptides. This interaction leads to the formation of arsenic–thiol complexes, which significantly

reduce the chemical reactivity and toxicity of arsenic by limiting its interaction with essential cellular components, particularly proteins and enzymes. Glutathione plays a central role in this process, acting both as a redox buffer and as a chelating agent. The formation of As(III)–GSH complexes facilitates intracellular detoxification by stabilizing arsenic in a less reactive form and preparing it for subsequent transport or compartmentalization. In addition, these complexes may serve as intermediates for further detoxification steps, including efflux or vacuolar sequestration. Metallothionein-like proteins, characterized by their high cysteine content, also contribute to arsenic binding. These proteins provide additional binding sites for As(III), enhancing the cell's capacity to sequester arsenic and mitigate its toxic effects. Overall, thiol-mediated complexation represents a crucial detoxification strategy that minimizes cellular damage by controlling arsenic reactivity and facilitating its safe handling within the intracellular environment (Robinson et al., 2021; Salama, 2025).

Ars system-mediated efflux. Fungal cells possess Ars-like detoxification systems that play a central role in maintaining low intracellular arsenic concentrations. These systems typically consist of regulatory proteins and membrane-associated transporters that coordinate arsenic sensing, transformation, and export. Once As(V) has been reduced to As(III), this more reactive form becomes the primary substrate for efflux mechanisms. Arsenite is actively transported out of the cell through specific efflux pumps, which may belong to families of membrane transporters such as ATP-dependent pumps or secondary transport systems. These transporters recognize As(III), often in its free or thiol-complexed form, and facilitate its extrusion across the plasma membrane, thereby reducing its intracellular accumulation and associated toxicity. The expression of Ars-related genes is commonly regulated in response to arsenic exposure, allowing cells to rapidly activate detoxification pathways under stress conditions. This regulatory component ensures an efficient and adaptive response to fluctuating arsenic levels in the environment. Importantly, the coupling of enzymatic reduction and active efflux constitutes one of the most effective detoxification strategies. While reduction converts As(V) into a transportable form, efflux systems complete the detoxification process by removing arsenic from the intracellular environment, thus preserving cellular integrity and function (Li et al., 2021; William; Magpantay, 2023; Sanaa-Qasem, 2025).

Methylation and volatilization pathways. In some fungal species, arsenic can undergo enzymatic methylation, leading to the formation of organic arsenic compounds such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). These transformations are typically mediated by methyltransferase enzymes that use S-adenosylmethionine (SAM) as a methyl donor. Through this process, inorganic arsenic species are converted into forms that are generally less reactive and, in some cases, less toxic. Methylation can also facilitate the volatilization of arsenic, generating gaseous compounds such as trimethylarsine. This volatilization process represents a potential detoxification route, as it enables the removal of arsenic from the cellular environment into the

atmosphere. However, the ecological implications of arsenic volatilization remain complex, as these compounds can be redistributed in the environment. Although these pathways are less characterized compared to reduction and efflux mechanisms, they contribute to the overall arsenic biotransformation capacity of fungi. The extent and efficiency of methylation and volatilization are influenced by environmental conditions, including substrate availability, redox state, and metabolic activity. Thus, these processes represent complementary detoxification strategies that may play a significant role in arsenic cycling and attenuation in contaminated environments (Naseem et al., 2021; Hamza et al., 2022; Mohsin et al., 2023).

Antioxidant enzymatic response and oxidative stress regulation. Arsenic exposure induces the generation of reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide and hydroxyl radicals, which disrupt cellular redox balance and lead to oxidative stress. These reactive molecules can damage essential biomolecules such as lipids, proteins and nucleic acids, ultimately impairing cellular function and viability. In response, fungal cells activate a coordinated antioxidant defense system to counteract oxidative damage. Key enzymatic components of this system include superoxide dismutase (SOD), which catalyzes the conversion of superoxide radicals into hydrogen peroxide; catalase (CAT), which decomposes hydrogen peroxide into water and oxygen; and glutathione-related enzymes such as glutathione peroxidase and glutathione reductase, which maintain intracellular redox homeostasis. GSH plays a dual role as both an antioxidant and a critical molecule in arsenic detoxification, linking oxidative stress response with thiol-mediated sequestration mechanisms. The regulation of these antioxidant pathways is essential for maintaining cellular integrity under arsenic stress conditions. Overall, the activation of antioxidant enzymatic systems represents a fundamental adaptive response that mitigates arsenic-induced damage and supports the functionality of other detoxification mechanisms within the cell (Hu et al., 2020; Yaakoub et al., 2022; Park; Son, 2024).

These mechanisms operate in an integrated manner, forming a coordinated detoxification network that enables fungi to survive under arsenic stress conditions. However, the efficiency and regulation of these pathways are highly dependent on environmental and cultivation conditions, including pH, oxygen availability, nutrient composition and arsenic speciation.

These limitations highlight the need for controlled experimental systems that allow precise manipulation of environmental variables and reproducibility of results.

In this context, bioreactor engineering emerges as a key strategy to bridge the gap between biological processes and experimental control. The following section presents the principles and design considerations of bioreactor systems tailored for the study of fungal-based arsenic bioremediation, emphasizing their role in improving the analysis of enzymatic detoxification mechanisms.

Bioreactor Engineering for Fungal-Based Arsenic Bioremediation

The conceptual design of the proposed bioreactor represents a key step toward bridging the gap between fungal physiology and controlled experimental conditions required for arsenic bioremediation studies. The system was conceived as a modular, low-cost, and scalable platform that integrates standard laboratory materials with essential process control capabilities. At its core, the design employs GL45 glass bottles (1–2 L) as cultivation vessels, enabling flexibility in experimental setups and facilitating replication. The modified caps incorporate multiple access ports that allow the integration of critical components, including temperature (PT100), pH, and dissolved oxygen sensors, as well as aeration inputs and a mechanical agitation system. The impeller ensures homogeneous mixing, which is essential for maintaining consistent nutrient distribution, oxygen transfer, and arsenic exposure within the culture medium. Additionally, the inclusion of a heating pad beneath the vessel allows precise temperature regulation, a critical parameter influencing fungal metabolism and enzymatic detoxification pathways. The vertical support structure with adjustable height enables adaptability to different vessel configurations and experimental requirements. Overall, this conceptual configuration ensures direct interaction between biological processes and controlled physical variables, thereby reducing environmental variability and improving reproducibility in fungal arsenic bioremediation experiments (Figure 2).

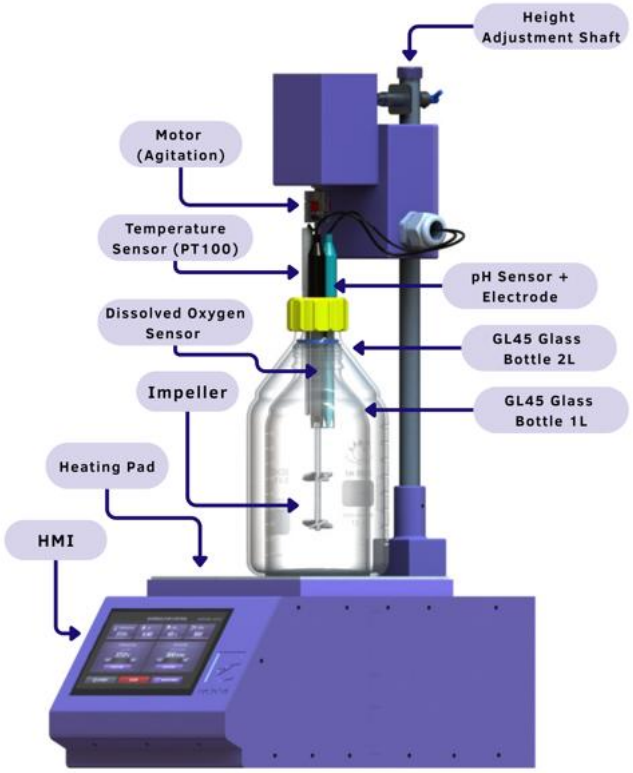


Figure 2. Conceptual design of the modular bioreactor system for fungal cultivation, illustrating the integration of sensing elements, mechanical agitation, and thermal regulation within a GL45-based vessel, along with its structural support and user interface for operation.

Building upon the physical configuration, the electronic architecture of the system establishes the functional backbone required for real-time monitoring and control of the bioprocess. The architecture is organized into four main subsystems: electrical power, control, sensors, and actuators. The power subsystem includes a regulated power supply with protective elements such as fuses, ensuring safe operation of the system. The control subsystem is centered on a microcontroller responsible for processing sensor signals and executing control algorithms, complemented by a Raspberry Pi module that enables higher-level monitoring, data visualization, and user interaction through the HMI interface. Sensor integration includes pH, temperature (PT100), dissolved oxygen, and motor encoder feedback, allowing continuous acquisition of key process variables. These signals are conditioned through an ADC/temperature amplifier before being interpreted by the control unit. On the actuation side, the system incorporates an H-bridge motor driver for agitation control and a MOSFET-based driver for the heating element, enabling precise regulation of mixing and temperature. This closed-loop configuration allows dynamic adjustment of operating conditions based on sensor feedback, ensuring stable and reproducible environments necessary for studying fungal enzymatic responses to arsenic stress (Figure 3).

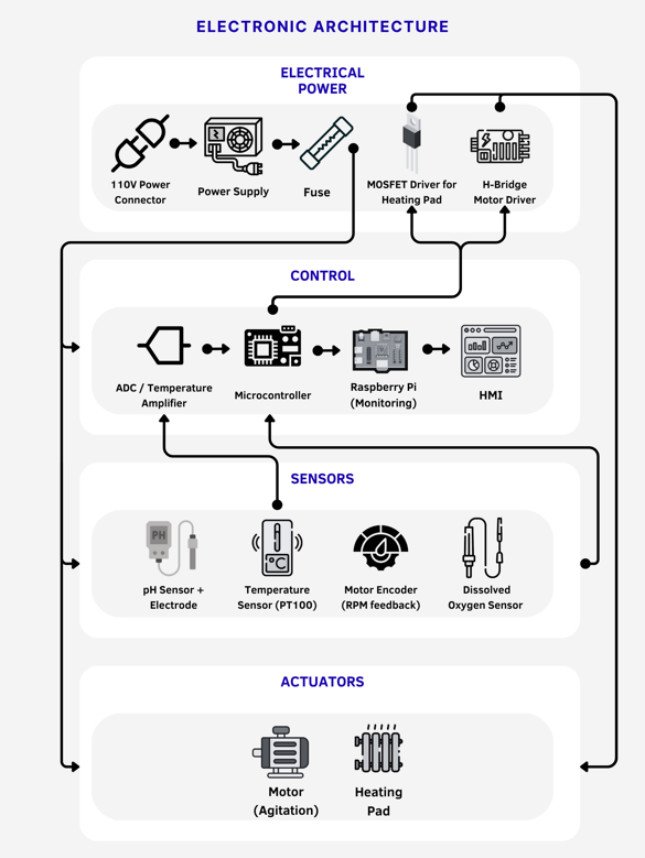


Figure 3. Electronic architecture of the bioreactor system, showing the organization of power supply, control units, sensor interfaces, and actuator drivers that enable signal acquisition, processing, and coordinated system operation.

Finally, the integration of an IoT-based monitoring and data acquisition system significantly enhances the analytical capabilities of the proposed bioreactor. The system enables continuous collection, storage, and visualization of process data, which are transmitted from the sensors through the control unit to a digital interface accessible via the HMI or remote devices. This architecture allows real-time tracking of key parameters such as temperature, pH, and dissolved oxygen, facilitating immediate detection of deviations and enabling timely corrective actions. Moreover, the implementation of remote monitoring supports experimental supervision without the need for constant physical presence, improving operational efficiency. The data acquisition system also enables the generation of structured datasets that can be used for advanced analyses, including pattern recognition, correlation of environmental conditions with enzymatic activity, and potential integration with predictive models. This digital layer not only reduces experimental variability but also strengthens the reproducibility and scalability of fungal bioremediation studies. By linking sensor data, control actions, and user interaction within a unified platform, the IoT design transforms the bioreactor into a smart system capable of supporting high-resolution studies of arsenic detoxification mechanisms (Figure 4).

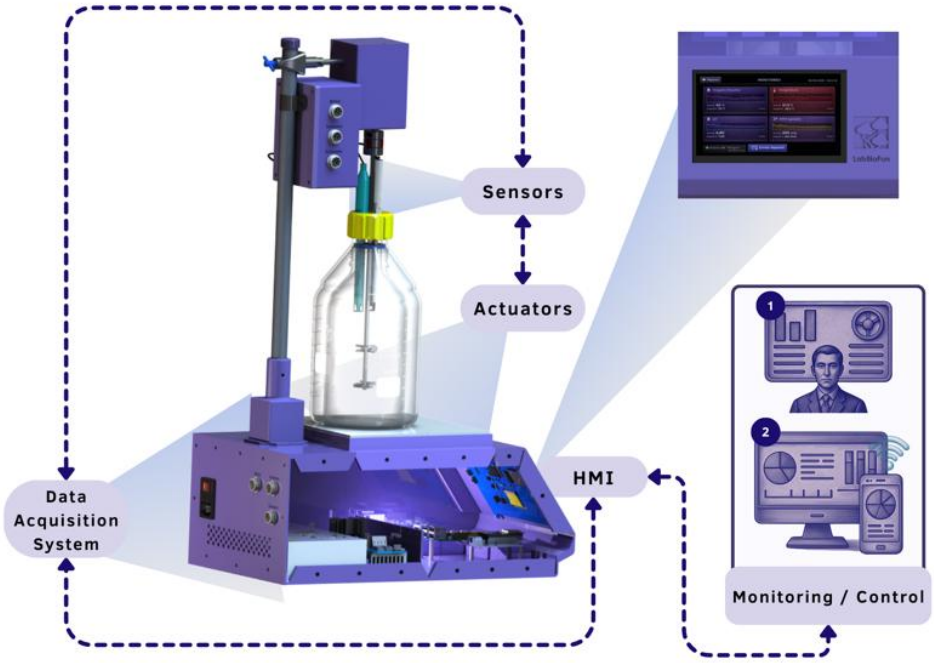


Figure 4. IoT-based system design illustrating the integration of data acquisition, real-time monitoring, and remote interaction between sensors, actuators, and user interfaces for process supervision and control.

CONCLUSIONS

This work establishes an integrative framework linking fungal arsenic detoxification mechanisms with controlled bioreactor engineering. The proposed design enables precise regulation of critical

variables and systematic data acquisition, creating conditions suitable for reproducible and high-resolution studies. By combining modular hardware, real-time monitoring, and IoT-based control, the system provides a robust platform to explore the relationship between environmental parameters and enzymatic responses. This approach not only strengthens experimental reliability but also supports the development of scalable and efficient strategies for arsenic bioremediation in complex environmental contexts.

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Chapter 23

Simplified dynamic modeling for scenario analysis of the anaerobic digestion of fruit and vegetable waste

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ABSTRACT

Particle size and the substrate-to-inoculum (S/I) ratio are key factors influencing methane production during the anaerobic digestion (AD) of fruit and vegetable waste (FVW). In this study, a simplified dynamic model was used to analyze process behavior under batch conditions through two-dimensional parametric analysis, with varying particle sizes (0.01–5 mm) and S/I ratios (2–4). The model successfully captured key dynamic behaviors, including volatile fatty acid (VFA) accumulation and substrate limitation, and enabled the identification of favorable operating regions. The results showed that methane production reached its highest values at intermediate particle sizes (0.75–2 mm), where a balance between substrate availability and microbial conversion rates was achieved. Smaller particle sizes promoted rapid hydrolysis and VFA accumulation, leading to process imbalance, whereas larger particles limited methane production because of slower substrate release. Similarly, increasing the S/I ratio reduced the methane yield because of insufficient microbial capacity to assimilate intermediate products. Although quantitative predictions depend on parameter calibration, the proposed approach provides a useful framework for analyzing process dynamics and supporting the assessment of AD operating conditions for this type of waste.

Keywords: anaerobic digestion, fruit and vegetable waste, dynamic simulation.

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INTRODUCTION

Fruit and vegetable waste (FVW) is particularly important because of its high generation volume, as it is estimated that approximately 44% of fruits and vegetables produced are lost along the supply chain (Ambuko et al., 2025). When these residues are disposed of in landfills or other final disposal sites, they present environmental and public health problems because of the release of greenhouse gases and the generation of leachates, which may infiltrate soil and water bodies, in addition to creating favorable conditions for the proliferation of sanitary vectors and pathogens (Sagar et al., 2018).

Nevertheless, a technology that promotes the valorization of these residues within the circular economy framework is anaerobic digestion (AD), which enables the production of biogas rich in methane, suitable for energy generation, and a stabilized digestate rich in nutrients, with potential use as an organic fertilizer (Ganesh et al., 2022).

During the AD process, FVW is disintegrated and hydrolyzed through extracellular enzymes, releasing soluble compounds such as amino acids, sugars, and long-chain fatty acids. These compounds are subsequently converted into volatile fatty acids (VFAs), which are further transformed into acetate and ultimately into methane (Mata-Alvarez, 2002). These stages are carried out by different microbial groups; therefore, a proper balance among them is required to ensure the efficient production and consumption of intermediates, thereby maintaining process stability and efficiency (Xu et al., 2018).

In general, in the AD of solid waste, the rate-limiting step is disintegration–hydrolysis (Vavilin et al., 2008). However, since FVW is rich in readily biodegradable carbohydrates, it can be rapidly converted into VFAs. In this context, reducing the particle size (< 0.5 mm) in this type of waste may inhibit methanogenesis because of the accumulation of VFAs (Izumi et al., 2010; Vigueras-Carmona et al., 2025).

On the other hand, system stability has been reported to improve when a high proportion of microorganisms relative to the substrate is present, which can be achieved by applying low substrate-to-inoculum (S/I) ratios (Assis; Gonçalves, 2022; Okoro- Shekwaga et al., 2020; Zhang et al., 2019). Therefore, analyzing the combined effect of these two factors on process performance is of interest.

A suitable approach for studying these factors is modeling and simulation. One of the most widely used models is the ADM1 (Batstone et al., 2002); however, its structural complexity, representing the process through 19 processes and 52 parameters, limits its direct application to specific cases. Consequently, systematic model reductions have been proposed (Weinrich; Nelles, 2021), or simpler earlier models have been used instead (Hill; Barth, 1977; Moletta et al., 1986).

In this context, the objective of this study was to analyze operational scenarios of the anaerobic digestion of fruit and vegetable waste through simulation, with an emphasis on identifying the effects of particle size and the S/I ratio. For this purpose, a mathematical model reported in the literature (Vian et al., 2024) was implemented and integrated in MATLAB.

MATERIALS AND METHODS

Mathematical model

A simplified anaerobic digestion model reported by Vian et al. (2024) was used in this study. The model describes the process in three stages: disintegration, acetogenesis, and methanogenesis.

