

RESEARCH PAPER

Microbial inoculation enhances photosynthetic efficiency in the vegetative stage of blue native corn

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Academic editor: Giuseppe Pulighe ♦ Received 17 June 2025 ♦ Accepted 14 January 2026 ♦ Published 12 March 2026

Abstract

Maize (*Zea mays* L.) is a key part of the daily food in Mexico and holds the title of the most widely consumed cereal in the nation. The aim of this study was to evaluate the photosynthetic efficiency of blue native maize during its vegetative stage through the inoculation with beneficial microorganisms. Four treatments were applied: control, *T. asperellum*, *Bacillus* sp., and a combination of both. Several parameters, including SPAD values (which indicate chlorophyll levels), the concentration of photosynthetic pigments, the activity of the nitrate reductase enzyme, and the levels of soluble amino acids were evaluated. The results showed that SPAD values increased when either microorganism was applied alone or together, compared to the control group. Additionally, the combination treatment significantly boosted *in vivo* nitrate reductase activity, while the *Bacillus* sp. treatment led to the highest levels of soluble amino acids. Although there weren't significant differences in photosynthetic pigment levels across the treatments, the fact that these microbial treatments were used alongside a 50% reduction in chemical fertilizers indicates that they can effectively enhance plant performance even with lower fertilizer inputs. Overall, these findings highlight the potential of *T. asperellum* and *Bacillus* sp. as biofertilizers in the production of native maize and emphasize their importance in promoting more sustainable farming practices.

Keywords

Bacillus sp., Biofertilizers, Plant physiology, Sustainable agriculture, *Trichoderma asperellum*, *Zea mays* L.

Introduction

Corn (*Zea mays* L.), a member of the *Poaceae* family (Wellhausen et al. 1951), is the staple food in Mexico (Hernández-Casillas and Esquivel-Esquivel 2004), standing out as the most consumed cereal. Mexico leads the world in per capita corn consumption, reaching an impressive 336.6 kg per year (SIAP 2018).

In 2024, the area planted with blue corn in Mexico was 9,331.00 ha, with a total yield of 11,820.75 t ha⁻¹. Only two states in the entire country produced this type of corn in 2024, with Puebla ranking first at 7,446.38 t ha⁻¹ and Chihuahua second at 4,374.37 t ha⁻¹ (SIAP 2025).

Pigmented corn varieties have played a vital role in various cultures and myths throughout history, religious practices, and diet of indigenous communities in Mexico.

Among the Aztecs, yellow, blue, and red corn were recognized as linked to the cult of “Chicomecoatl,” the goddess of nutrition and food (Sahagún 1975).

Anthocyanins in aleurone are responsible for the blue color found in the kernel of maize (Betrán et al. 2001). These water-soluble pigments possess a number of unique characteristics: they may lower blood plasma cholesterol and triglyceride levels and, thus, help prevent cardiovascular diseases. Their widespread agency in the food industry is as a natural colorant for products that include wine, jam, and fruit juice (Maltros et al. 1999). In the blue maize, anthocyanins are derived mainly from cyanidin, whereas with red maize, they originate mostly from pelargonidin (Salinas et al. 1999).

The increase in agricultural production while reducing the use of chemical fertilizers is essential for achieving more sustainable and environmentally friendly agricultural systems. In this context, the use of plant growth-promoting microorganisms (PGPMs) represents a promising strategy to enhance crop productivity, as well as food quality and safety, within sustainable and eco-friendly agricultural frameworks (Asghari et al. 2020; Etesami 2020).

These microorganisms contribute to plant performance by improving nutrient availability in the soil, regulating phytohormone production, and enhancing resistance against pathogens. Furthermore, they mitigate the effects of both biotic and abiotic stresses, reduce dependence on chemical fertilizers, and ultimately increase crop productivity (Abhilash et al. 2016; Etesami 2020). The most well-known genera containing PGPM species include *Azospirillum*, *Bacillus*, *Pseudomonas*, *Rhizobium*, *Trichoderma*, *Beauveria*, and *Streptomyces*, among others (Abhilash et al. 2016; Gouda et al. 2018).