Disintegration is modeled as an extracellular process in which enzymes colonize the surface of the complex substrate and degrade it uniformly, generating soluble compounds (Sanders et al., 2000). These compounds are subsequently consumed by acidogenic bacteria, which produce acetate (used as a lumped variable representing the total VFAs). Finally, acetate is consumed by methanogenic archaea for methane production. It is further assumed that decaying biomass is reincorporated into the system as a soluble substrate.

a) Disintegration

The disintegration process was described using the model proposed by Esposito et al. (2008). The particles were assumed to be spherical, allowing the change in particle size to be expressed in terms of the variation in the particle radius:

$$R(t) = R_0 - \frac{k_1}{\rho} t \qquad \text{Eq. (1)}$$

where $R(t)$ is the particle radius (dm) at time t, R_0 is the initial radius (dm), k_1 is the disintegration rate constant ($g \cdot dm^{-2} \cdot d^{-1}$), and ρ is the solid density ($g \cdot dm^{-3}$). The variation in the particle radius leads to a change in the specific surface area, defined as follows:

$$a^* = \frac{3}{\rho R(t)} \qquad \text{Eq. (2)}$$

where a^* is the specific surface area ($dm^2 \cdot g^{-1}$).

The disintegration rate is then expressed as follows:

$$-r_{dis} = k_1 \cdot a^* \cdot S_c \qquad \text{Eq. (3)}$$

where r_{dis} is the disintegration rate and S_{sc} is the complex substrate concentration ($g \cdot L^{-1}$), corresponding to FVW. For a batch system, the dynamic behavior of the complex substrate is described as follows:

$$\frac{dS_c}{dt} = -k_1 \cdot a^* \cdot S_c \qquad \text{Eq. (4)}$$

b) Acidogenesis and methanogenesis

For the description of the acidogenesis and methanogenesis stages, it is assumed that the process rates are governed by microbial growth kinetics. Acidogenic microorganisms are described using Monod kinetics, whereas methanogenic microorganisms follow Haldane kinetics to account for substrate inhibition by volatile fatty acids (VFAs). Biomass decay is modeled using first-order kinetics.

Accordingly, the dynamic behavior of the concentration of soluble substrate (S_s), acidogenic microorganisms (X_1), VFA (S_{agv}), methanogenic microorganisms (X_2), and methane production (P_{ch4}) are described by the following set of equations:

$$\frac{dS_s}{dt} = k_1 \cdot a^* \cdot S_c - \frac{1}{Y_1}\frac{\mu_{max1} S_s}{K_{s1} + S_s} X_1 + k_{d1} X_1 + k_{d2} X_2 \quad \text{Eq. (5)}$$

$$\frac{dX_1}{dt} = \frac{\mu_{max1} S_s}{K_{s1} + S_s} X_1 - k_{d1} X_1 \quad \text{Eq. (6)}$$

$$\frac{dS_{agv}}{dt} = \frac{\mu_{max1} S_s}{K_{s1} + S_s} X_1 Y_2 - \frac{1}{Y_3}\frac{\mu_{max2} S_{agv}}{K_{s2} + S_{agv} + \frac{S_{agv}^2}{K_i}} X_2 \quad \text{Eq. (7)}$$

$$\frac{dX_2}{dt} = \frac{\mu_{max2} S_{agv}}{K_{s2} + S_{agv} + \frac{S_{agv}^2}{K_i}} X_2 - k_{d1} X_1 \quad \text{Eq. (8)}$$

$$\frac{dP_{ch4}}{dt} = \frac{\mu_{max2} S_{agv}}{K_{s2} + S_{agv} + \frac{S_{agv}^2}{K_i}} X_2 Y_4 \quad \text{Eq. (9)}$$

where $\mu_{max,1}$ and $\mu_{max,2}$ are the maximum specific growth rates (d^{-1}), $K_{s,1}$ and $K_{s,2}$ are the half-saturation constants ($g \cdot L^{-1}$), Y_1 and Y_3 are the biomass yield coefficients, and Y_2 and Y_4 are the product yield coefficients for acidogenic and methanogenic microorganisms, respectively.

Dynamic analysis

To analyze the temporal evolution of the model variables over a 15-day period, the system was solved numerically in MATLAB R2025b using the *ode15s* solver, which is suitable for stiff systems. The parameter values used in the simulations are presented in Table 1.

Table 1. Model parameters and their values.

Parameter	Value	Parameter	Value
μ_{max1}	1.5 d^{-1} [a]	Y_1	0.2 (-) [d]
μ_{max2}	0.138 d^{-1} [a]	Y_2	2.45 (-) [d]
K_{s1}	0.26 $g \cdot L^{-1}$ [a]	Y_3	0.06 (-) [d]
K_{s2}	0.003 $g \cdot L^{-1}$ [a]	Y_4	18.26 $g \cdot L^{-1}$ [d]
K_i	0.064 $g \cdot L^{-1}$ [b]	k_{d1}	0.01 μ_{max1} [b]
k_1	0.4 $g \cdot dm^{-2} \cdot d^{-1}$ [c]	k_{d2}	0.05 μ_{max2} [b]

a Parameter taken from Moletta et al. (1986).
b Values from Vian et al. (2024).
c Value reported by Vigueras-Carmona et al. (2025).
d Yield coefficients from Hill & Barth. (1977).

The analysis was conducted from two perspectives. First, the effect of particle size on the performance of the AD of FVW was evaluated. Simulations were performed using an initial total inoculum concentration of 2.5 g $VS \cdot L^{-1}$, consisting of 66% acidogenic and 34% methanogenic microorganisms (Razaviarani; Buchanan, 2015; Vian et al., 2024), and an initial substrate concentration of 7.5 g $VS \cdot L^{-1}$, resulting in an S/I ratio of 3. The particle size was varied by considering diameters of

0.5, 1, and 2 mm. The evaluated variables were cumulative methane production, soluble substrate concentration, and VFA concentration.

Second, the effect of the S/I ratio was evaluated. In this case, the particle diameter was fixed at 0.5 mm, and the total inoculum concentration was maintained at 2.5 g VS·L^{-1} (with the previously defined microbial distribution). The initial substrate concentration was varied (5, 7.5, and 10 g VS·L^{-1}), corresponding to S/I ratios of 2, 3, and 4, respectively. The same response variables as in the previous analysis were considered.

Operational scenarios

The combined effect of the particle size and S/I ratio was evaluated through two-dimensional parametric analysis. A simulation grid was defined in which the initial particle diameter ranged from 0.01 to 5 mm, while the initial S/I ratio was varied between 5 and 6.

The analysis was carried out in MATLAB by numerically solving the system of ordinary differential equations using the *ode15s* solver, which is suitable for stiff systems. For each combination of initial conditions, the system was integrated over a time interval of 0 to 10 days, using relative and absolute tolerances of 10^{-6} and 10^{-9}, respectively, to ensure numerical stability and solution accuracy.

Finally, the results were arranged into a response matrix to construct response surfaces, enabling the identification of optimal operating conditions. The response variable was the methane yield (mL·g^{-1} VS), defined as the ratio between the cumulative methane produced and the initial mass of substrate added.

RESULTS AND DISCUSSION

Effect of particle size on process dynamics

The effect of the initial particle size on the performance of the AD of the FVW is shown in Figure 1. The highest cumulative methane production was obtained at 1 mm (317 mL), followed by 2 mm (307 mL) and 0.5 mm (57 mL) (Figure 1a).

For larger particles, the lower methane production is attributed to a reduced substrate availability rate due to slower hydrolysis, resulting in more gradual methane accumulation. Therefore, although comparable methane yields were observed for particle sizes of 1 and 2 mm after 15 days, the 1 mm condition achieved its maximum production within the first 6 days, whereas for 2 mm particles, the maximum was reached on day 15. This finding indicates that the process proceeded at a faster rate for the 1 mm particle size. In contrast, the smallest particle size (0.5 mm) led to a sharp decrease in methane production after day 1, suggesting an imbalance between acidogenic and methanogenic activities.

This behavior is supported by the data in Figures 1b and 1c. At 0.5 mm, a rapid increase in soluble substrate is observed, followed by its rapid conversion into VFAs, reaching 3.5 g·L^{-1}. Under these conditions, the consumption of VFAs is limited, and their accumulation suggests a potential inhibitory

effect on methanogenic activity. Conversely, at larger particle sizes, lower substrate release rates result in reduced VFA production, allowing their efficient consumption and maintaining concentrations below 0.0035 $g \cdot L^{-1}$.

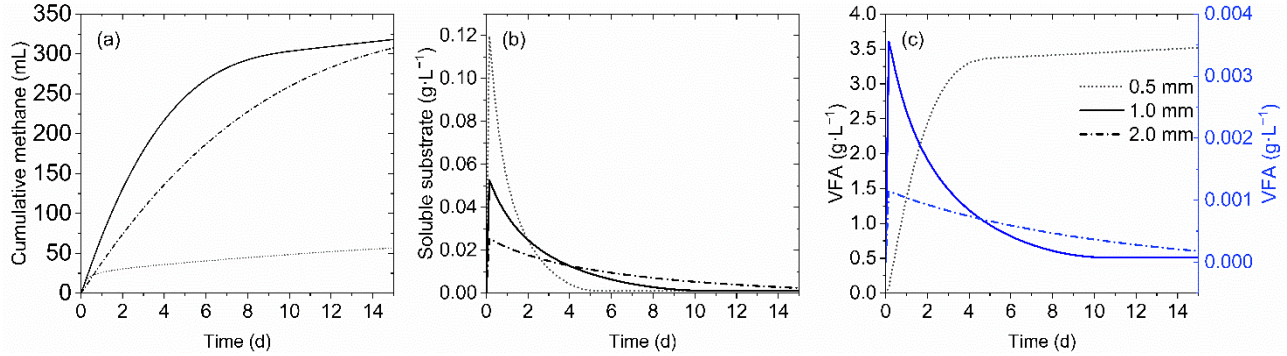


Figure 1. Effects of particle size on (a) cumulative methane production, (b) soluble substrate concentration, and (c) total VFA concentration at an S/I ratio of 3.

Overall, these results confirm that hydrolysis is the rate-limiting step (Vavilin et al., 2008). While larger particles slow down the process, smaller particles increase the risk of intermediate accumulation and process imbalance (Agyeman; Tao, 2014; Izumi et al., 2010; Vigueras-Carmona et al., 2025), with an optimum observed at intermediate particle sizes.

Effect of the S/I ratio on process dynamics

In addition, the effect of the initial substrate-to-inoculum (S/I) ratio was evaluated by comparing scenarios at constant particle size. As shown in Figure 2a, the highest cumulative methane production was obtained at an S/I ratio of 2 (223 mL), whereas significantly lower values were observed at higher S/I ratios (57 and 41 mL for S/I = 3 and 4, respectively). These findings indicate that increasing the S/I ratio negatively affects methane production.

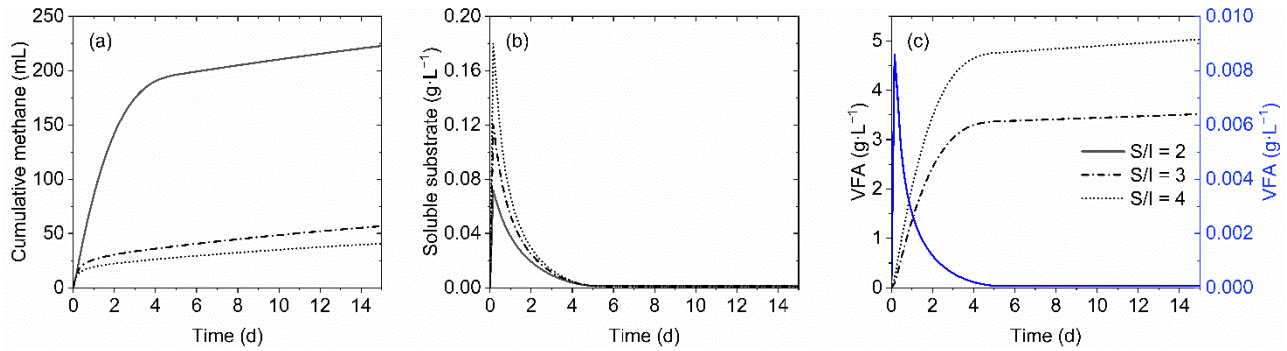


Figure 2. Effects of the S/I ratio on (a) cumulative methane production, (b) soluble substrate concentration, and (c) total VFA concentration for a particle size of 0.5 mm.

The presence of soluble substrate peaks within the first 6 days in all the cases is shown in Figure 2b, with slightly higher magnitudes at higher S/I ratios, indicating rapid substrate consumption by acidogenic microorganisms. However, as shown in Figure 2c, efficient VFA consumption is only observed at the lowest S/I ratio, where the VFA concentration remains below 0.01 $g \cdot L^{-1}$. In contrast, for higher S/I ratios, VFA accumulation reaches approximately 3.5 and 5 $g \cdot L^{-1}$ (for S/I = 2 and 3, respectively), suggesting an imbalance between acidogenic production and methanogenic consumption.

This behavior is consistent with previous studies on the anaerobic digestion of food waste, which reported that low S/I ratios increase methane production because of the relatively high abundance of microorganisms, providing an effective sink for metabolic intermediates (Okoro- Shekwaga et al., 2020; Zhang et al., 2019). Accordingly, S/I values less than 0.5 are often recommended for readily biodegradable substrates such as FVW, whereas values of approximately 1 are suggested for more slowly degradable materials (Assis; Gonçalves, 2022).

In this study, the simulation results suggest that stable operation may still be achieved under intermediate conditions (e.g., a particle size of 0.5 mm and S/I = 2), as indicated by the fact that most methane production occurs within the first 6 days (Figure 2a). This observation is consistent with previous reports indicating that up to 80% of methane production from fresh organic waste occurs during the initial days of digestion (Martínez et al., 2022).

Overall, these results highlight the critical importance of properly balancing the S/I ratio to prevent intermediate accumulation and ensure stable and efficient methane production.

Operational scenarios

The methane yield as a function of both the particle size and the S/I ratio are shown in Figure 3. A region of high performance (470–581 $mL \cdot g^{-1}$ VS) is observed within a particle size range of 0.75 to 2 mm across all evaluated S/I ratios. However, within this range, smaller particle sizes are associated with higher yields at lower S/I ratios, whereas larger particle sizes achieve higher yields at higher S/I ratios. This behavior can be explained by the need to avoid VFA accumulation at smaller particle sizes, whereas at larger particle sizes, higher S/I ratios help prevent limitations in soluble substrate availability and subsequent VFA production.

As observed and discussed in the previous sections, the model successfully captures both VFA accumulation effects and substrate availability limitations associated with particle size. The range of maximum methane yield predicted by the simulations lies at the upper end and is partially beyond the values reported in the literature for the anaerobic digestion of FVW (207–523 $mL \cdot g^{-1}$ VS) (Agrawal et al., 2022; Yan et al., 2021). Such variability in reported yields is attributed mainly to differences in substrate composition (Edwiges et al., 2018, 2020).

These results suggest that a more accurate prediction of methane yield would require calibration of the yield coefficients, as these parameters directly control biomass growth and product formation.

Nevertheless, the simplified model proved adequate for capturing the system dynamics and evaluating the effects of the studied factors.

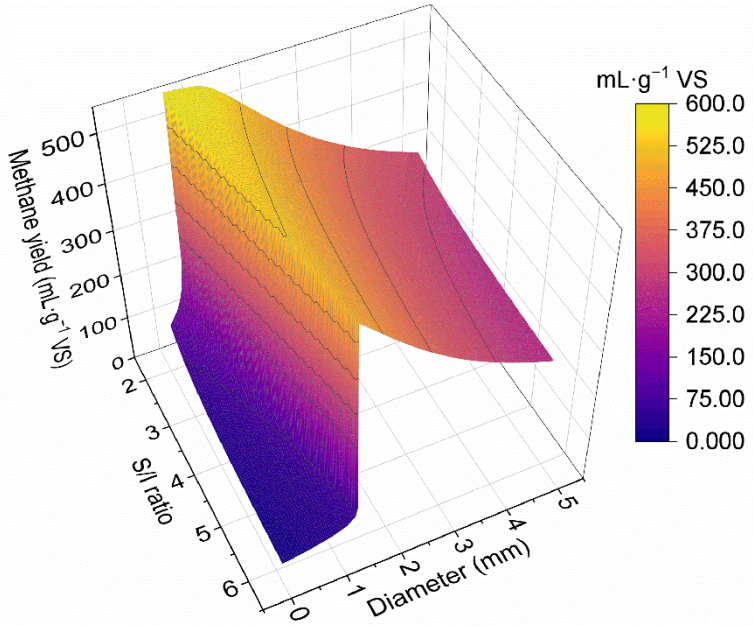


Figure 3. Effects of the S/I ratio and particle size on the methane yield during the anaerobic digestion of fruit and vegetable waste.

CONCLUSIONS

Through simulation analysis of the AD of FVW, it was determined that the methane yield is strongly influenced by the combined effect of particle size and the substrate-to-inoculum (S/I) ratio. The highest yields were obtained at intermediate particle sizes (0.75–2 mm), where a balance between substrate availability and microbial activity was achieved. Smaller particle sizes promote rapid hydrolysis and the accumulation of VFAs, which may lead to process imbalance, whereas larger particles limit methane production because of a lower substrate conversion rate.

Similarly, low S/I ratios favor stable conditions by ensuring sufficient microbial capacity to assimilate intermediate products, whereas higher ratios increase the risk of VFA accumulation. Although quantitative predictions require further parameter calibration, particularly because the model does not explicitly account for substrate composition, the simplified model proved effective at capturing key system dynamics and identifying favorable AD processes.

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Chapter 24

Impact of biofertilizers on wheat (*Triticum aestivum* L.) yield in different environments of the Bajío region of Guanajuato

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ABSTRACT

The excessive use of synthetic chemical fertilizers in wheat cultivation increases production costs, degrades the physical and chemical properties of the soil, and affects crop profitability. The objective of this study was to identify the effects of combining synthetic chemical fertilizers with biofertilizers on wheat yield, yield components, and physiotechnical variables (NDVI and SPAD values) under conditions in Bajío, Guanajuato, Mexico. The experiments were conducted at two groups of locations during the 2017–2018 fall–winter growing season. The first group consisted of four locations, and the second consisted of two locations. Two microbial compounds were evaluated: 1) the first formulated on the basis of *Azospirillum brasilense* + a consortium of the arbuscular fungus *Rhizophagus irregularis* (BIO1) and 2) the second on the basis of *Streptomyces* spp., *Bacillus thuringiensis, Trichoderma longibrachiatum, Pseudomonas, Mycorrhiza*, and *Azospirillum* (BIO2). In the first group of locations, the following treatments were evaluated: a) application of 100% NPK chemical fertilizer (280–98–20); b) application of 75% NPK chemical fertilizer (210–74–15); c) application of 75% NPK chemical fertilizer + BIO1; d) application of 75% NPK chemical fertilizer + BIO2; and e) a control (no application of chemical fertilizer or biofertilizer). In the second group of locations, the following treatments were evaluated: a) application of 100% of the NPK chemical fertilizer dose (280–98–20); b) application of 50% of the NPK chemical

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fertilizer dose (140–49–10); c) application of 50% of the NPK chemical fertilizer dose + BIO1; d) application of 50% of the NPK chemical fertilizer dose + BIO2; and e) the control plot. In the first group of locations, the results of the SREG analysis revealed statistically significant differences because of the interaction between environments and treatments, where treatments explained 97.5% of the variance in grain yield, whereas locations accounted for 1.64%. Two contrasting response patterns were observed, which were associated mainly with the environmental conditions at each location. In terms of the first response pattern, the yield was greater at the three sites; the yield was particularly enhanced by 100% in response to chemical fertilization and the two types of biofertilizers. A second response pattern was observed at a single location, which was situated in a different sector from the rest of the sites and where the treatment with 75% chemical fertilization yielded the highest results. In general, the treatments with the highest yields were the treatments with 100% chemical fertilization (6.6 t ha-1) and those with 75% chemical fertilization supplemented with BIO2 and BIO1 (6.4 and 6.5 t ha^{-1}, respectively). In the second group of sites, fertilization with 50% chemical fertilizer was evaluated; however, no significant interaction was observed. The BIO2 + 50% chemical fertilizer treatment performed statistically the same as the 100% treatment and better than the 50% chemical fertilizer treatment did.

Keywords: interactions between locations and treatments, microbial compounds, synthetic chemical fertilization.

INTRODUCTION

Fertilizers significantly increase global food production. Research shows that between 30% and 50% of crop yields are attributed to fertilizer application (Yan et al., 2022). This contribution explains why many farmers believe that applying more fertilizer will lead to higher yields (Verhulst et al., 2010). In the Bajío region of Guanajuato, over the past ten years, an average annual production of 318,988.43 t has been achieved, representing 10% of national production (SIAP, 2026). A large proportion of producers in the region make excessive use of inorganic fertilizers, which increases production costs (Gonzales-Figueroa et al., 2022). The use of excessive doses of inorganic fertilizer contributes to environmental impacts that harm the physical, chemical, and biological properties of the soil (Orosco-Corral et al., 2016). The amount of fertilizer applied per unit of cultivated land has increased nearly eightfold since 1961 (Lu; Tian, 2017), and excessive nitrogen input has led to significant losses in the soil through ammonia (NH3) volatilization, nitrous oxide emissions, surface runoff, and nitrate leaching (Zhou et al., 2016; Huddell et al., 2020). Therefore, it is necessary to utilize technological innovations that help reduce environmental impact as well as lower production costs. One alternative is the use of biofertilizers made from bacteria or fungi, which enable plants to acquire nutrients to improve their growth and yield (Aghili et al., 2014). The term "biofertilizer" has a very broad interpretation, encompassing the effects of microorganisms, green manure, manure, and even plant extracts containing microorganisms. When inoculated, these

agents can live in association or symbiotically with plants, aiding in nutrient assimilation, protection, and increased yield across multiple crops (Nagrale et al., 2023). The objective of this chapter was to identify the effects of the use of inorganic fertilizers combined with biofertilizers on wheat yield, yield components, and physiotechnical variables (NDVI and SPAD values) in the Guanajuato Bajío region.

MATERIALS AND METHODS

Study sites

The evaluation was conducted in five municipalities within the Bajío Guanajuatense region. The evaluation sites were plots of land belonging to cooperating farmers who participated in the MasAgro Guanajuato Project. During the 2017–2018 fall–winter growing season, trials were conducted at various locations within the municipalities of Irapuato (20°30'33"N, 101°17'46"W, 1,730 m), Pénjamo (20°18'53'N, 101°50'09'W, 1,689 m), Salamanca (20°30'31.1868'N, 101°13'27.066"W, 1,721 m), Yuriria (20°20'72.059"N, -101°16'79.928"W, 1,776 m) and Villagrán (20°16'52"N, 101°10'04"W, 1,726 m).

Soil sampling and conditions

Prior to setting up each experiment, soil samples were collected using the "zigzag" method, with 15 subsamples taken to a depth of 30 cm. The subsamples were mixed to obtain a 1-kilogram composite sample, which was sent to the FERTILAB laboratory in the city of Celaya, Guanajuato. In general, the soils are characterized as vertisols with a clay texture; the organic matter concentration ranges from low to moderately high, the chemical reaction is moderately alkaline (with the exception of Irapuato), and the nitrate concentration ranges from low to moderately high; the phosphorus concentration is extremely high except in Villagrán and Yuriria, and the potassium concentration ranges from moderate to very high. The remaining soil fertility parameters are shown in Table 1.

Table 1. Soil fertility parameters of the evaluation sites in the autumn–winter cycle from 2017–2018 in the Bajío region, State of Guanajuato, Mexico.

Site	pH	NO3 (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	POM (%)	HC (cm/h)	PSI (%)	PTC (%)
Irapuato	6.4	14.8	98	622.8	3.2	0.4	3	0
Pénjamo	7.6	4.4	66.5	793.8	3.2	0.1	6.7	2
Salamanca	8	22.5	95.8	1179.3	3.2	0.1	18	4.6
Villagrán	7.7	6.2	41.5	578.2	1.3	0.5	4	2.3
Yuriria	8	3.9	2	699	1.4	0.2	6.7	2.2

pH = Hydrogen potential; NO3 = Nitrates; P = Phosphorus; K = Potassium; POM %= Percentage of organic matter; HC= Hydraulic conductivity; ESP = Exchangeable sodium percentage; PTC % = Percentage of total carbonates.

Soil management

Conservation agriculture (CA) was implemented in the Irapuato, Villagrán, and Salamanca experiments in 2013, as well as in Pénjamo and Yuriria in 2014. Planting was carried out in 150 cm wide permanent raised beds, each with six planting rows spaced 15 cm apart. Before planting, the beds were reshaped by reworking the furrows. The crop rotation at the evaluation sites consisted of corn during the spring–summer cycle and wheat during the fall–winter cycle. On average, 13.2 t ha^{-1} of corn residue and 7.15 t ha^{-1} of wheat residue were produced per cycle. All of this crop residue was left on the soil surface.

Weather conditions

The average maximum, minimum, and mean temperatures at the monitoring sites were 26.6 °C, 8.2 °C, and 17.2 °C, respectively (Table 2). The cumulative precipitation during the cycle was 25.5 mm, and the cumulative solar radiation was 2494.4 Mj m^{-2}.

Table 2. Climate conditions at the evaluation sites during the 2017–2018 fall–winter cycle in the Bajío region, State of Guanajuato, Mexico.

Site	Temperature (°C)			Accumulated precipitation (mm)	Accumulated solar radiation (Mj m^{-2})
	Maximum	Minimum	Mean		
Irapuato	27.6	8.3	18.0	19.6	2530.1
Salamanca	26.9	8.0	17.4	2.2	2754.1
Pénjamo	25.9	8.4	17.1	20.8	2299.1
Yuriria	26.6	5.4	16.0	51.8	2305.5
Villagrán	26.2	10.9	18.6	33.2	2583.0

Experimental design

A completely randomized block design with three replicates was used. The experimental unit (EU) had an area of 24 m^2 and consisted of three beds that were 1.6 m wide and 5 m long. Two microbial mixtures were evaluated: 1) a mixture based on *Azozpirillum brasilense* + a consortium of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* (BIO1) and 2) a mixture based on *Streptomyces* spp., *Bacillus thuringiensis*, *Trichoderma longibrachiatum*, *Pseudomonas*, mycorrhizal fungi, and *Azospirillum* (BIO2, CreCR®) (Tierra del Monte®, Querétaro, Mexico). The microbial mixtures were evaluated under the following treatments: a) application of 100% NPK chemical fertilizer (280–98–20); b) application of 75% NPK chemical fertilizer (210–74–15); c) application of 75% NPK chemical fertilizer + BIO1; d) application of 75% NPK chemical fertilizer + BIO2; and e) a control (no application of chemical fertilizer or biofertilizer). In the localities of Yuriria and Villagrán, the following were evaluated: a) application of 100% NPK chemical fertilizer (280–98–20); b) application of 50% NPK chemical fertilizer (140–49–10); c) application of 50% NPK chemical fertilizer + BIO1; d) application of 50% NPK chemical fertilizer + BIO2; and e) the control treatment.

The arbuscular mycorrhizal consortium (BIO1) consists of *Glomus dimorphicum, Glomus monosporum, Rhizophagus proliferus, Rhizophagus fasciculatus, Funneliformis mosseae*, and *Scutellospora cerradensis*, formulated as a soil-like granule, and *Azospirillum brasilense*, formulated as a granular inoculant. The microbial compound (BIO2) consists of *Streptomyces* spp., *Bacillus thuringiensis, Trichoderma longibrachiatum, Pseudomonas*, mycorrhizal fungi, and *Azospirillum*, formulated as a powdered suspension.

Management of treatments using microorganisms

Treatment BIO1: Inoculation was performed prior to sowing. A wetting agent solution (water + wetting agent contained within the product) was prepared and poured over the seed; subsequently, *Azospirillum brasilense* and the arbuscular mycorrhizal consortium were added to the seed and mixed until the biofertilizer dose adhered to the seed. A foliar booster application of the same mixture was performed after the second supplementary irrigation (Table 3).

BIO2: The first application was made 15 days after planting and was applied as a drench, targeting the base of the plant. A second application was made 60 to 70 days after planting, also as a drench.

Table 3. Method and dose of application of treatments at the evaluation sites in the autumn–winter cycle 2017–2018 in the Bajío region, State of Guanajuato, Mexico.

Treatments	Application mode	Dose	Water (L)
mycorrhizal fungi + *Azospirillum brasilense* (BIO1)	Inoculation	1 kg of mycorrhizal fungi+ 380 gr of *Azospirillum brasilense* ha^{-1}	12
mycorrhizal fungi + *Azospirillum brasilense* (BIO1)	Drench	3 kg of mycorrhizal fungi + 1 L of *Azospirillum brasilense* ha^{-1}	200
Microbial formulation (BIO2)	Drench	1 kg ha^{-1}	200

FERTILIZATION MANAGEMENT

The fertilizer application rate ranged from 280–98–20 (NPK). The fertilizer sources were ammonium sulfate [$(NH_4)2SO_4$ (20.5-0-0)], MAP [$NH_4H_2PO_4$ (11-52-0)], and potassium chloride [KCl (0-0-60)]. The fertilizer was applied manually and incorporated using a hoe, which was then covered with the same soil.

Test run

Sowing was carried out dry; the seed was placed by hand at a seeding rate of 130 kg ha^{-1}. The genetic material used was the wheat variety Cortazar S94. Weed control was achieved by applying 30 g ha^{-1} of sodium flucarbazone 30 days after sowing. Pest control was carried out by applying 300 ml ha^{-1}

imidacloprid at stage Z4.3 to control aphids [*Diuraphis noxia* (Mordvilko)]. To prevent yellow rust (*Puccinia striiformis* f. sp. tritici), 1 L ha^{-1} tebuconazole was applied at the Z4.3 stage.

Variables evaluated

Harvesting was conducted when the grain reached physiological maturity. All spikes were manually harvested from a plot measuring 1.5 m wide by 2 m long. A one-meter-long section of the remaining experimental plot was selected to cut the plants at ground level; 100 stems were randomly selected to estimate yield components (Pietragalla; Pask, 2013). The stems were dried to constant weight in an oven at a temperature of 80 °C and subsequently threshed in a single-plant threshing machine. The grain yield was adjusted to 12% moisture content (yield, kg ha^{-1}); grains per square meter (Grains, m^{-2}); ears per square meter (Ears m^{-2}); grains per ear (Grains ear^{-1}); thousand-grain weight (TGW, g); harvest index (HI); biomass (Biomass, t ha^{-1}); and grain protein content (PCG, %). In addition, the plant height (cm) and SPAD® value (Soil and Plant Analysis) were evaluated at 57, 64, and 86 days after sowing (das, SPAD57, SPAD64, and SPAD6, respectively). The SPAD value was obtained using a SPAD-502 Plus optical sensor (Konica Minolta Inc., Tokyo, Japan). Ten readings were taken per experimental unit in the middle adaxial zone of the last ligulate leaf of the measured plants. The normalized difference vegetation index (NDVI) values were recorded at 39, 57, and 86 DAS. The NDVI value was obtained using the Green Seeker® optical sensor. Two readings were taken along the central furrows of each experimental unit.

Statistical analyses

The data were subjected to a combined analysis of variance, and tests were conducted to compare means using the least significant difference (LSD, $P \leq 0.05$). Principal component analyses were performed to reduce the dimensionality of the original set of variables (Greenacre et al., 2022); these analyses were conducted using SAS 9.3 software (SAS Institute, 2002). Treatment*environment interaction analysis (IGA) for yield was used to identify the response to treatments across environments (Alvarado et al., 2015), for which the site regression model (SREG) was employed. A stability analysis was conducted for grain yield across locations to identify the yield response to each treatment and across locations (Dyke et al., 1995).

RESULTS AND DISCUSSION

Evaluations with 75% chemical fertilization at four locations

Analysis of variance

Statistical analysis (Tables 4 and 5) revealed that the variability in yield and yield components generated by each study site significantly differed across locations and treatments, except for grain weight per 1,000 grains and the harvest index. With respect to the physio-technical variables, significant

differences were found across locations for the NDVI 39 and the NDVI 86, as well as for the SPAD 64 and the SPAD 86. Furthermore, there was a significant interaction between location and treatment for yield and the NDVI 39, indicating that the behavior of these two variables differed among the evaluation sites. In terms of yield, the treatments accounted for 63% of the total variance, while the evaluation environments accounted for 14%. These findings indicate that the effects of the treatments were more significant than those of the evaluation environments were.

Treatments

When chemical fertilization was reduced to 25%, a statistically significant 5.2% decrease in yield was observed (Figure 1). However, the treatments with 75% chemical fertilization and the addition of biofertilizer (BIO1 and BIO2) performed statistically the same as the previous treatments did. In other words, the incorporation of microorganisms tended to improve yield despite the overall reduction in fertilization. On the other hand, omitting fertilization during the growing cycle resulted in a significant 42.4% reduction in production compared with the maximum dose evaluated during the study.

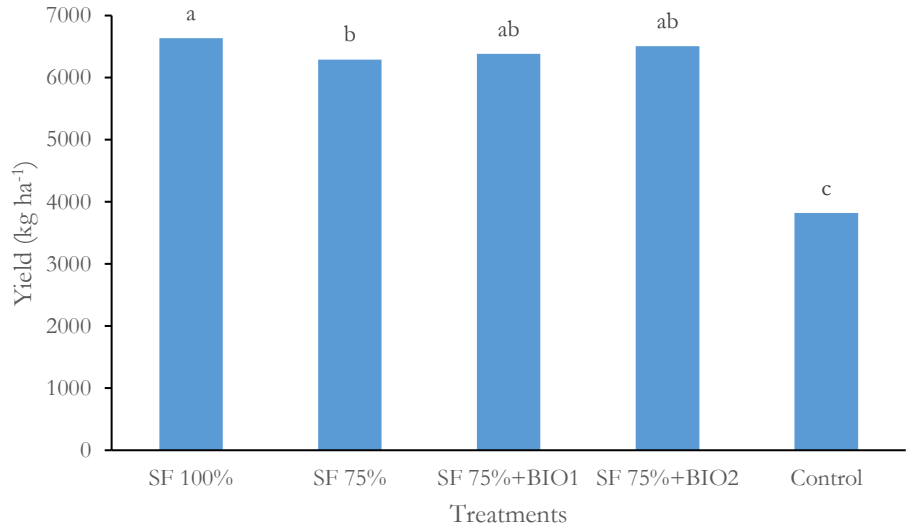


Figure 1. Wheat grain yield of treatments with synthetic fertilization and biofertilizers in four locations in the Bajío region, State of Guanajuato, Mexico. SF: synthetic fertilization; BIO1: microbial formulation 1; BIO2: microbial formulation 2.

Table 4. Mean squares of the yield variables, i.e., Grains m^{-2}, Ears m^{-2}, Grains ear^{-1}, TGW, HI and Biomass, in treatments with a 75% reduction in chemical fertilization at four locations in the Bajío region, State of Guanajuato, Mexico.

Source of variation	DF	Yield	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}	TGW	HI	Biomass
Sites	3	3839013.15**	8049355.08**	18680.167*	376.1**	55.381	0.0066	18.569**
Blocks (Blk)	2	5513680.53**	15899788.7**	14631.875*	0.686	11.204	0.0054	24.648**
Blk/Site	6	52717.56	432597.8	2537.16	20.859	17.998	0.0025	0.916
Treatments (Treats)	4	16843569.43**	66598368.6**	9772.644*	285.228**	19.618	0.0056	52.336**
Treats*Sites	12	429255.64**	1577984.5	14631.875	13.342	11.204	0.0031	2.099
Residual error	32	162026	1850308.6	3378.697	18.3	25.732	0.0036	1.744
CV Treatments (%)		6.8	12.5	17.2	13.1	9.7	12	11.8
CV Sites (%)		3.9	6.1	14.9	13.9	8.1	9.9	8.5

DF: degrees of freedom; Yield: grain yield; Grains m^{-2}: grains per square meter; Ears m^{-2}: ears per square meter; Grains ear^{-1}: grains per ear; TGW: thousand-grain weight; HI: harvest index; * and **: significant differences with $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 5. Mean squares of the variables NDVI at 39, 57 and 86 days after sowing (das) and SPAD values at 57, 64 and 86 DAS in treatments with a 75% reduction in chemical fertilization in the Bajío region, State of Guanajuato, Mexico.

Source of variation	DF	NDVI 39 das	NDVI 57 das	NDVI 86 das	SPAD 57 das	SPAD 64 das	SPAD 86 das
Sites	3	0.38315819**	0.00728222	0.02859278**	39.6626667	35.5237778*	201.6575556*
Blocks (Blk)	2	0.00121625	0.001185	0.00018667	3.9161667	4.322	27.6551667
Blk/Sites	6	0.00272069	0.00286722	0.00171111	10.6801667	4.2237778	23.0473889
Treatments (Treats)	4	0.03061729**	0.08228917**	0.0593875**	126.7139167**	128.0179167**	176.73725**
Treats * Sites	12	0.0077634*	0.00088917	0.00079972	3.9178056	8.9539167	9.5365833
Residual error	32	0.00336281	0.00139667	0.00233417	3.9583333	9.449792	9.160375
CV Treatments (%)		9.6	4.7	7.2	4.4	6.6	6.3
CV Sites (%)		8.6	6.7	6.2	7.2	4.4	10

DF: degrees of freedom; Yield: grain yield; Grains m^{-2}: grains per square meter; Ears m^{-2}: ears per square meter; Grains ear^{-1}: grains per ear; TGW: thousand-grain weight; HI: harvest index; * and **: significant differences with $P \leq 0.05$ and $P \leq 0.01$, respectively.