Native maize varieties of different colors are recognized as an important genetic and cultural resource, but their physiological performance is still not well researched. The majority of studies on maize physiology, and especially those assessing photosynthetic parameters, have been concentrating on yellow or commercial hybrid cultivars, leaving a clear gap in understanding how native pigmented varieties respond to agronomic or microbial inoculation. This gap in the literature initiated a strong curiosity in the utilization of effective beneficial microorganisms namely *Bacillus* spp. and *Trichoderma* spp. Among the many Plant Growth-Promoting Microorganisms, *Bacillus*—a genus of Gram-positive, aerobic, and ubiquitous bacteria—stands out for its agricultural relevance (Grover et al. 2011; Vejan et al. 2016).

A key characteristic of these bacteria (*Bacillus* spp.) lies in their capability to form spores, a defensive strategy that allow them tolerate severe environmental challenges (Siddikee et al. 2010). Such bacteria in crops foster growth in plants by direct mechanisms for example, nitrogen fixation (N_2), siderophore production, nutrient solubilization, and phytohormone synthesis; and indirectly generate hydrogen cyanide, exopolysaccharides, enzymes with lytic activities, and biofilms, among other things, under diverse environmental settings (Sarmiento-López et al. 2022; Mejía-Bautista et al. 2022).

Similarly, research on *Trichoderma* spp. shows that certain species act not only as antagonists against phytopathogens (Cabrefiga et al. 2023) but also offer significant ad-

vantages to crops. These benefits include promoting plant growth, improving seed germination, and increasing emergence of seedling (Chagas et al. 2016). *Trichoderma* spp. serve as growth regulators by producing phytohormones (Jaroszuk-Ścisł et al. 2019), like IAA (indole-3-acetic acid) (Sabre et al. 2017) and GA3 (gibberellic acid) (Turaeva et al. 2020), which are crucial for plant development. These compounds aid in root formation and structure, boost germination of seed and also, viability, enhance photosynthesis, flower formation, and overall yield quality (Halifu et al. 2019).

These microorganisms have been extensively documented to enhance nutrient uptake, stimulate chlorophyll biosynthesis, and increase photosynthetic efficiency under both optimal and stress conditions, as demonstrated by numerous recent studies across economically important crops specifically maize. For example, the maize plants inoculated with *Bacillus* sp. showed a highly positive response in their crop growth with a plant density higher after emergence and just before harvesting. However, on the other hand, the bacterium treated plants provided grain yields higher than 10 t ha^{-1} , and protein content; in the control plots (without the bacteria), yields were 9.7 t ha^{-1} (Jarecki 2023). Ijaz et al. (2021) and Ahmad et al. (2023), inoculated maize seeds with a mix of *Bacillus* spp. and *Paenibacillus polymyxa* and a notable boost in the SPAD (Soil Plant Analysis Development) index when compared to untreated plants was observed, leading to impressive improvements in plant growth.

Regarding the fungus *Trichoderma* spp. in the evaluation of four species in maize (*T. asperellum*, *T. melanomagnum*, *T. spirale* and *T. reesei*), Cuenca-Sedamano et al. (2022) found that the inoculation of the plants with *T. spirale* produced the tallest plants, *T. melanomagnum* had the largest ear diameter, and *T. asperellum* - *T. melanomagnum*, especially at concentrations of 1×10^{10} and 1×10^{11} CFU, significantly increased yield. Evidence from another study suggested that the application of *T. harzianum* T-22 at a concentration of 1×10^8 CFU in combination with mineral (NPK) and manure fertilization resulted in enhanced photosynthetic efficiency in *Z. mays* plants under drought stress (Radzikowska-Kujawska et al. 2025).