Principal component analysis

Locations

The responses of all the variables evaluated across the four locations are shown in Figure 2. The first two principal components explained 87.8% of the total variance contributed by the thirteen variables. Using the eigenvectors of each principal component (CP) revealed that the most important variables for CP1 were the GPMC, GPE, and NDVI at 57 and 86 days and that for CP2, the most important variables were the Mil, NDVI at 39 days, yield, and EPMC. Therefore, (Figure 3) Pénjamo stood out for having higher GPMC, GPE, and NDVI values at 57 and 86 days. Moreover, Villagrán stood out for having higher Mil and NDVI values at 39 days and EPMC.

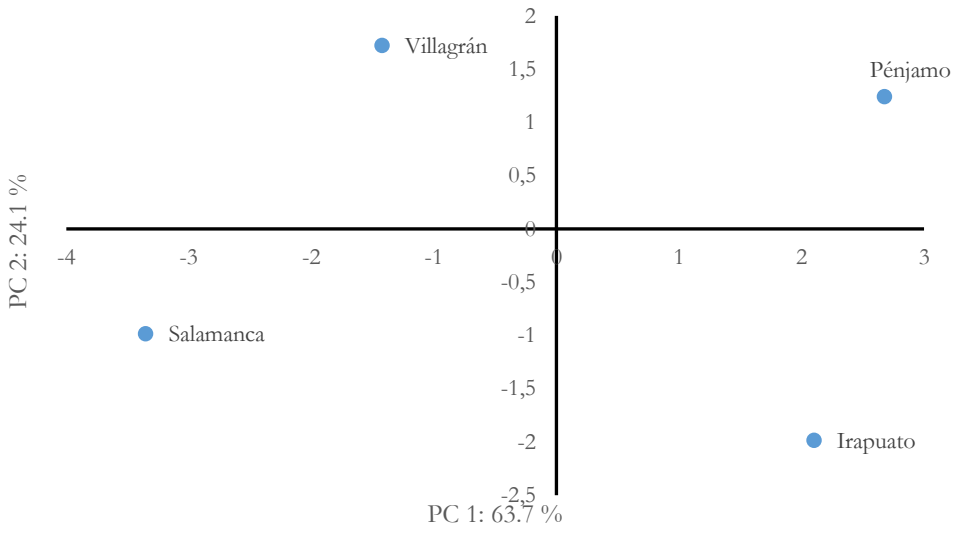


Figure 2. Principal component analysis for the four wheat assessment locations in the Bajío region, State of Guanajuato, Mexico. PC 1 and 2: principal components 1 and 2.

Treatments

The responses of the 13 variables evaluated under the four environmental conditions, as well as the dispersion of the treatments, are shown in Figures 3 and 4. The analysis revealed that the first and second principal components together explained 92.25% of the total variance contributed by the 13 variables studied. The behavior of the variables within the principal components is shown in Figure 3. The variables Mil, IC, and EPMC are more dispersed within the quadrants, indicating that their importance is lower than that of BIO, GPMC, GPE, and the physicochemical variables (NDVI and SPAD). These same variables were distributed within an angle of less than 45°, so their responses were similar, indicating that each exhibited high interdependence because of the magnitude and direction of their vectors with grain yield. Therefore,

when the characteristic vectors of each principal component (CP) were used, most variables, with the exception of IC and EPMC, were the most important for CP1, whereas for CP2, it was IC; however, the same analysis allows us to identify that this component—and thus, the dispersion of IC—was not particularly significant (0.05%).

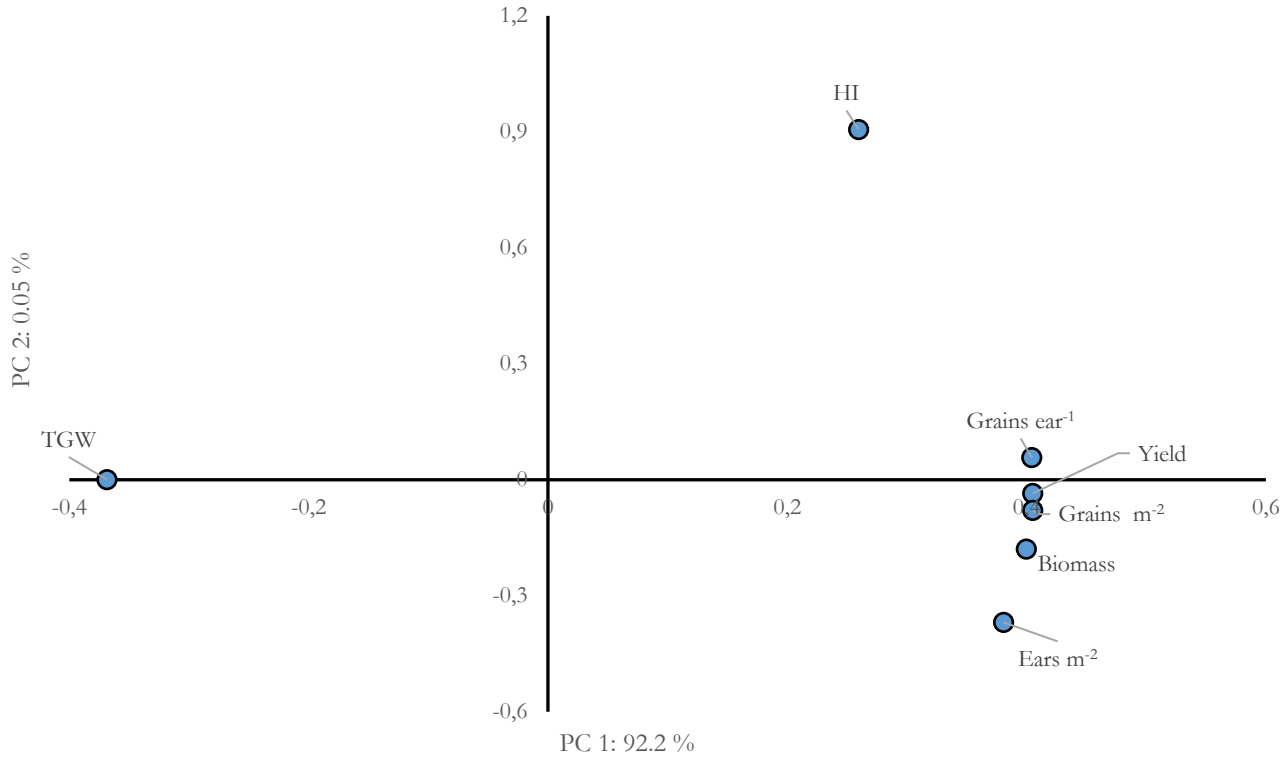


Figure 1. Eigenvectors of the variables evaluated at four locations in the Bajío region, state of Guanajuato, Mexico. PC 1 and 2: Principal component 1 and 2; Yield: grain yield; Grains m^{-2}: grains per square meter; Ears m^{-2}: ears per square meter; Grains ear^{-1}: grains per ear; TGW: thousand-grain weight; HI: Harvest index.

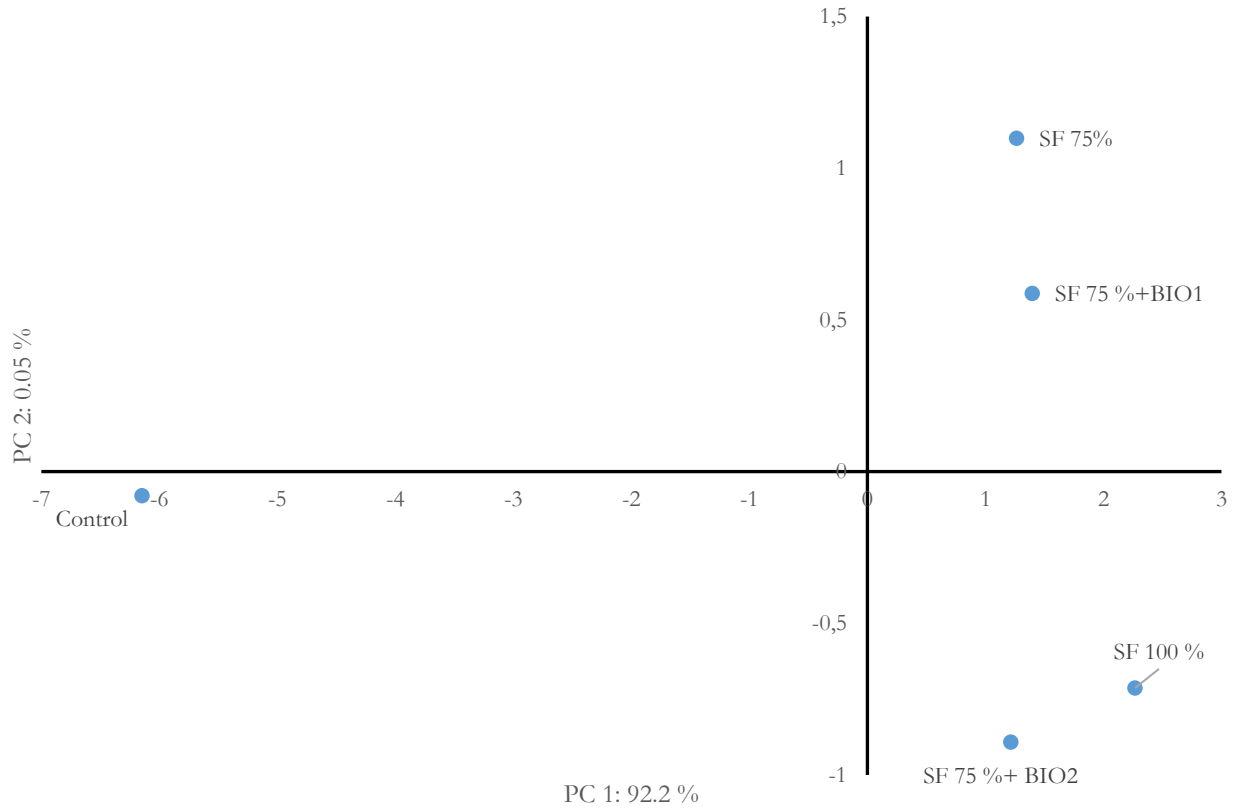


Figure 4. Dispersion analysis of synthetic and biofertilizer fertilization treatments in the Bajío region, State of Guanajuato, Mexico. PC 1 and 2: Principal component 1 and 2; SF 100%: Synthetic fertilization 100%; SF 75%: Synthetic fertilization 75%; SF 75%+BIO1: Synthetic fertilization + Microbial formulation 1; SF75%+BIO2: Synthetic fertilization + Microbial formulation 2.

Interaction between treatments and the environment

As shown in Figure 5, of the total variance observed in the yield data, the first factor—or principal component—related to the treatments explained 97.5%, and the second factor, related to the evaluation environments, explained 1.64%; thus, the SREG model explained almost all of the variation in the treatments and environment (99.1%). Three main sectors were observed, one of which included all the locations except Pénjamo, which was located in another sector of the Cartesian plane. The soil conditions of the first group of locations were characterized by a high percentage of organic matter in two locations (Irapuato and Salamanca), whereas in Villagrán, it was low. The soil pH in these locations was moderately alkaline, unlike that in Irapuato, which was moderately acidic. The phosphorus supply was high except in Villagrán, whose level was moderately low. In all three cases, the potassium supply was moderately high. The salinity percentage was low at two locations (Salamanca and Villagrán), while Irapuato was free of salinity. In terms of the percentage of sodium saturation, two locations had a level less than 5% (Villagrán and Irapuato), whereas Salamanca had a level of 18%.

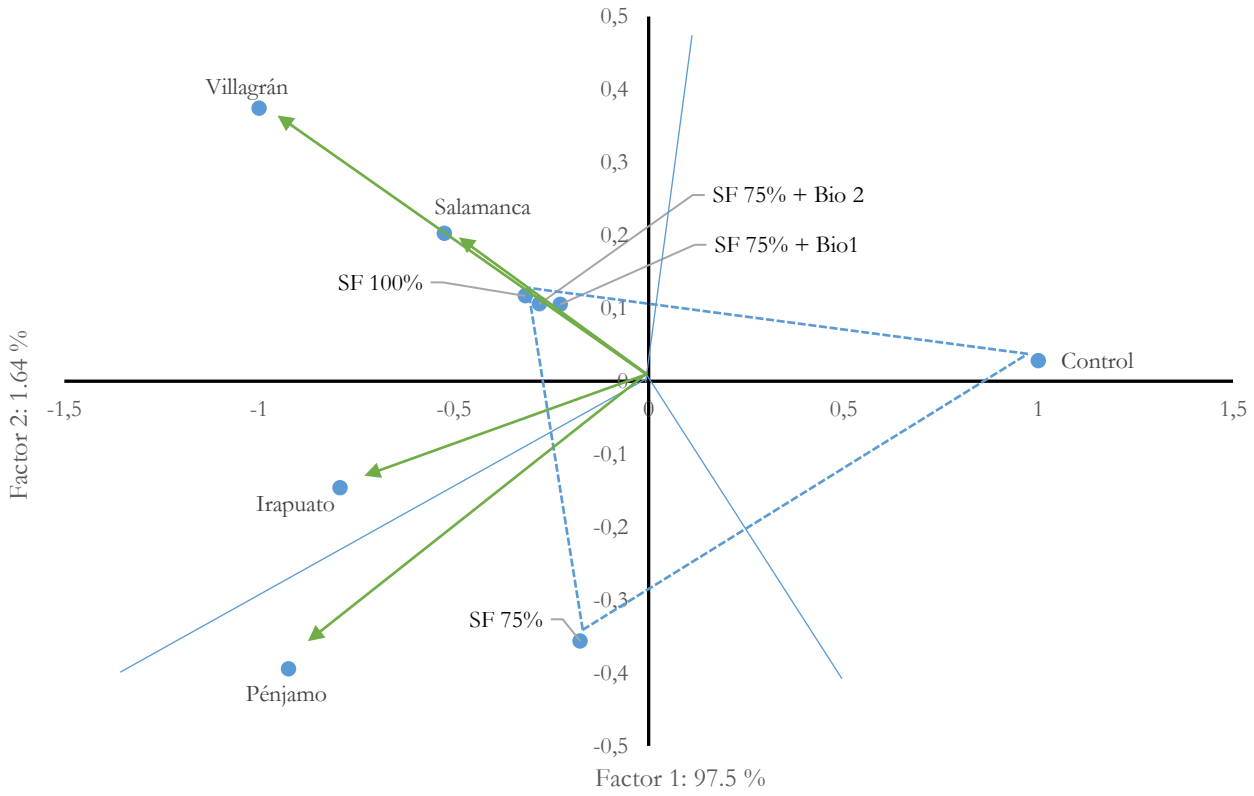


Figure 5. Treatment * environment interaction analysis for wheat yield at four locations in the Bajío region, State of Guanajuato, Mexico. SF 100% and 75%: synthetic fertilization at 100% and 75%, respectively; SF 75%+BIO1: synthetic fertilization at 75%+ microbial formulation 1; and SF 75%+BIO2: synthetic fertilization at 75%+ microbial formulation 2.

On the Cartesian plane, the treatments with 100% chemical fertilization and the two with biofertilizers favored yield performance in this set of locations. On the other hand, the location of Pénjamo was situated in a different sector from the other locations, where the yield of the treatment with 75% chemical fertilization performed best. Climate conditions may be key to understanding these results, as compared with the other locations, Pénjamo had the lowest maximum temperature, lower average temperature and radiation, and the lowest accumulated radiation (Table 2). Among the locations, those with the highest yield on the basis of the length of their vectors were Villagrán and Pénjamo. The treatments with the highest yields were the treatments with 100% chemical fertilization, as well as those with 75% chemical fertilization supplemented with BIO2 and BIO1.

Stability analysis

Although the control treatment was the most stable, it also had the lowest yield compared with all the other treatments and evaluation sites (Figure 6). On the other hand, compared with the regression line, the treatments with 100% chemical fertilization and both treatments incorporating biofertilizer exhibited similar behavior; consequently, their stability across the evaluation sites was similar in terms of regression deviations and higher yields.

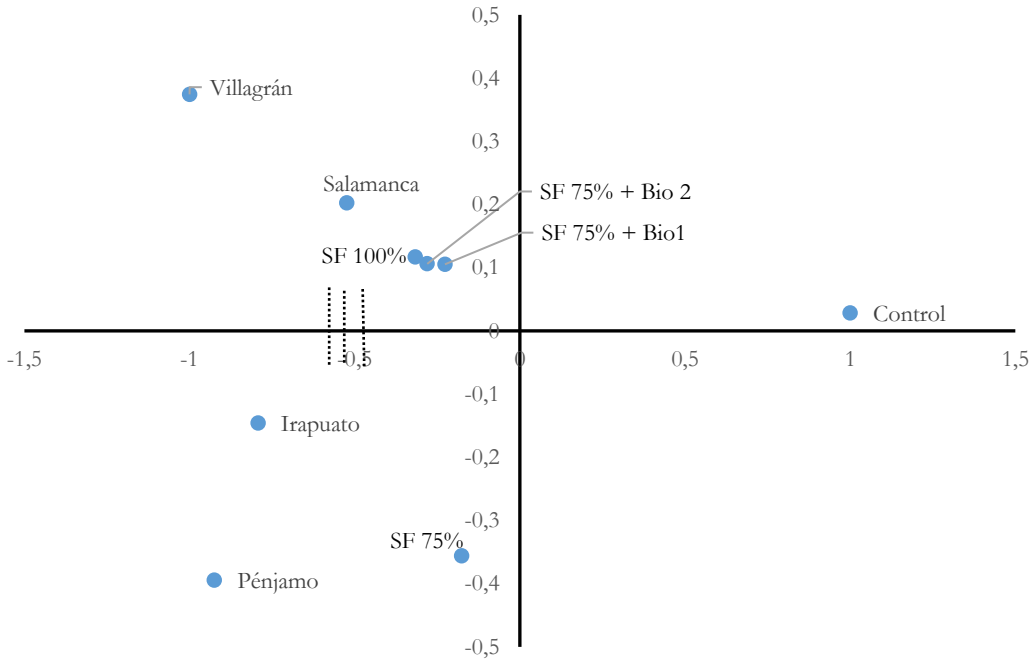


Figure 6. Performance stability analysis in biofertilizer evaluation environments for wheat yield at four locations in the Bajío region, State of Guanajuato, Mexico. SF 100% and 75%: synthetic fertilization at 100% and 75%, respectively; SF 75%+BIO1: synthetic fertilization at 75%+ microbial formulation 1; and SF 75%+BIO2: synthetic fertilization at 75%+ microbial formulation 2.

The treatment with the greatest instability was the 75% chemical fertilization treatment; this means that its performance across the sites is unpredictable, which is likely due to the different soil fertility conditions at the sites. In terms of the sites, Irapuato was the site with the lowest regression deviation, while Pénjamo and Villagrán exhibited contrasting and somewhat unpredictable performance.

Evaluations using 50% chemical fertilizer at two locations.

Analysis of variance

Among the locations (Table 6), significant differences were observed in yield and its components, with the exception of the numbers of GMC, GPE, and IC, whereas for the physicochemical variables (Table 7), significant differences were observed among the locations for all the variables, with the exception of SPAD at 42 and 57 days after sowing. For the treatments, statistical significance was observed for all the evaluated variables except for the IC (Table 6). With respect to the interaction between treatments and study sites, no significant differences were observed except for the NDVI at 57 days after sowing; therefore, the response of the treatments across locations was similar.

Treatments

The results of the multiple comparison test for means (least significant difference) revealed (Tables 8 and 9) that the grain yield was significantly greater when 100% chemical fertilizer was applied than when 50% was applied (Figure 7); however, when BIO2 was added to this fertilization rate (Tables 8 and 9), the yield was statistically the same as that in the 100% treatment and was greater than that in the 50% chemical fertilization treatment.

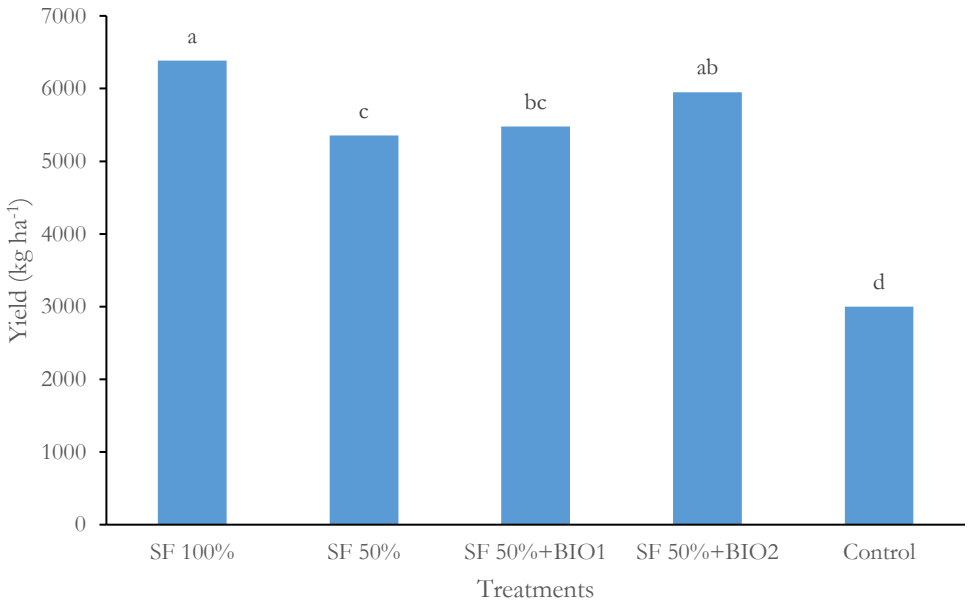


Figure 7. Wheat grain yield in response to chemical fertilization and biofertilizer treatments at two locations in the Bajío region, State of Guanajuato, Mexico. SF: synthetic fertilization; BIO1: microbial formulation 1; BIO2: microbial formulation 2.

Table 6. Mean squares of the yield variables, i.e., Grains m^{-2}, Ears m^{-2}, Grains ear^{-1}, TGW, HI and Biomass, in treatments with a 50% reduction in chemical fertilization at two locations in the Bajío region, State of Guanajuato, Mexico.

Source of variation	DF	Yield	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}	TGW	HI	Biomass
Sites	1	7090740.833**	3714374.907	42007.692*	131.8803333	246.5333333**	0.00147	16.1773633*
Blocks (Blk)	2	454343.09	1823490.5	2530.489	31.759	13.075	0.00013	0.7235833
Blk/Sites	2	314629.49	1064684.3	881.239	27.0223333	0.0583333	0.00063	0.4425633
Treatments (Treats)	4	10345878.06**	1064684.3**	10083.20417*	136.4553333**	6.575*	0.00038	37.79480**
Treats*Sites	4	154752.08	656876.8	1498.3245	15.0086667	3.9083333	0.00155333	2.484955
Residual error	16	218105.51	596164	3266.9636	9.664	2.0979167	0.00108417	1.2557025
CV Treatments (%)		8.9	8.1	17.7	10.5	2.8	6.7	11.1
CV Sites (%)		10.7	10.9	9.2	17.6	0.5	5.1	6.6

DF: degrees of freedom; Yield: grain yield; Grains m^{-2}: grains per square meter; Ears m^{-2}: ears per square meter; Grains ear^{-1}: grains per ear; TGW: thousand-grain weight; HI: harvest index; * and **: significant differences with $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 7. Mean squares of the NDVI and SPAD values measured at 42, 59 and 94 days after sowing (das) in treatments with a 50% reduction in chemical fertilization at two locations in the Bajío region, State of Guanajuato, Mexico.

Source of variation	DF	NDVI 42 das	NDVI 59 das	NDVI 94 das	SPAD 42 das	SPAD 59 das	SPAD 94 das
Sites	1	0.83000333**	0.08008333*	0.22360333**	11.163	6.075	769.1203333*
Blocks (Blk)	2	0.00036333	0.00624333	0.00175	1.8253333	1.393	6.4543333
Blk/Sites	2	0.00996333	0.00384333	0.00196333	4.404	6.871	14.2263333
Treatments (Treats)	4	0.04727167**	0.09378833**	0.03496167**	102.5211667**	41.0555*	163.9353333**
Treats*Sites	4	0.001045	0.03704167**	0.00192833	11.9871667	7.7058333	27.3853333
Residual error	16	0.00308833	0.0068475	0.002715	7.0784167	12.5261667	16.655333
CV Treatments (%)		9.5	11.4	9.7	6.0	7.8	9.7
CV Sites (%)		17.1	8.5	8.3	4.8	5.8	9.0

DF: degrees of freedom; Yield: grain yield; Grains m^{-2}: grains per square meter; Ears m^{-2}: ears per square meter; Grains ear^{-1}: grains per ear; TGW: thousand-grain weight; HI: harvest index; * and **: significant differences with $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 8. Comparison of the means of the minimum significant difference for yield, Grains m^{-2}, Ears m^{-2}, Grains ear^{-1}, TGW, HI and biomass in treatments with a 50% reduction in chemical fertilization at two locations in the Bajío region, State of Guanajuato, Mexico.

Treatments	Yield (kg ha^{-1})	Grains m^{-2} (No.)	Ears m^{-2} (No.)	Grains ear^{-1} GPE (No.)	TGW (g)	HI	Biomass (t ha^{-1})
SF 100%	6384.7 a	11891.4 a	365.02 a	33.467 a	50.75 b	0.48833	12.3683 a
SF 50%	5352.8 c	9556.9 c	323.63 ab	30.817 a	52.9167 a	0.48833	10.545 b
SF 50%+BIO1	5476.3 bc	9702.7 c	319.32 ab	30.767 a	53.5 a	0.505	10.3133 b
SF 50%+BIO2	5950.8 ab	10843.8 b	346 a	31.533 a	51.9167 ab	0.48833	11.515 ab
Control	3000.9 d	5403.2 d	256.53 b	21.267 b	52.4167 ab	0.485	5.88 c
DMS	430	143	4.1	0.03	0.4		0.23

DMS: least significant difference; means with the same letters per column are not significantly different (LSD, $P \leq 0.05$); Yield: grain yield; Grains m^{-2}: grains per square meter; Ears m^{-2}: ears per square meter; Grains ear^{-1}: grains per ear; TGW: thousand-grain weight; HI: harvest index.

Table 9. Comparison of means using the minimum significant difference for the NDVI and SPAD values at 42, 59 and 94 days after sowing (das) in treatments with a 50% reduction in chemical fertilization at two locations in the Bajío region, State of Guanajuato, Mexico.

Treatments	NDVI 42 das	NDVI 59 das	NDVI 94 das	SPAD value 42 das	SPAD value 59 das	SPAD value 94 das
FS 100%	0.65167 a	0.84333 a	0.60333 a	47.2 a	48.483 a	49.117 a
FS 50%	0.615 a	0.77833 a	0.56167 a	45.317 a	45.333 ab	39.483 bc
FS 50%+BIO1	0.61 a	0.80833 a	0.56167 a	46.517 a	46.1 a	42.383 b
FS 50%+BIO2	0.42833 b	0.535 c	0.405 b	36.933 b	41.25 b	34.95 c
Control	0.61667 a	0.67333 b	0.55333 a	44.35 a	45.783 a	43.583 b
DMS	0.0017	0.03	0.0007	0.55	0.25	1.1

DMS: least significant difference; means with the same letters per column are not significantly different (LSD, $P \leq 0.05$); NDVI: normalized differential vegetation index.

This finding indicates that the incorporation of BIO2 increased the yield. There were no significant differences between BIO1 and BIO2; however, BIO2 performed similarly to the 50% chemical fertilization treatment. With respect to yield components, the number of GMCs increased when 100% chemical fertilization was applied. For EPMC and GPE, the treatments performed similarly, with the exception of the control. With respect to the thousand-kernel weight (TKW), all the treatments performed similarly, with the exception of the 100% chemical application treatment, which had the lowest values and was similar to the 50% treatment with BIO2. For IC, no significant differences were observed among the treatments.

DISCUSSION

Statistical analyses revealed that the treatments accounted for the greatest percentage of the observed variability in yield (80% and 58%, explaining 75% and 50% of the variation in fertilizer application, respectively), whereas the evaluation sites accounted for 18% and 40.3% (evaluating 75% and 50% of the fertilizer dose, respectively), and the interaction between the two accounted for 2% and 0.9% (evaluating 75% and 50% of the fertilizer dose, respectively). These results indicate that fertilizer rate management was more important than environmental conditions for yield expression in the evaluated treatments, which is consistent with the findings of Alotaibi et al. (2024). When 100% and 75% chemical fertilization was evaluated, the effects of biofertilizers on grain yield tended to increase yield; this finding was similar to the results reported by Rangel et al. (2014) and Sánchez de la Cruz et al. (2008) in México and by Marcandalli -Boleta et al. (2020), Alhammad et al. (2023), and Iqbal et al. (2024) in Brazil and the latter in Pakistan and in contrast with the results of Báez-Pérez et al. (2020) in Mexico's Bajío region, as they reported significantly higher yields in treatments with inoculants than in treatments without inoculation, although these latter results were based on evaluations conducted at a single location. Shahwar et al. (2023) reported significant responses in yield components to inoculation with various growth promoters in various crops and at various latitudes. On the other hand, Lemos et al. (2013) reported that inoculation with *Azospirillum brasilense* in conjunction with nitrogen fertilization was successful, as bacterial inoculation contributes to plant development; however, it did not replace nitrogen fertilization but rather eventually allowed a reduction in the nitrogen dose to be applied.

The effect of biofertilizers on the productivity of the treatments was not significant under the conditions in which they were evaluated. These findings suggest the need for further investigation into the behavior of soil microorganisms and their interactions with the environment. Studying soil microorganisms could aid in identifying their abundance in the soil associated with each treatment, which could aid in understanding the effects of biofertilizers on yield (Asoegwu et al., 2020). Shakir et al. (2012) and Jalal et al. (2023) reported that the yield response to inoculation is more pronounced in plants under adverse conditions, such as water deficit and low soil nutrient concentrations; however, Ferreira et al.

(2019) and Tabassum et al. (2017) reported that inoculation with *Azospirillum brasilense* does not always result in a favorable yield response under water stress or low soil nutrient concentrations.

By reducing chemical fertilization by 50%, it was found that the application of BIO 2 biofertilizer significantly increased yield by 10% compared with that in the treatment without inoculation (50% chemical fertilization), a result similar to that reported by El-Akhdar et al. (2024), Shintate-Galindo et al. (2019), and Nunes et al. (2015), who reported significant yield increases in contrast to those in treatments without biofertilizer inoculation. The BIO2 treatment performed statistically the same as the treatment with full fertilization (100%); this result is similar to that reported by Grageda-Cabrera et al. (2018), who reported that mycorrhizal inoculation with 50% chemical fertilization yielded the same yield as full fertilization (100%). The yield tended to improve in the BIO1 treatment; however, it was statistically inferior to full fertilization. This suggests the possibility of conducting evaluations in environments with adverse conditions to identify alternatives that optimize production, as well as the use of chemical fertilizers (Singh et al., 2023).

Furthermore, the results of the periodic measurements of physiotechnical variables such as the chlorophyll concentration and the SPAD and NDVI values revealed that all the treatments with biofertilizers resulted in similar physiological behaviors. The information provided by these variables served as the basis for identifying the effects of biofertilizers on the treatments. These results are consistent with those reported by Quintarelli et al. (2025), who reported no differences among different biofertilizers but reported that these treatments outperformed the absolute control treatment.

Statistical differences in the interactions between treatments and study sites indicated that the effects of biofertilizers varied across different environments; the study by Santillano-Cázares et al. (2022) helps explain these findings, since when 14 biofertilizers were evaluated across five states in Mexico, they reported that the effects of treatments inoculated with biofertilizers were not the same across the evaluated locations and reported a significant yield response to inoculation with *Azospillum brasilense* and Glomus spp. in only two of six locations in Mexico. On the other hand, these results contrast with those reported by Moradi and Siosemardeh (2025), who reported no statistically significant differences in the interaction between environments. The interaction analysis revealed that climatic conditions were also important, with Pénjamo location presenting better conditions for yield expression; this finding is similar to the results of Chavez-Díaz et al. (2020), who highlighted that the treatments evaluated with microorganisms in wheat differed because of interactions among plants, microorganisms, and the environment.

CONCLUSIONS

The interaction effect between location and treatment on yield was significant, indicating that yield performance differed across the evaluation sites. The treatments accounted for 63% of the total

variance, while the evaluation sites accounted for 14%, indicating that the effect of the treatments was more significant than that of the evaluation sites.

The analyses revealed that the treatment with 100% chemical fertilizer significantly outperformed the 75% and 50% treatments in terms of yield. However, when BIO2 was added to the 50% fertilizer treatment, the yield was statistically equivalent to that of the 100% control and greater than that of the 50% baseline. Similarly, when BIO1 or BIO2 was added to the 75% fertilizer treatment, a grain yield comparable to that of the full treatment was achieved (100%).

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Chapter 25

Genomic Reprogramming and Epigenetic Memory: Transforming Somaclonal Variation into a Tool for Climate Resilience

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ABSTRACT

This chapter examines the conceptual transition of somaclonal variation from "biological noise" to an adaptive breeding tool during the Agricultural Anthropocene. Given the increase in abiotic stress, particularly soil salinity, which affects 10.7% of the global land surface, and the structural bottlenecks associated with conventional breeding in perennial crops, plant biotechnology has emerged as a critical solution. Somatic embryogenesis (SE) is characterized by the ultimate expression of totipotency, unlocked through extensive chromatin remodeling mediated by the BRAHMA ATPase and the hierarchical activation of master genes such as BABY BOOM (BBM) and WUSCHEL (WUS). Experimental evidence in blackberry (*Rubus* spp.) has demonstrated that overcoming biological recalcitrance requires specific strategies, such as substituting 2,4-D with thidiazuron (TDZ) and organic nitrogen (L-glutamine), to transition from disorganized proliferation to morphogenetic autonomy. This process is governed by a critical "oxidative window" of reactive oxygen species (ROS) signaling that act as a master switch for cell fate. Furthermore, this chapter explores the phenomenon of "epigenetic memory" or priming, which allows for the generation of "epitypes" with constitutive tolerance to future stressors without exogenous gene introgression. Finally, the implementation of high-precision aeroponic systems under LED lighting is discussed as an indispensable technological bridge for validating adaptive vigor under ex vitro conditions. By integrating cellular biotechnology with advanced cultivation systems, this work provides a framework for engineering plant resilience and ensuring global food security under environmental volatility.

Keywords: Somatic embryogenesis, Abiotic stress, Totipotency. Aeroponic culture

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INTRODUCTION: THE CONVERGENCE OF CAUSES IN THE AGRICULTURAL ANTHROPOCENE

During the early expansion of the commercial micropropagation industry, somaclonal variation was universally perceived as a technical failure—a form of "biological noise" that needed to be silenced. The promise of *in vitro* cloning rested on the exponential multiplication of elite genotypes identical to those of the original donor. In this context, any deviation from the desired phenotype represented an unacceptable economic risk (Karp, 1994; Bairu et al., 2011).

This negative perception was not unfounded; it was grounded in large-scale agronomic disasters that traumatized entire sectors of tropical agribusiness. The most emblematic and devastating case involved the oil palm (*Elaeis guineensis*) in Southeast Asia and West Africa. The massive introduction of clones propagated via somatic embryogenesis resulted in a 30% increase in yield because of the heterosis of the tenera hybrid (Corley; Law, 1997). However, upon reaching the productive phase, millions of palms exhibited a "mantled" floral anomaly. This condition is characterized by the homeotic transformation of stamens into carpeloid structures, resulting in partial sterility or fruit abortion (Jaligot et al., 2000; Ong-Abdullah et al., 2015). This phenomenon, which affected thousands of hectares, led to catastrophic financial losses and lasting distrust of tissue culture products among farmers and corporate planters (Rival et al., 2013).

Historically, plant breeding has prioritized pest resistance and yield under optimal conditions, a focus that has inadvertently eroded genetic diversity; for instance, modern wheat has lost a significant portion of the diversity found in its wild relatives, such as wild emmer. Climate change has shifted the risk landscape, elevating abiotic stresses—drought, heat, and salinity—to primary threats. The scientific literature indicates that developing crops capable of withstanding these stressors by utilizing biological models of extremophile and halophytic plants is the most promising path to securing the future food supply (Razzaq et al., 2021; Zhang et al., 2018).

Soil salinization represents one of the most insidious threats to sustainable agriculture, as it can compromise soil fertility, ecosystem health, and the economic stability of key producing regions. The Food and Agriculture Organization (FAO) of the United Nations recently presented a global assessment of salt-affected soils. The report, *Global Status of Salt-Affected Soils* (2024), reveals that approximately 1.4 billion hectares (1,381 Mha) of land are affected by salinity, constituting 10.7% of the total land surface. Critically, 10% of both irrigated and rainfed croplands are already compromised. This distribution is asymmetric, with ten countries—including major producers such as the United States, China, Australia, and Argentina—accounting for 70% of these soils. The economic impact is devastating, with estimated annual losses to global agricultural productivity totaling $27.3 billion USD (FAO, 2024; Qadir et al., 2014).