There is no literature addressing the microbial inoculation in native maize to study the crop's photosynthetic activity, for this motive the aim of this study was to evaluate the photosynthetic efficiency of blue native maize during its vegetative stage through the inoculation with beneficial microorganisms.

Materials and methods

Crop management

The planting of blue corn took place in a field at the Facultad de Ciencias Agrícolas y Forestales (FCAyF) of the Universidad Autónoma de Chihuahua (UACH), while the analysis of the physiological parameters was conducted at the Centro de Investigación en Alimentación y Desarrollo (CIAD) AC, Unidad Delicias. The maize was cultivated from August to December 2023.

Before sowing, the blue corn seeds were soaked for 24 h in 100 mL of either *T. asperellum* (1×10^{10} UFM mL⁻¹) (Martínez et al. 2023) and/or *Bacillus* sp. (1×10^9 CFU mL⁻¹) (Dong et al. 2023), depending on the treatment. The seeds were then planted in sandy-clay soil, arranged in furrows that were 80 cm apart, with three seeds per linear meter (about 33.3 cm between each plant). Fertilization occurred in three stages: the first two applications were spaced one month apart, and the third was applied 22 d after the second. The initial fertilizer application included 300 kg ha⁻¹ of urea and 50 kg ha⁻¹ of commercial MAP® (11-52-00), following that, 20 L ha⁻¹ of commercial Nitro-Sul® (12% of nitrogen and 26% of sulphur), and the final application consisted of 150 kg ha⁻¹ of urea dissolved in water, which was delivered through irrigation. The plants received irrigation every three days using a drip tape system.

Study design and treatment conditions

The investigation followed a completely randomized design with four treatments, in which T1 = control; T2 = *T. asperellum*; T3 = *Bacillus* sp.; T4 = T2 + T3. Ten replications per treatment were used (Fig. 1).

In this study, Treatment 1 acted as the control group, relying solely on traditional commercial fertilizers without any biofertilizer, for Treatment 2, the biofertilizer *T. asperellum*, was introduced following the guidelines set by Razafintsalama et al. (2022), using a fungal concentration of 1×10^{10} CFU mL⁻¹. The *T. asperellum* utilized here is part of the collection of the Molecular Genetic Laboratory of FCAyF, Mexico and the GenBank accession number is MN950427. Moving on to Treatment 3, root nodules of common bean (*Phaseolus vulgaris* L.) collected in Delicias, Chihuahua, Mexico, served as the source for isolating *Bacillus* sp. strains. The method described by Bakuei et al. (2015) was followed for disinfection of the nodules and bacteria culture. These bacterial isolates were then cultured in a LB (Luria Bertani) liquid medium at 30 °C for 72 h and inoculated at a concentration of 10^6 – 10^7 CFU mL⁻¹. Treatment 4 combined T2 and

T3. *Trichoderma asperellum* and *Bacillus* sp. were applied to the soil around the rhizosphere every 15 d. In all treatments that included biofertilizers, chemical fertilizer dosage was reduced to 50% of the standard application rate.

Plant sampling

Before plants started reproductive phase, the above-ground parts were harvested and thoroughly rinsed with distilled water, then the fresh tissue was used to evaluate a range of physiological parameters, such as the chlorophyll index (SPAD values), the content of photosynthetic pigments, the activity of the nitrate reductase enzyme, and the concentration of soluble amino acids.

Chlorophyll index (SPAD values)

To estimate chlorophyll content, a SPAD-502 portable chlorophyll meter from Konica Minolta Sensing, Inc., Japan was used. This handy device gives a quantitative measure of how green the leaves are (Díez-Méndez and Menéndez 2021). The measurements were taken around midday when the sunlight was at its peak, collecting three random readings from each plant to ensure the representative values.

Photosynthetic pigments

The method outlined by Wellburn (1994) was used for the analysis of photosynthetic pigments. For this leaf samples were used with a stalk diameter of about 7 mm and a weight of 0.125 g per treatment, placing them in tubes with 10 mL of methanol, kept them in the dark for 24 h until pigments extraction. Later, at 470, 653, and 666 nm the absorbance was determined using a GENESYS™ 10S UV-VIS spectrophotometer from Thermo Fisher Scientific in Madison, WI, USA. The pigment concentrations

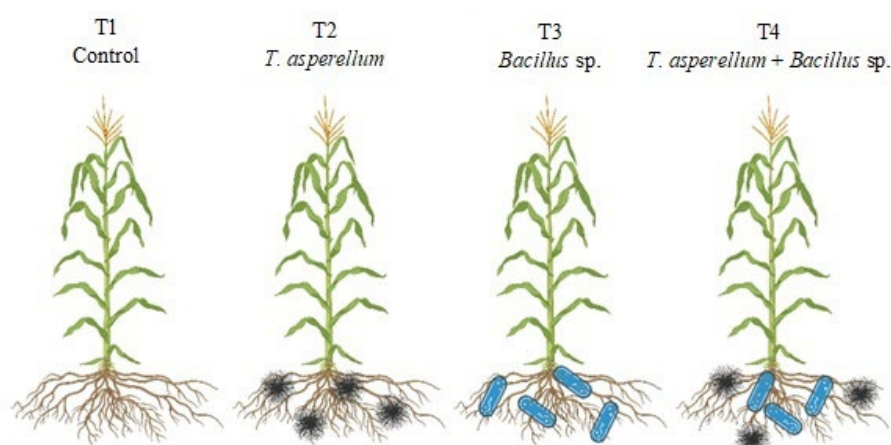


Figure 1. Treatments used on blue native corn plants in maize plants. T1 = control; T2 = *Trichoderma asperellum*; T3 = *Bacillus* sp.; T4 = T2+T3.

were calculated using specific equations (Equations 1–4) and expressed them as micrograms per square centimeter of fresh leaf weight:

$$\text{Chl } a^* = [15.65(A666) - 7.34(A653)] \quad (1)$$

$$\text{Chl } a = (\text{Chl } a^* \times V_f \times W_1) / (W_2 \times \pi \times r^2 \times n)$$

$$\text{Chl } b^* = [27.05(A653) - 11.21(A666)] \quad (2)$$

$$\text{Chl } b = (\text{Chl } b^* \times V_f \times W_1) / (W_2 \times \pi \times r^2 \times n)$$

$$\text{Carotenoids}^* = [(1000 \times A470) - 2.86(\text{Chl } a) - 129.2(\text{Chl } b)] / (221) \quad (3)$$

$$\text{Carotenoids} = (\text{Carotenoids}^* \times V \times W_1) / (W_2 \times \pi \times r^2 \times n)$$

$$\text{Total chlorophyll} = \text{Chl } a + \text{Chl } b \quad (4)$$

where V_f : final volume; W_1 : weight per leaf disc; W_2 : total weight of leaf discs; r : radius of the leaf discs; n : number of leaf discs.

Nitrate reductase enzyme activity (NR activity) "in vivo"

The activity of NR (EC 1.7.1.1) was quantified following the method outlined by Kandil and Özdamar (2023). Fresh leaves were used, from which discs with a diameter of 7 mm and weight of 0.1 g were obtained and placed in tubes with 10 mL of an incubation buffer [100 mM potassium phosphate (pH 7.5) and 1% (v/v) propanol]. The samples were vacuum filtered in a NAPCO 5851 oven (USA) and placed at 30 °C in the dark for one hour in a digital incubator WIG-50 (DAIHAN SCIENTIFIC, Seoul, Republic of Korea). The samples were placed for 15 min in a FELISA FE-371 water bath (Mexico) to stop the enzymatic activity. Nitrite was determined colorimetrically at 540 nm using a GENESYS™ 10S UV-VIS spectrophotometer (Thermo Fisher Scientific, Madison, WI, USA) by mixing 1 mL of the extract with the enzyme, 2 mL of 1% (w/v) sulfanilamide in 1.5 M HCl, and 2 mL of 0.02% (w/v) N-(1-naphthyl)-ethylenediamine dihydrochloride (NNE-DA). Finally, the results were expressed as $\mu\text{mol NO}_2^-$ per gram of fresh weight per hour ($\mu\text{mol NO}_2^- \text{ g}^{-1} \text{ FW h}^{-1}$).