Climate change acts as a threat multiplier, potentially expanding the area affected by salinity to encompass between 24% and 32% of the total land surface by the end of the century. The primary mechanisms include increased evapotranspiration in arid zones, which concentrates salts at the surface; seawater intrusion into fertile deltas because of rising sea levels; and the forced use of marginal or wastewater for irrigation during droughts, which accelerates secondary salinization (Corwin, 2021; FAO, 2024; Tarolli et al., 2023).

Understanding why current crops fail is fundamental to developing new varieties. The plant response to salinity is complex and occurs in two phases: rapid osmotic stress that limits water uptake, and slow ionic toxicity caused by sodium (Na+) accumulation in the tissues.

There is a fundamental physiological trade-off in plants. Modern elite crops have been selected to maximize yield traits, such as high stomatal conductance, thereby losing the plasticity required to downregulate their metabolism under adverse conditions. Often, tolerance mechanisms—such as active salt exclusion or the synthesis of compatible osmolytes—are so energetically expensive that they compete directly with biomass and grain production, resulting in what is known as a "yield penalty" in tolerant varieties (Dalal et al., 2017; Munns; Tester, 2008; Razzaq et al., 2021).

In the banana industry (*Musa* spp.), the situation was analogous. Micropropagation, which is essential for obtaining disease-free planting material, frequently generates "off-type" variants with undesirable traits, such as dwarfism, leaf variegation, deformed bunches, or changes in fruit hand orientation (Israeli et al., 1991). The export industry, governed by the strict aesthetic standards of European and North American supermarkets, severely penalized these deviations. Consequently, the scientific literature from the late 20th century focused obsessively on protocols to minimize somaclonal variation, optimize plant growth regulators (PGRs) and reduce the number of subculture cycles to ensure genetic stability (Sahijram et al., 2003).

Limitations of Conventional Plant Breeding: Critical Biological and Economic Barriers to Conventional Breeding

Modern agriculture exhibits a sharp technological dichotomy. While annual field crops have accelerated their genetic gains through rapid cycling and hybrid vigor, fruit production remains hindered by structural barriers inherent to the biology of perennial species. Conventional breeding in fruit trees—based on sexual hybridization and phenotypic selection—faces an innovation bottleneck defined by the complex interaction between protracted life cycles, a highly heterozygous genetic architecture, and restrictive reproductive mechanisms. These limitations not only extend varietal development timelines to decades but also impose an economic burden that threatens the viability of breeding programs in the face of urgent climate change and emerging phytosanitary pressures (Flachowsky et al., 2011; Migicovsky; Myles, 2017; van Nocker; Gardiner, 2014).

Physiological and Reproductive Barriers: The "Time Wall" and Genetic Complexity

The most formidable restriction for fruit breeders is juvenility, a mandatory ontogenetic phase during which the plant is physiologically incapable of flowering, regardless of environmental stimuli. Unlike annual crops that complete their cycle in months, woody species require extensive periods of vegetative growth before fruit quality can be evaluated. This juvenile period varies drastically across species: in apple (*Malus domestica*) and pear (*Pyrus communis*), seedlings typically take 4 to 8 years to flower; in citrus (*Citrus* spp.), this span often extends from 5 to 12 years and is accompanied by undesirable juvenile traits such as large thorns; and in avocado (*Persea americana*), the wait can exceed 15 years. This biological latency implies that the introgression of a single trait through six generations of backcrossing would theoretically require more than half a century, rendering the development of pure lines practically unfeasible (Hackett, 1985; Hanke et al., 2007; Lahav et al., 1986; Peña et al., 2007).

In addition to the temporal barrier, the genetic architecture of fruit trees prevents the fixation of traits through conventional inbreeding strategies. Most fruit species are obligate outcrossers and maintain extreme levels of heterozygosity. Unlike in maize or rice, where it is possible to create homozygous inbred lines to exploit heterosis in the F1 generation, self-fertilization in fruit trees triggers severe inbreeding depression. Attempts to "purify" the genome in species such as apple, cherry, or almond result in a drastic loss of vigor, low fertility, and the frequent appearance of lethal or sublethal phenotypes—such as albinism or extreme dwarfism—due to the historical accumulation of deleterious recessive alleles that were masked in the heterozygous state. This forces breeders to work with genetically "noisy" parents, where offspring segregate unpredictably. Consequently, the probability of recovering a superior genotype that combines all desired quality attributes is infinitesimally low, necessitating massive selection (Brown, 1973; McClure et al., 2014; Rahemi et al., 2011; Wu et al., 2016).

Reproductive biology imposes further constraints through self-incompatibility (SI) systems that prevent self-pollination and limit possible crosses. In the Rosaceae (apple, cherry, almond) and Solanaceae families, gametophytic self-incompatibility, which is dominated by the S-locus, prevails. In this mechanism, S-RNases (ribonucleases) in the style arrest pollen tube growth if the pollen has an allele identical to that of the maternal tissue. This not only restricts the breeder's crossing designs but also complicates agronomic management by necessitating the planting of compatible pollinator varieties. Furthermore, in genera such as *Citrus* and *Mangifera* (mango), nucellar polyembryony and apomixis add a layer of logistical difficulty: seeds produce multiple clonal embryos from the mother that compete with and often eliminate the sexual hybrid embryo. This necessitates the use of costly molecular markers or delayed morphological evaluations to distinguish true hybrids from unwanted maternal clones (De Franceschi et al., 2012; Fujii et al., 2016; Koltunow et al., 1996; Sassa et al., 1997).

Global Food Security and Environmental Pressures

Contemporary agriculture stands at an existential crossroads, defined by the convergence of unprecedented demographic pressures, climatic volatility, and the accelerated degradation of basic edaphic resources. This report addresses the critical issue of global food security through the lens of an urgent need for new cultivars capable of thriving in marginal environments, with a specific focus on soil salinization induced and exacerbated by climate change. As the global population increases, the demand for calories and, crucially, quality nutrition will grow exponentially, challenging current production systems that already show signs of exhaustion.

Modern food security is not limited to supply availability; it depends intrinsically on physical and economic access. Accelerated urbanization is a determining factor in this equation; it is estimated that by 2050, two-thirds of the world's population will reside in urban areas, consuming the vast majority of food produced while creating a geographical dissociation between production and consumption. This dynamic, coupled with water scarcity, constitutes a formidable barrier to sustainable development goals (SDGs). Given that agriculture already occupies 38% of global land and utilizes 70% of freshwater, the expansion of the agricultural frontier is unsustainable; the necessary increases must stem from efficiency and resilience (Tuffrey; Espeut, 2015; van Zutphen et al., 2023; Zhang et al., 2018).

Plant Biotechnology as a Breeding Tool: Tissue Culture Beyond Clonal Micropropagation

Plant breeding has evolved from conventional phenotypic selection toward integration with advanced biotechnological tools. *In vitro* tissue culture has transitioned from being merely a method for mass multiplication to becoming a critical experimental platform. This platform enables the induction of genetic variability, the selection of resilient genotypes, and the circumvention of biological barriers inherent to traditional sexual hybridization (Gulzar et al., 2024).

Somaclonal variation is not a purely stochastic event; rather, it is a genomic adaptive response to culture-induced stress. This response encompasses point mutations, changes in ploidy, and, fundamentally, epigenetic alterations such as DNA hypomethylation and the activation of transposable elements (Ramos-Sánchez; Dela-Peña, 2025). In species characterized by obligate vegetative propagation or protracted life cycles—such as pineapple (*Ananas comosus*) or species within the Rubus genus—the selection of somaclonal variants has facilitated the development of genotypes with superior agronomic traits. These include resistance to fungal pathogens, modified plant architecture, and enhanced secondary metabolite profiles, all of which are achieved without the need for exogenous gene introgression (Ramos-Sánchez; Dela-Peña, 2025).

Historical Context and Paradigm Shift

Historically, the micropropagation industry perceived somaclonal variation as a technical failure and a source of unacceptable economic risk due to undesirable phenotypic deviations (Karp, 1994; Bairu et al., 2011). This stigma was reinforced by agronomic disasters, such as the "mantled" floral anomaly in oil palm (Elaeis guineensis), where the homeotic transformation of stamens resulted in partial sterility, and the emergence of "off-type" variants in banana (Musa spp.) exhibiting dwarfism and deformed bunches (Israeli et al., 1991; Jaligot et al., 2000).

Today, this paradigm has shifted toward the deliberate exploitation of somaclonal variation as an adaptive genomic response to culture stress. In long-lived species such as pineapple and Rubus, this induced variability enables the identification of genotypes with improved resilience and biochemical profiles, thereby consolidating tissue culture as a vital tool to overcome the bottlenecks of conventional breeding (Gulzar et al., 2024; Ramos-Sánchez; Dela-Peña, 2025).

Cell Selection: Capitalizing Induced Variability

This induced variability is harnessed through cell selection, a technique that involves subjecting cell cultures to specific selective pressures that mimic environmental stress.

Biotic and Heavy Metal Stress: Pathogen culture filtrates or sublethal concentrations of heavy metals have been utilized to isolate resistant cell lines. Recent studies emphasize the use of *in vitro* cellular models to select genotypes possessing mechanisms for the exclusion or vacuolar sequestration of toxic metals such as cadmium (Cd) and lead (Pb), thereby contributing to food safety (Nik Yusoff et al., 2024).

Abiotic Stress (Salinity and Drought): The addition of osmotic agents, such as polyethylene glycol (PEG) or NaCl, to the culture medium enables the recovery of drought- and salinity-tolerant variants. These phenotypes, selected at the cellular level, frequently maintain their tolerance following the regeneration of the whole plant, validating the physiological correlation between *in vitro* and *ex vitro* performance (Turfan, 2025).

Physiological and molecular bases of SE

SE represents the ultimate expression of plant totipotency, a potential that is unlocked through dedifferentiation and extensive chromatin remodeling (Fehér, 2019; Ishiuchi; Sakamoto, 2023). Although SE morphologically recapitulates the stages of zygotic embryogenesis, phylotranscriptomic studies suggest that SE activates a primordial developmental program. In species such as the grapevine (*Vitis vinifera*), the heart stage of somatic embryos expresses the phylogenetically oldest transcriptome of the lineage (Tikhonova et al., 2024).

Table 1. SEQ Table * ARABIC 1. Documented cases of somaclonal variation-mediated induction of abiotic stress tolerance: Selection criteria and metabolic-genomic findings in diverse plant species.

Author(s)	Species	Selection Criteria or Detected Variable	Metabolic, fenotipic and Genomic Findings
Marček et al. (2018)	*Rubus* spp.	Chlorophyll fluorescence and ionic leakage	Somaclones selected under NaCl showed stabilized Photosystem II and reduced membrane damage.
Rai et al. (2011)	Citrus sinensis	Free proline accumulation	Cell lines showed enhanced Na+ exclusion and osmoprotectant synthesis via the glutamate pathway.
Lutts et al. (1998)	Oryza sativa	K+/Na+ selectivity ratio	Somaclones exhibited higher affinity for K+ uptake, mitigating ionic toxicity in the cytoplasm.
Patade et al. (2008)	Saccharum spp.	Antioxidant enzyme activity (APX, CAT)	Upregulation of the ascorbate-glutathione cycle to neutralize ROS generated by salt and drought stress.
Morpurgo (1991)	Solanum tuberosum	Cellular osmotic adjustment	Stable tolerance to 150 mM NaCl was linked to increased intracellular solute concentration.
Cano et al. (1996)	Lycopersicon esculentum	Polyamine biosynthetic levels	Increased putrescine and spermidine levels served as protective agents against saline-induced protein degradation.
Winicov (1991)	Medicago sativa	Alfin1 transcription factor expression	Identification of a specific somaclonal mutation leading to over-expression of genes regulating root growth under stress.
Troncoso et al. (1999)	Vitis vinifera	Vacuolar chloride compartmentation	Somaclonal variants demonstrated enhanced Cl- sequestration, preventing leaf tissue necrosis.
D'Onofrio et al. (2016)	Fragaria x ananassa	Photosynthetic pigment stability	High-temperature selection yielded somaclones with thermally stable light-harvesting complexes.
Badawy et al. (2021)	*Musa* spp.	NHX1 gene upregulation	Enhanced activity of vacuolar Na+/H+ antiporters, essential for maintaining cytoplasmic ionic balance.
Ben-Hayyim (1987)	Citrus aurantium	Intracellular K^+concentration	Confirmed that somaclonal salt tolerance is primarily a function of maintaining high potassium levels.
Rahman et al. (2018)	Solanum tuberosum	Stomatal density and root biomass	Selection using PEG-induced osmotic stress led to variants with optimized water-use efficiency.
Gurel et al. (2008)	Beta vulgaris	BADH enzyme synthesis	Increased betaine aldehyde dehydrogenase activity, crucial for the synthesis of glycine-betaine.

This genomic "reset" toward an ancestral state requires the removal of repressive marks, such as histone H3 lysine 27 trimethylation (H3K27me3), from the promoters of regenerative genes (Li et al., 2025). Recent research has demonstrated that this process is mediated by the BRAHMA (BRM) ATPase, which antagonizes Polycomb-mediated repression to facilitate the transcriptional activation necessary for embryo regeneration. This allows somatic cells to bypass the maturation constraints inherent to adult tissues (Li et al., 2025).

The stress applied during induction not only has an immediate effect on embryo formation but can also leave a lasting imprint on the regenerated plant through the phenomenon of priming or epigenetic memory (Yakovlev; Fossdal, 2017; Kvaalen; Johnsen, 2008). In conifers such as *Picea abies* and *Pinus radiata*, it has been demonstrated that stress conditions during somatic embryogenesis modulate the epigenetic machinery, altering DNA methylation patterns and small RNA (sRNA) expression (Yakovlev; Fossdal, 2017; Kvaalen; Johnsen, 2008).

These stable epigenetic changes generate "epitypes" that exhibit enhanced constitutive tolerance to future stressors, allowing somatic embryogenesis to evolve from a mere cloning technique into a sophisticated tool for adaptive breeding (Yakovlev; Fossdal, 2017). These findings validate the utility of *in vitro* culture for the epigenetic engineering of plant resilience, enabling the generation of individuals "primed" to face climate change scenarios and soil salinization without the need for exogenous gene introgression (Ramos-Sánchez; Dea-Peña, 2025).

Cellular plasticity and totipotency

Totipotency is not a constitutive state of all living plant cells but rather a potential state that must be unlocked through drastic shifts in cell fate.

This process begins with dedifferentiation, in which specialized cells lose their tissue identity and re-enter the cell cycle, where they acquire a state of "competence" or transient pluripotency. This phase requires extensive chromatin remodeling to silence tissue-specific genes and activate proliferation genes. Under specific inductive stimuli (generally auxins), redifferentiation subsequently occurs, where competent cells execute the embryonic developmental program (Fehér, 2019). Recent studies suggest that regeneration involves epigenetic reprogramming that restores an "open" chromatin state, similar to that of meristematic cells (Li et al., 2025).

Differences between Zygotic and Somatic Embryogenesis

Although SEs morphologically recapitulate the stages of zygotic embryogenesis (ZE)—globular, heart, and torpedo—there are significant molecular differences. Physiologically, somatic embryos often

lack the natural desiccation and dormancy phase and may exhibit anomalies in nutrient reserve accumulation (Méndez-Hernández et al., 2019).

From an evolutionary perspective, a striking finding from phylotranscriptomics in Vitis vinifera revealed that, while ZE follows an "hourglass model" (where the mid-embryonic stage is the most evolutionarily conserved), SE presents the phylogenetically oldest transcriptome during its "heart" stage. These findings suggest that SE may represent a more "ancestral" or primordial developmental program than sexual embryogenesis does (Tikhonova et al., 2024).

TRANSCRIPTIONAL REPROGRAMMING MECHANISMS

The conversion of a somatic cell into an embryo is governed by a hierarchical network of master transcription factors (TFs) that orchestrates a complete overhaul of the cellular transcriptome.

Expression of Master Genes in Induction: WUSCHEL (WUS), BABY BOOM (BBM), and LEAFY COTYLEDON (LEC)

Ectopic overexpression of BABY BOOM (BBM), an AP2/ERF family transcription factor, is sufficient to trigger spontaneous somatic embryogenesis (SE). BBM functions by directly activating the LAFL gene network (*LEC1, ABI3, FUS3, and LEC2*), which is responsible for embryonic identity and maturation (Horstman et al., 2017).

In parallel, WUSCHEL (WUS), a homeobox gene, is critical for maintaining the stem cell niche during initial division and promoting vegetative-to-embryonic transition. Recently, the coexpression of *BBM* and *WUS* has been used to overcome biological recalcitrance in the genetic transformation of cereals such as maize (*Zea mays*). This synergy induces a robust embryogenic response in tissues that typically fail to regenerate under standard conditions (Wu et al., 2025).

Role of the Somatic Embryogenesis Receptor Kinase (SERK) Complex

Embryogenic competence is molecularly marked by the expression of the SERK1 gene. The SERK1 protein is a leucine-rich repeat receptor-like kinase (LRR-RLK) that functions as a versatile coreceptor. It forms heterodimeric complexes with the brassinosteroid receptor BRI1 and other receptors involved in stress and developmental signaling (Albrecht et al., 2008).

Signaling through the SERK complex integrates hormonal and environmental stimuli, acting as a molecular hub. This signaling center is indispensable for the acquisition of the morphogenetic capacity required for embryo formation by somatic cells (Fontes, 2024).

The reprogramming of the transcriptome during SE depends on chromatin remodeling, which allows the transcriptional machinery to access "locked" totipotency genes.

A key mechanism involves the removal of the repressive H3K27me3 mark (trimethylation of histone H3 at lysine 27) from the promoters of regenerative genes. This process is mediated by the ATP-dependent SWI/SNF chromatin-remodeling complex, specifically by the BRAHMA (BRM) ATPase. Recent investigations in *Arabidopsis* have shown that BRM antagonizes polycomb-mediated repression, facilitating the erasure of H3K27me3 and enabling the transcriptional activation of genes necessary for both shoot and embryo regeneration (Li et al., 2025).

In addition to histone modifications, DNA methylation acts as a stable epigenetic lock that maintains differentiation. The transition toward SEs requires global DNA hypomethylation. In studies of *Coffea canephora* and *Medicago truncatula*, the application of demethylating agents such as 5-azacytidine induced the expression of *LEC1* and *BBM*, promoting embryo formation. These findings demonstrate that a reduction in DNA methylation is a functional prerequisite for unlocking the embryonic genetic program in somatic cells (Nic-Can et al., 2013; Orłowska; Kępczyńska, 2020).