Soluble amino acids

To determine the soluble amino acid content, the method developed by Yemm et al. (1955), was used with some modifications from Sánchez et al. (2004). The procedure was started by homogenizing about 0.5 g of plant tissue in 5 mL of 50 mM phosphate buffer (pH 7.0) on ice to keep the sample intact. After that, the homogenate was filtered through a mesh and centrifuged it at 12,000 g for 15 minutes. The supernatant was used for the quantification of

amino acids, with the addition of 100 μL of it and 1.5 mL of ninhydrin reagent in a test tube, placed for 20 min in a water bath at 100 °C to develop the color, while a glycine standard curve for reference was prepared. After the incubation period, both the samples and standards were cooled in an ice bath. Next, 8 mL of 50% propanol were added to each tube and let the mixtures sit for 30 min. The amino acid concentrations were measured with an absorbance at 570 nm, and were reported as $\text{mg g}^{-1} \text{ FW}$.

Statistical analysis

To analyze the data statistically, the SHAPIRO–WILK test ($p \leq 0.05$) was applied to verify normal distribution, while Levene's test at the same significance level was used to examine variance homogeneity. A one-way ANOVA ($\alpha = 0.05$) was then performed to detect significant differences among treatments. DUNCAN'S multiple range test ($p \leq 0.05$) was employed to compare means across different factor levels, such as dosage and application timing. All analyses were carried out using SAS software, version 9.0.

Results

Chlorophyll index (SPAD values)

In the case of blue native corn, the chlorophyll index (SPAD values) was influenced by microbial inoculation (Fig. 2). This measurement simplifies the determination of leaf chlorophyll content and allows for estimating the efficiency of the photosynthetic process.

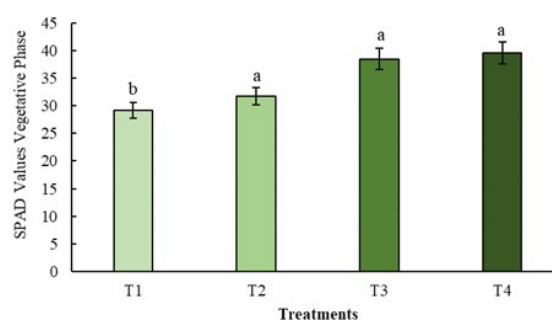


Figure 2. Effects of microorganism applied to blue native corn (*Z. mays*) on SPAD values: T1 = control; T2 = *T. asperellum*; T3 = *Bacillus* sp.; T4 = T2 + T3. Bars represent the mean values $\pm$ standard error. Different letters indicate statistically significant differences according to Tukey ($p \leq 0.05$).

Photosynthetic pigments

The influence of the microorganisms used in this research on the chlorophyll and carotene pigments in blue native maize is showed in Fig. 3.

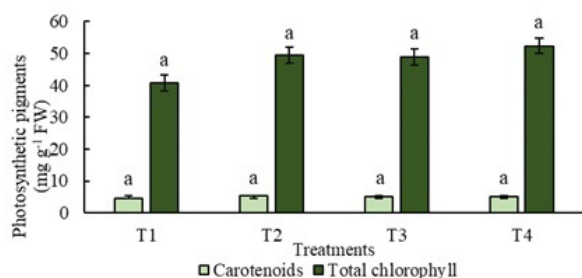


Figure 3. Effect of microorganisms applied to blue native corn (*Z. mays*) on photosynthetic pigment content, where: T1 = Control; T2 = *T. asperellum*; T3 = *Bacillus* sp.; T4 = T2 + T3. Bars represent the mean values $\pm$ standard error. Different letters indicate statistically significant differences according to Tukey's test ($p \leq 0.05$).

All the treatments were statistically equal so the use of microorganisms didn't really change this parameter. That said, given that chemical fertilization was reduced by 50% when microorganisms were applied, it is a good idea to reduce chemical fertilizer inputs and start using microbial inoculants.

Nitrate reductase enzyme activity (NR activity) "in vivo"

The activity of NR activity "in vivo" in the different treatments is shown in Fig. 4.

NR activity increased by 20.22% in the treatment combining both microorganisms (T4) related to the control. Alternatively, when *T. asperellum* (T2) was used decrease a of 9.20% was observed, although this difference was not statistically significant with T1 (control) and T3 (*Bacillus* sp.).

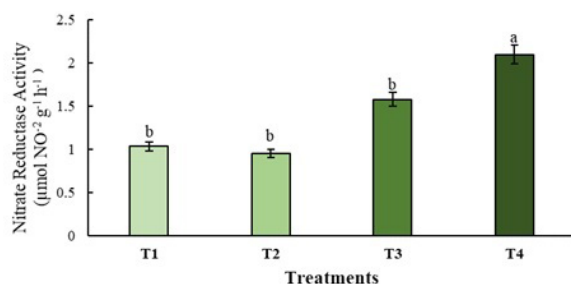


Figure 4. Effect of microorganisms applied to blue native corn (*Z. mays*) crops on the *in vivo* nitrate reductase enzyme activity, where: T1=Control; T2= *T. asperellum*; T3= *Bacillus* sp.; T4= T2+T3. Bars represent the mean values $\pm$ standard error. Different letters indicate statistically significant differences according to Tukey ($p \leq 0.05$).

Soluble amino acids

In this study, the treatment using *Bacillus* sp. (T3) led to the highest levels of soluble amino acids, showing a notable 17% increase compared to the control group (Fig. 5).

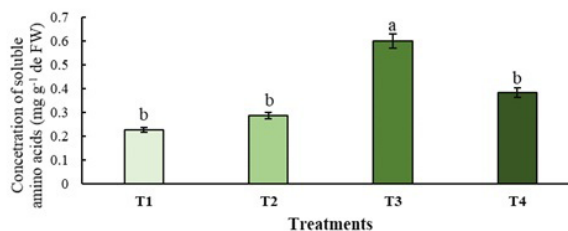


Figure 5. Concentration of soluble amino acids with different microorganism treatments applied to the blue native corn crop (*Z. mays*) where: T1 = Control; T2 = *T. asperellum*; T3 = *Bacillus* sp.; T4 = T2 + T3. Bars represent the mean values $\pm$ standard error. Different letters indicate statistically significant differences according to Tukey's test ($p \leq 0.05$).

The lowest results were obtained in the control treatment, although without significant differences with treatments 2 and 4.

Pearson correlation heatmap

The correlation heatmap revealed significant relationships among the physiological and biochemical parameters evaluated in blue native corn during the vegetative phase under microbial treatments. Notably, SPAD values—used as an indicator of chlorophyll content—were positively and significantly correlated with total chlorophyll ($r = 0.71$, $p < 0.01$) and amino acid content ($r = 0.72$, $p < 0.01$). This suggests that enhanced chlorophyll indices are closely associated with improved N_2 assimilation and metabolic activity in the plant.

Total chlorophyll exhibited a robust positive correlation with carotenoids ($r = 0.89$, $p < 0.001$), reflecting coordinated accumulation of photosynthetic pigments, even though pigment levels were not statistically different across treatments. This highlights that microbial inoculation, despite reducing fertilizer input, maintained functional pigment biosynthesis pathways.

The moderate correlations between amino acid content and carotenoids ($r = 0.50$), and with total chlorophyll ($r = 0.53$), further support the hypothesis that *Bacillus* sp., which promoted higher amino acid levels, may be influencing both N_2 metabolism and pigment stability.