Methodology: Factors Regulating Induction (Herbaceous vs. Woody Species): The Role of Stress as a Developmental Switch

The technical foundation of plant biotechnology rests on the ability to regenerate whole plants from *in vitro-selected* somatic cells. However, the efficiency of these technologies is intrinsically tied to the ability of the plant cell to revert to its differentiated state—a phenomenon that is frequently hindered by biological recalcitrance, particularly in long-cycle woody species.

Contemporary understanding has shifted from a focus solely on hormonal balance to a paradigm in which abiotic stress acts as the master developmental switch. In this context, physical and chemical stress signals, mediated by reactive oxygen species (ROS) such as H2O2 and O2, unlock cellular plasticity. This mechanism contrasts the fluid developmental dynamics of herbaceous models with the complex physiological barriers found in woody species (Fehér, 2015; Guan et al., 2016).

Genotypic Determinants and the Maturation Barrier in Woody Species

The induction of somatic embryogenesis (SE) is a multifactorial process in which the genotype of the explant determines the threshold for responding to inductive signals. In herbaceous model species, such as *Arabidopsis thaliana* or *Brassica napus*, there is broad regenerative plasticity that allows the reprogramming of various types of somatic tissue, even at advanced stages of development. In contrast, woody species—including fruit trees and economically vital forest species such as conifers—exhibit strict genotypic dependence and a rapid loss of embryogenic competence with age, a phenomenon known as phase maturation or cyclophilins.

This biological constraint forces breeders to work almost exclusively with juvenile material, such as immature zygotic embryos. Consequently, the ability to clone and improve elite adult trees that have already demonstrated superior field performance is severely limited. Recalcitrance in woody plants is not merely a failure of culture techniques but also a reflection of a repressive epigenetic architecture and robust cellular stability that evolved to support the longevity of the organism. Therefore, compared with their herbaceous counterparts, dedifferentiation in these species requires much more precise and often aggressive stress stimuli (Isah, 2016; Bonga et al., 2010).

Stress as a Primary Signal: Mechanics of Cellular Reprogramming

The transition from a somatic cell to an embryogenic cell requires a violent disruption of cellular homeostasis to force the abandonment of the current gene expression program.

1. Wounding Stress

Mechanical wounding represents the primary and universal inductive stimulus. The physical damage inflicted during explant preparation triggers instantaneous changes in turgor pressure gradients and systemic ion fluxes, most notably calcium (Ca^2+) waves that propagate through the tissue.

In herbaceous species, this signaling rapidly activates key transcription factors, such as *WOUND INDUCED DEDIFFERENTIATION 1 (WIND1)*, which coordinates healing and promotes the dedifferentiation required for callus formation and subsequent regeneration. In woody species, the activation of these regenerative pathways often involves direct competition with conserved defense mechanisms, such as rapid lignification and phenolic compound synthesis. While these responses seal the wound to prevent infection, they paradoxically block the cell proliferation necessary for embryogenesis (Iwase et al., 2011; Isah, 2016).

2. Osmotic Stress and Nutrient Starvation

Osmotic stress and nutrient deprivation act as potent inducers by simulating adverse environmental conditions. Plasmolysis induced by high concentrations of osmotic agents—such as sucrose or polyethylene glycol (PEG)—causes protoplast retraction and the rupture of plasmodesmata (intercellular connections).

> This physiological isolation is considered a fundamental prerequisite for the cell to "forget" its original tissue function and acquire the autonomy needed to initiate a new embryogenic program. In microspore systems (e.g., tobacco or barley), sugar and nitrogen starvation induce autophagy, which recycles cytoplasmic components and eliminates proteins associated with the differentiated state, effectively "cleaning" the cellular environment to prepare for totipotency. In conifers, while osmotic stress is primarily used for embryo maturation, its controlled application during induction can modulate endogenous abscisic acid (ABA) levels and promote the formation of embryogenic tissue (Fehér, 2015; Stasolla; Yeung, 2003).

Thermal and Chemical Stress: Catalysts for Cellular Reprogramming

Thermal and chemical stresses are equally relevant, as they disrupt the stability of cellular structures and chromatin architecture. Thermal shock—whether induced by heat or cold—triggers the massive synthesis of heat shock proteins (HSPs) and reorganizes the cytoskeleton. This structural reorganization can reorient the plane of cell division toward an embryogenic pathway, a phenomenon clearly observed during androgenesis in *Brassica* species.

Furthermore, chemical agents such as colchicine, often used in Vaccinium breeding to induce polyploidy, act as severe stressors. At sublethal doses, colchicine drastically reprogrammed the cell cycle by disrupting microtubule polymerization. Similarly, the application of high concentrations of synthetic auxins, such as 2,4-D, should not be interpreted merely as a form of hormonal supplementation. Instead, it constitutes a chemical stress ("auxinic shock") that alters DNA methylation patterns and disrupts endogenous auxin homeostasis, thereby unlocking the expression of master embryogenesis genes such as *WUSCHEL (WUS)* and *BABY BOOM (BBM)* (Seguí-Simarro, 2010; Hernández et al., 2024).

Reactive oxygen species (ROS) signaling and the "oxidative window"

At the molecular core of the stress response lies the generation of reactive oxygen species (ROS). These molecules are no longer viewed solely as toxic metabolic byproducts but are recognized as essential second messengers in developmental signal transduction.

The successful induction of somatic embryogenesis depends on the existence of a critical "oxidative window": a specific concentration range of ROS—particularly hydrogen peroxide (H2O2) needed to modify the redox state of transcription factors and activate the embryogenic gene cascade. While basal ROS levels maintain quiescence or a normal cell cycle, a transient and controlled increase acts as a reprogramming signal.

ROS interact closely with hormone signaling, modulating polar auxin transport and the stability of transcriptional regulators. This interaction creates the morphogenetic gradients necessary for the formation of the new embryo. However, if ROS production exceeds the capacity of cellular antioxidant systems, the signaling threshold is bypassed, leading to lethal oxidative stress, macromolecular damage, and programmed cell death (PCD) (Zhou et al., 2016; Bailly et al., 2008).

Epigenetic Memory: A Bridge toward Climate Adaptation

It is critical to emphasize that the stress applied during induction does not merely have an immediate effect on embryo formation; it can leave a lasting "imprint" on the regenerated plant—a phenomenon with profound implications for the improvement of forest cultivars. In conifers such as Norway spruce (*Picea abies*) and Radiata pine (*Pinus radiata*), temperature and stress conditions during

somatic embryogenesis modulate the epigenetic machinery, altering DNA methylation patterns and the expression of small RNAs (sRNAs).

These stable epigenetic changes generate “epitypes” that exhibit adapted phenologies and enhanced constitutive tolerance to future stressors, a process known as priming or epigenetic memory. These findings increase the use of somatic embryogenesis from a mere cloning technique to a sophisticated tool for adaptive breeding, enabling the generation of trees "primed" to address climate change scenarios. These findings validate the importance of *in vitro* culture not only for genetic selection but also for the epigenetic engineering of plant resilience (Yakovlev; Fossdal, 2017; Kvaalen; Johnsen, 2008).

MECHANISM OF PLANT GROWTH REGULATORS (PGRS)

Auxins: Mechanism of action of 2,4-D, picloram, and Dicamba

The use of synthetic auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D), picloram, and Dicamba is fundamental for inducing cellular dedifferentiation through an “auxinic shock” that reprograms the transcriptome toward totipotency.

At the molecular level, these auxins saturate the receptors of the TIR1/AFB family, triggering the ubiquitination and subsequent degradation of Aux/IAA repressor proteins via the 26S proteasome. Once these repressors are removed, auxin response factors (ARFs) are free to dimerize and activate the expression of master embryogenesis genes, such as *WUSCHEL (WUS)* and *BABY BOOM (BBM)*. Furthermore, these supraphysiological concentrations strongly interfere with polar auxin transport by delocalizing PIN efflux carrier proteins, which disrupts endogenous indole-3-acetic acid (IAA) gradients and forces the establishment of new cellular organizing centers necessary for somatic embryo polarity (Fehér, 2015; Horstman et al., 2017; Seguí-Simarro, 2010; Wu et al., 2025).

Cytokinins: The Importance of TDZ and the SERK Complex

The induction of embryogenic competence depends critically on the auxin/cytokinin ratio, which dictates the transition from a somatic cell to a proembryogenic state. In recalcitrant species, the use of thidiazuron (TDZ) is important because it uniquely inhibits the enzyme cytokinin oxidase (CKX). This inhibition results in a massive accumulation of endogenous cytokinins that are difficult to degrade.

Biochemically, TDZ acts as a potent inductor that facilitates the formation of the SERK1 (Somatic Embryogenesis Receptor Kinase 1) complex. This receptor kinase functions as an indispensable signaling “hub”, allowing somatic cells to acquire morphogenetic competence and execute the embryonic developmental program under stress conditions (Albrecht et al., 2008; Fontes, 2024; Isah, 2016).

The practical application of transcriptional reprogramming concepts is clearly illustrated by the response of blackberry (*Rubus* spp.) anther cultures. Initially, the application of the synthetic auxin 2,4-D induced an "auxinic shock" that promoted massive cellular dedifferentiation; however, in this specific system, the stimulus resulted in spherical, hardened, and vacuolated cells (hollow cells) lacking organized embryonic identity (Figure 2A). In this state, the repressive H3K27me3 marks likely remained prevalent at regenerative loci, preventing the hierarchical activation of master genes and maintaining a state of robust biological recalcitrance where the tissue exhibited a total dependency on exogenous auxins, leading directly to necrosis upon their removal (Chen et al., 2024; Fehér, 2015; Liu et al., 2023; Seguí-Simarro, 2010).

This breakthrough was achieved by substituting 2,4-D with thidiazuron (TDZ) at 1 mg/L for 15 days in MS medium supplemented with 400 mg/L L-glutamine. As shown in Figure 2B, this transition triggered the emergence of organized, chlorophyllous exophytic structures ("green bumps"), marking the transition toward proembryogenic mass formation. This shift is consistent with the role of TDZ as a powerful inducer of morphogenic regulators capable of bypassing the metabolic bottlenecks of conventional auxins in woody species, while L-glutamine acts as a critical signaling molecule, providing the organic nitrogen necessary to stabilize cellular metabolism and enhance embryo quality (Aliu et al., 2024; Ara et al., 2020; Jiang et al., 2024).

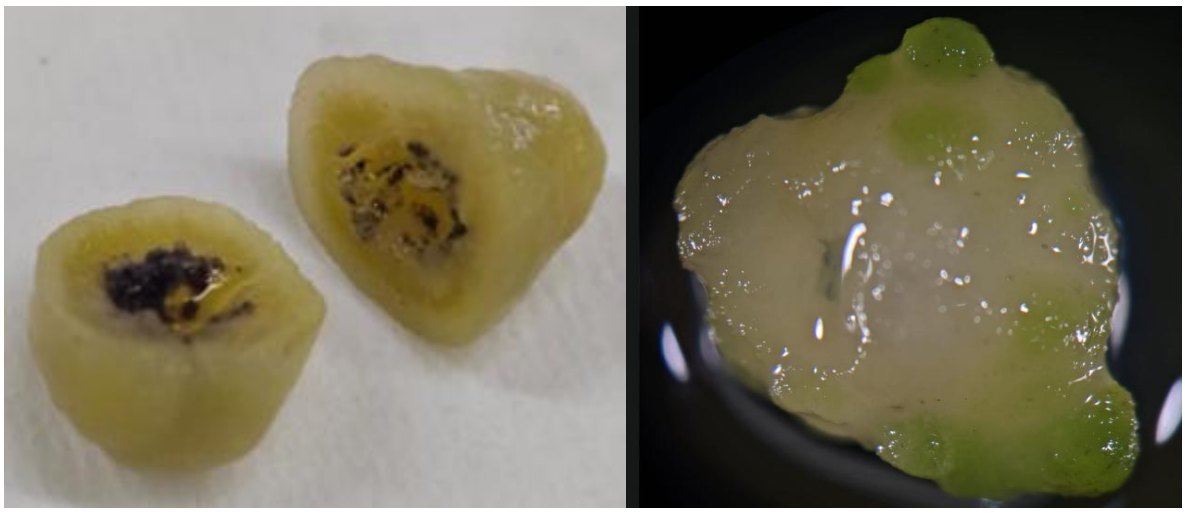


Figure 1. Morphogenetic evolution of blackberry (*Rubus* spp.) anther cultures in suspension. Cellular morphology after prolonged 2,4-D 1 mg/L treatment: round, hardened, and vacuolated cells with no regenerative capacity. (B) Induction of morphogenetic competence after shock with 1 mg/L TDZ and L-glutamine, resulting in the formation of green proembryogenic centers.

Biochemically, the effectiveness of TDZ is attributed to the inhibition of cytokinin oxidase (CKX) activity and the facilitation of the SERK1 complex. This receptor kinase functions as a signaling hub for morphogenetic competence, integrating environmental stress signals into the developmental program.

The current ability of these cultures to maintain growth in hormone-free media validates the achievement of morphogenetic autonomy, suggesting the activation of self-sustaining feedback loops in the LAFL gene network facilitated by reactive oxygen species (ROS) acting as master switches for cell fate (Albrecht et al., 2008; Fontanet-Manzaneque et al., 2024; Fontes, 2024; Horstman et al., 2017; Isah, 2016; Vandeputte et al., 2024; Wu et al., 2025; Zhu et al., 2025).

Comparative Analysis: Herbaceous vs. Woody Models

Recalcitrance in woody species, such as berries, is defined as the inability of tissues to respond to *in vitro* morphogenetic stimuli, acting as a "bottleneck" for biotechnological innovation. Unlike herbaceous models, these species possess a repressive epigenetic architecture and robust cellular stability that evolved to support prolonged life cycles, hindering cellular dedifferentiation.

A critical factor is cyclophysis or phase maturation, a process by which tissue gradually loses its embryogenic competence as the plant ages. This forces researchers to rely almost exclusively on juvenile material, such as immature zygotic embryos or anthers at early developmental stages. Additionally, extreme heterozygosity and self-incompatibility systems complicate the fixation of traits, requiring much more precise and aggressive stress stimuli to unlock the master genes of totipotency (Bonga et al., 2010; Isah, 2016; van Nocker; Gardiner, 2014).

The initial response to culture stress in woody tissues triggers a massive release of secondary metabolites, specifically phenolic compounds, which function as a long-term defense strategy. When the explant is fragmented, the rupture of cellular compartmentalization releases these phenols, which are subsequently oxidized by enzymes such as polyphenol oxidase (PPO) and peroxidase (POD) in the presence of reactive oxygen species (ROS), generating highly reactive and toxic quinones. This process manifests macroscopically as tissue browning, leading to accelerated necrosis. This effectively blocks the constructive ROS signaling required for cellular reprogramming and ultimately triggers programmed cell death (PCD) (Bailly et al., 2008; Isah, 2016; Tang et al., 2004).

To mitigate this impact, the methodology requires the integration of antioxidant strategies and strict control of physical culture conditions. The use of adsorbing agents such as activated charcoal is imperative to sequester quinones and other inhibitors before they affect the explant. Simultaneously, exogenous antioxidants such as ascorbic acid help maintain the redox environment within permissible physiological limits. Additionally, maintaining cultures in initial darkness is a standard practice to prevent the photolytic degradation of synthetic auxins and to inhibit the early synthesis of chlorophyll and other pigments, which could divert the metabolic pathway toward organogenesis rather than somatic embryogenesis (Fehér, 2015; Isah, 2016).

Metabolic Divergence: Herbaceous vs. Woody Species

The management of ROS signaling defines the fundamental difference between the high regenerative capacity of herbaceous plants and the recalcitrance of woody species.

Herbaceous Species: These plants possess dynamic and plastic antioxidant systems (e.g., SOD, CAT, and APX) that allow them to generate the oxidative "peak" required for initial signaling after stress and quickly neutralize it to preserve cell viability.

Woody species are characterized by the constitutive accumulation of secondary metabolites, particularly phenolic compounds, as a long-term defense strategy.

During stress induction in woody tissues, the rupture of cellular compartmentalization releases these phenols. In the presence of ROS, they are oxidized by enzymes such as polyphenol oxidase (PPO) and peroxidase (POD), generating highly reactive and toxic quinones. This process triggers an uncontrollable oxidative stress chain reaction that manifests macroscopically as tissue browning and necrosis.

In this scenario, the constructive signaling function of ROS is overwhelmed by chemical toxicity, effectively blocking the embryogenic pathway. Consequently, the methodology for woody species imperatively requires the use of exogenous antioxidants (e.g., ascorbic acid and citric acid) and adsorbent agents such as activated charcoal to maintain the redox environment within the physiological limits that allow for reprogramming without triggering cell death (Isah, 2016; Tang et al., 2004).

Challenges in Maturation and Conversion

Even when somatic embryo induction is successful, the conversion phase into whole plants presents significant challenges because of anomalies in the developmental program. Unlike zygotic embryogenesis, somatic embryos often lack a natural desiccation and dormancy phase, resulting in asynchronous maturation and deficient reserve accumulation. This asynchrony prevents all the embryos from simultaneously reaching the vigor necessary for germination, increasing the incidence of recalcitrant dormancy and reducing survival rates during *ex vitro* acclimatization. Therefore, optimizing embryo quality through the regulation of osmotic potential and the control of abscisic acid (ABA) levels is crucial for ensuring a successful transition toward physiological validation systems (Méndez-Hernández et al., 2019; Stasolla; Yeung, 2003).

Metabolic Divergence in Hormonal Stimulation

There is a marked metabolic dichotomy in how herbaceous and woody models process hormonal signals for regeneration. In herbaceous models (such as Arabidopsis or tobacco), the response is rapid and fluid because of the high sensitivity of TIR1/AFB receptors and a more "open" epigenetic architecture that allows for the immediate degradation of Aux/IAA proteins.

Conversely, woody models (such as *Rubus* or *Vaccinium*) exhibit recalcitrance linked to high oxidase activity and the presence of phenolic compounds that act as competitive inhibitors of hormonal perception. While conventional cytokinins (such as BAP or kinetin) are usually sufficient for herbaceous plants, woody species require "supercytokinins" such as thidiazuron to overcome enzymatic degradation by cytokinin oxidase (CKX) and to unlock totipotency loci that are silenced by repressive histone marks (Bonga et al., 2010; Isah, 2016).

Differences in Response Times and Nutritional Requirements

The temporal dynamics of somatic embryogenesis (SE) vary drastically between herbaceous and woody models. Herbaceous species can reach the globular stage within a few days, whereas woody species require prolonged induction periods, often under conditions of total darkness, to prevent the photolytic degradation of synthetic auxins.

From a nutritional perspective, woody species demand more complex media formulations, with a particular emphasis on organic nitrogen sources such as L-glutamine and the strict regulation of osmotic potential to prevent the premature plasmolysis of proembryogenic cells. Furthermore, in woody systems, the use of adsorbing agents such as activated charcoal is imperative to neutralize quinones generated by phenolic oxidation. These quinones directly interfere with the reactive oxygen species (ROS) signaling required for cellular reprogramming (Isah, 2016; Tang et al., 2004).

Influence of Photoperiod and Physical Culture Conditions

The physical environment of the culture acts as a modulator of the redox state and hormonal stability, simultaneously impacting embryogenesis and the emergence of somaclonal variation.

During the induction phase in woody species, the photoperiod is typically eliminated to mimic the endogenous tissue environment and prevent chlorophyll synthesis. Early chlorophyll production can divert the metabolic pathway toward organogenesis rather than somatic embryogenesis. However, once the embryos reach the torpedo or cotyledonary stage, a transition to a light photoperiod (16 h light/8 h dark) is vital:

1. Activate the photosynthetic apparatus.
2. Facilitate the accumulation of nutrient reserves.

This physical alternation is less critical in herbaceous models, which maintain greater plasticity in terms of light variation during the early stages of *in vitro* development (Fehér, 2015; Isah, 2016; Seguí-Simarro, 2010).

Agronomic Validation and the Aeroponic System as a Technological Bridge

The transition from the *in vitro* environment to final establishment is a critical juncture in plant breeding, as the stability of induced somaclonal variations must be rigorously corroborated. Although molecular techniques such as AFLP provide a genomic perspective of variability, ex vitro testing is indispensable to validate that these mutations translate into a real physiological advantage, such as tolerance to salinity or sodicity stress. Agronomic evaluation allows for the discarding of genetic instabilities or transient epigenetic effects that would not persist throughout the productive cycle.

In this context, the implementation of aeroponic systems with controlled LED lighting emerges as a superior verification technique compared with conventional field methods. This methodology facilitates precise evaluation by nebulizing the nutrient solution directly onto the root system, eliminating the soil's buffering effect and allowing absolute control over variables such as electrical conductivity and pH. At the metabolic level, the use of LED spectra optimizes the photosynthetic rate and pigment biosynthesis, reducing acclimation stress and accelerating the expression of key morphological traits, such as stem architecture and thorn formation.

The benefits of this technique include real-time monitoring of root kinetics, maximum rhizome oxygenation, and the ability to induce controlled abiotic selection pressures without the risks of substrate contamination. This expertise in managing high-precision aeroponic systems for the validation of micropropagated plants has been successfully documented, demonstrating that the integration of aeroponics and LED light is a robust platform for obtaining genotypes with high genetic fidelity and adaptive vigor (Avila-Avila et al., 2024).

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CONCLUSIONS

The conceptual transition of somaclonal variation, from being perceived as “biological noise" to becoming an adaptive breeding tool, represents an essential paradigm shift for addressing the challenges of the Agricultural Anthropocene. These findings confirm that the success of SE depends on unlocking totipotency through extensive chromatin remodeling and the hierarchical activation of master genes such as *BBM* and *WUS*.

As critical considerations for recalcitrant woody species, the case of *Rubus* spp. demonstrates that overcoming physiological and epigenetic barriers requires specific strategies, such as the use of thidiazuron (TDZ) and the supply of organic nitrogen via L-glutamine, to transition from disorganized proliferation toward morphogenetic autonomy.

With respect to applications, leveraging priming or epigenetic memory enables the generation of “epitypes” with constitutive tolerance to abiotic stresses, particularly soil salinity, without resorting to the introgression of exogenous genes. Finally, the use of high-precision validation systems, such as aeroponics with LED lighting, acts as an indispensable technological bridge to confirm that these variations translate into real adaptive vigor under *ex vitro* conditions. This integration between cellular biotechnology and advanced cultivation systems is the most promising path to ensuring food security and resilience in the face of the global climate crisis.

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Chapter 26

Research on the use of rhizobacteria for the biodegradation of herbicides and insecticides in agriculture and their effects on environmental protection

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ABSTRACT

In modern agriculture worldwide, increased crop yields are associated with increased doses of chemical products, such as pesticides and herbicides, to promote better crop development, reduce competition, and decrease the number of pests that negatively affect yield. This situation leads to a significant increase in the pollutant load. Although the biodegradation and neutralization capabilities of the residual effects of chemical products have been studied, this topic is still not fully understood. While many pesticides are selective, they can damage other crops and the environment. Considering the need to protect the environment and the existence of beneficial microbiological conditions in some areas, such as semideserts, alternative biodegradation methods using these microorganisms could be explored, which would have significant economic, ecological, and environmental implications. This study aims to conduct an updated literature review of recent advances in the use of microorganisms for the bioremediation of herbicides and insecticides to gather references and establish biodegradation trials under laboratory and field conditions. Studies confirm that the use of microorganisms is feasible for the biodegradation of herbicides and insecticides; therefore, in some regions, such as Yaqui Valley, Sonora, it could become a sustainable and ecologically viable option for environmental protection.

Keywords: beneficial microbiological conditions, sustainable, chemical products, pesticides

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INTRODUCTION

A wide variety of physical and chemical methods exist for treating contaminated soils and aquifers, but many of these treatments do not destroy hazardous compounds but rather transfer them from one phase to another. Therefore, biological treatment, in which these hazardous compounds are transformed into simpler compounds, is a significant challenge. These methods offer the advantages of operating at ambient temperature and pressure, reducing capital and operating costs. However, a limitation of biotreatment systems is the toxic potential of xenobiotic agents.

In practice, this means that great care must be taken with the microorganisms used, as they can be inhibited or killed by the toxic effects of organic contaminants (Beshay et al., 2002). For biodegradation, the pesticide can ideally serve as the sole source of carbon and energy for microorganisms, including the synthesis of the appropriate enzymes for its transformation, providing an important mechanism in the degradation of xenobiotic compounds in the environment (Murugesan et al., 2010).

Pesticides (herbicides, fungicides, and insecticides) are widely used in agriculture and industry worldwide because of their high activity. More than 55% of the land used for agricultural production in developing countries uses approximately 26% of the total pesticides produced globally.

However, the rate of increase in pesticide use in developing countries is considerably greater than that in developed countries. Pesticides are necessary to protect crops, as losses can constitute approximately 45% of total global food production. Although several classes of pesticides exist, pyrethroids are among the most important because they are highly effective even at very low concentrations. These are commonly used against flies, mosquitoes, and aphids.

The extensive and incorrect use of chemical products leads to greater risks to the health of plants, animals, and the human population. One of the serious problems with pesticides is their high persistence, which increases their toxicity and carcinogenicity (Dalvie et al., 2003; Holmes et al., 2008; Murugesan et al., 2010). In Mexico, the cultivated agricultural area over the last 20 years has totalled 20 million hectares, with rainfed agriculture being the most prevalent system. This area later decreased to 15.5 million hectares, while the area of irrigated agriculture remained at 5 hectares during this period; in total, this represents 75% of the planted area in the country.

The main problems for grain and vegetable crops have been the different types of diseases, pests, and weeds that damage everything from the seed to the plant and the fruit. These problems have been mitigated through the application of pesticides.

The use of pesticides, which are primarily applied in agricultural activities, has caused environmental damage. Soil contamination can lead to a loss of fertility, which can also affect beneficial microbial populations. These beneficial microorganisms can reach the air through the volatilization of

compounds and their dispersion into the atmosphere and surface water and groundwater through leaching and infiltration (Dhananjayan et al., 2019).

The role of bacteria in the biodegradation and transformation of pesticides in the soil varies and depends on the availability, mobility, and toxicity of the pesticide and the physicochemical properties of the soil and the pesticide, among other environmental factors (Racke; Coats, 1990). Among the microbial groups reported as pesticide-degrading organisms are many heterotrophic bacteria that are used as substrates, either by metabolizing the molecules or using them as nutrients and energy sources. This group of bacteria includes *Azotobacter* spp., *Pseudomonas* spp., *Agrobacterium* spp., *Bacillus* spp., *Clostridium* spp., and *Flavobacterium* spp., among others (Madigan et al., 2004).

Use of Pesticides

Pesticides are chemical compounds used to eliminate pests. They are chemical or biological agents that weaken, disable, and kill pests. Pesticides can be divided into several groups according to the type of pest they target: insecticides, herbicides, rodenticides, bactericides, fungicides, and larvicides. The literature mentions that during the 19th and 20th centuries, plant extracts, specifically pyrethrins, were used as insecticides, fungicides, and herbicides. The increased use of pesticides occurred with the advent of synthetic chemistry during the 1930s. During this period, inorganic chemicals such as arsenic and sulfur compounds were applied for crop protection. Arsenic poison was deadly to insects, while sulfur was used as a fungicide. At the beginning of World War II, numerous pesticides were synthesized, primarily organic chemicals such as dichlorodiphenyltrichloroethane (DDT), aldrin, and dieldrin, which were used as insecticides, while 2-methyl-4-chlorophenoxyacetic acid (MCPA) and 2,4-dichlorophenoxyacetic acid (2,4-D) were used as herbicides (Matthews, 2018).

Degree of Pesticide Contamination and its Effect on the Environment

Pesticides are intended to prevent, eliminate, and control harmful pests, which can be detrimental to the environment and human health. Their indiscriminate use can generate high concentrations of pollutants in the environment. Over the years, the World Health Organization has classified pesticides and reported their toxicity and effects on human health. Currently, several pesticides are banned in some countries because of their high toxicity. However, their production and use continue, especially in developing countries (Tudi et al., 2021).

Pesticides persist in the environment and can bioaccumulate and contaminate the food chain, affecting human health and the environment as a whole. Pesticides tend to retain their molecular integrity and their chemical, physical, and functional characteristics for a certain period after their release into the soil (Pérez-Lucas, et al., 2020).

Process and Mechanism of Bacterial Biodegradation of Pesticides

Over the years, several bacterial strains capable of degrading the pesticides present in soils have been identified. Each bacterium possesses a specificity that makes it particularly suitable for a particular degradation process. Operating conditions, such as temperature, pH, water content, and the types of contaminants, affect the adaptation, development, and function of a bacterial strain. Furthermore, metabolites can form during the degradation process and cause additional environmental problems, as they can be more difficult to remove than the original compound, which should be considered a disadvantage. For example, chlorpyrifos, an organophosphate used as an insecticide, is hydrolyzed by microorganisms, and the main degradation product is 3,5,6-trichloro-2-pyridinol (TCP). TCP is more water soluble than chlorpyrifos and causes widespread contamination in soils and aquatic environments. Few microorganisms can degrade pesticides and their metabolites, and among them, the bacterium *Ochabactrum* sp. JAS2 is capable of hydrolyzing both compounds (Abraham & Silambarasan, 2016). In many cases, degradation is easier when a bacterial consortium is used than when an isolated pure culture is used (Doolotkeldieva et al., 2021; Jariyal et al., 2018). In nature, bacteria coexist and depend on each other for their viability. In pesticide degradation metabolic pathways, each bacterium can generate metabolites that can be used as a substrate by others.

During biodegradation processes, pesticides are transformed into degradation products or completely mineralized by the action of microorganisms, which use polluting compounds as nutrients for their metabolic reactions. Enzymes such as hydrolases, peroxidases, and oxygenases play key roles in biotransformation mechanisms, influencing and catalyzing biochemical reactions.

The pesticide degradation process can be divided into three phases, which can be summarized as follows:

Phase 1: Pesticides are transformed into more water-soluble and less toxic products through oxidation, reduction, or hydrolysis reactions.

Phase 2: The products of Phase 1 are converted into sugars and amino acids, which have greater water solubility and lower toxicity.

Phase 3: Conversion of the metabolites from Phase 2 into less toxic secondary conjugates. The microorganisms involved in the degradation process are bacteria or fungi, which can produce intracellular or extracellular enzymes (Khajezadeh et al., 2020).

In many cases, biodegradation is easier when a bacterial consortium is used than when an isolated pure culture is used. In nature, bacteria coexist and depend on each other for their viability. In pesticide degradation metabolic pathways, each bacterium can produce metabolites that can be used as a substrate by others (Doolotkeldieva et al., 2026; Jariyal et al., 2018). Research has reported some organophosphate-degrading microorganisms, such as certain species of the genus Lactobacillus, and some fungi, such as

Hypholoma fasciculare, *Coriolus versicolor*, and *Phanerochaete chrysosporium*. and some bacteria, such as *Serratia liquefaciens*, *Serratia plymuthica*, *Pseudomonas putida* and *Pseudomonas radiora* (Kosimov et al., 2015).

National and international research on pesticide biodegradation

In Mexico, in the Yaqui Valley, Sonora, experiments were conducted in an area with a 15–25-year history of pesticide contamination and handling. Carrillo et al. (2004) isolated mixed bacterial cultures from contaminated water, soil, and sediment samples. This culture consisted of gram-negative bacilli belonging to the genera Pseudomonas, Neisseria, Moraxella, and Acinetobacter. It was evaluated in the presence of DDT (133 ppm) as the sole carbon source and degraded with 43% efficiency within 80 hours. Similarly, the concentrations of the metabolites DDD and DDE, impurities of commercial DDT, were completely degraded without any observed increase.

Another case study using bacteria to degrade contaminants was conducted by Mendoza et al. (2011). In this study, permethrin and cypermethrin were degraded at concentrations of 50 and 100 mg/L, respectively, over a period of 5 to 20 days using strains of *Pseudomonas putida*, *Pseudomonas mendocina*, *Chromobacterium violaceum*, and *Burkholderia cepacia* in batch reactors. These bacteria were isolated from hydrocarbon-contaminated soils in the municipality of Acatzingo, Puebla. This bacterial consortium achieved a degradation efficiency of more than 90% at a concentration of 50 mg/L, and at 100 mg/L, a degradation efficiency of 89% or higher was obtained. At both concentrations, the efficiency of permethrin degradation was greater than that of cypermethrin degradation. Despite these differences, the results demonstrate that these bacteria can be used individually or in consortia for the biodegradation of pyrethroid pesticides.

Degradation studies using bacteria were conducted at the Autonomous University of the State of Morelos. A bacterial consortium was isolated from agriculturally managed soils. This consortium contained strains identified as *Stenotrophomonas malthophilia*, *Proteus vulgaris*, *Vibrio metschinkouii*, *Serratia ficaria*, *Serratia* spp., and *Yersinia enterocolitica*. The experiments evaluated the ability of the bacteria to degrade tetrachlorvinphos (phosphoric acid, 2-chloro-1-(2,4,5-trichlorophenyl) ethenyldimethyl ester) as the sole carbon source. The consortium demonstrated the feasibility of using the product in the bioremediation of soils contaminated with this type of pesticide. The hydrolyzed compounds were analyzed using gas chromatography coupled with mass spectrometry (Ortiz; Sánchez, 2010).

Yah (2018) analyzed the degradation level of 2,4-dichlorophenoxyacetic acid by native bacteria from agricultural soils in the community of San Víctor in Corozal, Belize, Mexico. The results revealed that the degradation percentage by strains B01 and B03 was 32.31% and 29.87%, respectively, after 6 days.

Ortiz-Hernández et al., (2018). Strategies for biodegradation and bioremediation of pesticides in the environment. Strategies for bioremediation of organic and inorganic pollutants, 95-115. aimed to evaluate the effectiveness of atrazine degradation by bacteria isolated from agricultural soils. In this research, the herbicide GESAPRIM granular 90 WG was used. containing 90% atrazine. Selection of atrazine-degrading consortia was performed, along with rRNA gene amplification and molecular analysis. The results showed that the MIG-AI consortium exhibited the best performance, with a specific growth rate of 0.31 h^{-1}, solubilizing atrazine in 2 days and generating clearing zones in 4 days in liquid and solid media, respectively. Furthermore, 16S rRNA gene analysis revealed that the *Stenotrophomonas maltophilia* MIG-AI/2 strain was able to degrade atrazine. Finally, the selected consortia generated clearing zones after 6 days.

Kamika and Momba (2013) conducted a study in Witbank, Mpumalanga, South Africa, where they collected samples of industrial wastewater from an area with a mining history. These water samples underwent various characterization analyses (BOD, pH, and dissolved oxygen). Additionally, the attenuation efficiency of the bacteria *Pseudomonas putida*, *Bacillus licheniformis*, and *Peranema* spp. in water contaminated with heavy metals was evaluated. The results revealed the following removal rates for *Pseudomonas putida* (Co71%, Ni-51%, Mn-45%, V-83%, Pb-96%, Ti-100%, and Cu-49%), *Bacillus licheniformis* (Al-23% and Zn-53%), and Peranema spp. (Cd-42%), with *Pseudomonas putida* and *Bacillus licheniformis* being the most efficient strains.

Benitez et al. (2009) conducted research with various pesticides and reported that the toxicity of herbicides was moderate, above that of insecticides and below that of fungicides, indicating that herbicides were the most toxic to *Bacillus subtilis*. Furthermore, a difference was observed between Furadan and the herbicide Tordon 101. Furadan was described as having no significant toxicity to bacteria, whereas among its compounds, Tordon 101, which contains 2,4-D, was the most toxic herbicide reported (Benitez et al., 2009). These findings coincide with the results obtained in the present study, in which the herbicide Esteron (2,4-D) resulted in greater bacterial inhibition than the insecticides and acaricides Malathion and Furadan (Carbofuran).

This summary table presents the most relevant research and authors during the period 2024–2026 on the use of plant growth-promoting rhizobacteria (PGPR) for the biodegradation of agrochemicals and environmental protection.

In the past two years, research has shifted from studying individual bacteria to focusing on microbial consortia and gene editing (CRISPR) to accelerate soil biodegradation.

CONCLUSIONS

The bioremediation of agricultural soil for pesticide removal can be carried out by exploiting specific or native microorganisms (bacteria and fungi) or through enzymatic degradation. While

numerous studies on soil bioremediation at the laboratory level exist in the literature, little information is available on real-world, large-scale activities. unfortunately, this is due mainly to the lack of cooperation between research laboratories, local authorities that mandate specific soil cleanup measures, and companies involved in the bioremediation of pesticide-contaminated soils.

The use of soil microorganisms and their beneficial interactions with plants and the ecosystem is a promising strategy for achieving sustainable agriculture, especially given the high frequency of glyphosate detection in agricultural soils. It is necessary to gather and organize scientific references related to the microbial bioremediation of the main organophosphate pesticides to facilitate access. Given the significant increase in the use of these agrochemicals in recent years, there has also been a growing interest in understanding how they act in arable soils and how pesticide residues can be eliminated through bioremediation.

The success or failure of bioremediation depends on several factors, such as the competitive ability of the microorganisms, bioavailability, organophosphate concentration, moisture, pH, soil temperature, soil type, the presence or absence of nutrient supplements, and the inoculum concentration.

Microbial consortia have demonstrated greater potential for pesticide degradation than individual populations do since a consortium's effectiveness is the sum of its components. indeed, compatible physiological and ecological relationships between individuals allow them to coexist and develop complex functions that guarantee the metabolism of substrates that are difficult to assimilate in higher percentages for individual populations.

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