Interestingly, nitrate reductase activity showed only weak correlations with other variables, indicating that its activity might respond independently or be transient compared to the other metabolic markers analyzed (Fig. 6).

Radar chart: multivariate comparison by microbial inoculation

The radar chart shows the response of maize plants to different microbial inoculation treatments, considering five key variables: relative chlorophyll content (SPAD), total chlorophyll, carotenoids, soluble amino acids, and nitrate reductase activity (Fig. 7).

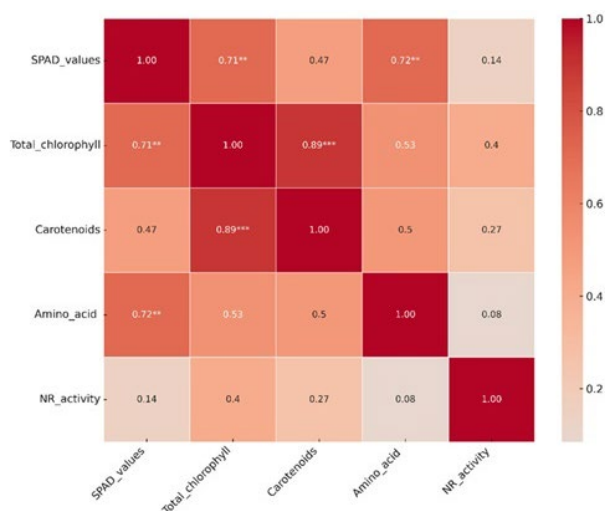


Figure 6. The heatmap displays the Pearson correlation coefficients among the physiological and biochemical variables measured in blue native corn (*Z. mays*) crops. The color gradient ranges from blue (indicating negative correlations) to red (indicating positive correlations), illustrating the strength and direction of the relationships between variables. Numerical values within each cell represent the correlation coefficient, and asterisks indicate the statistical significance of the correlation as follows: $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

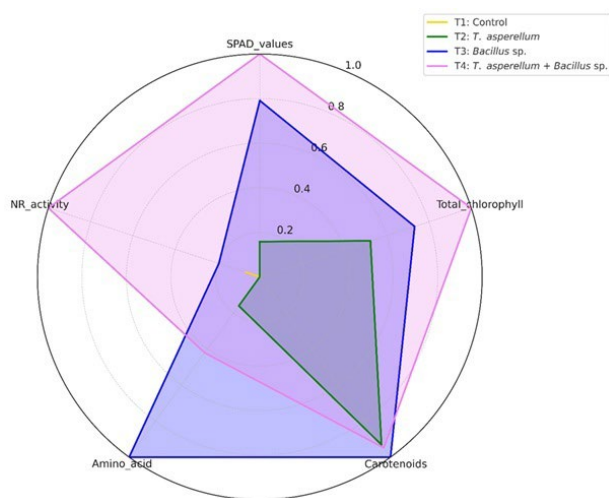


Figure 7. Radar chart illustrating the normalized physiological and biochemical responses of *Z. mays* seedlings under four treatments: T1 = Control; T2 = *T. asperellum*; T3 = *Bacillus* sp.; T4 = T2 + T3. The evaluated variables included relative chlorophyll content (SPAD), total chlorophyll, carotenoids, total free amino acids, and nitrate reductase (NR) activity. All values were normalized between 0 and 1 to facilitate multivariable comparison. The chart reveals distinct response patterns among treatments, with the microbial consortium (T4) showing a synergistic effect on most physiological and biochemical parameters.

The control treatment (T1) showed the lowest values across most variables, which suggests that there was limited physiological and biochemical growth. Conversely, the

inoculation with *T. asperellum* (T2) primarily boosted the levels of photosynthetic pigments like chlorophyll and carotenoids, although its impact on other factors was somewhat moderate. In contrast, the treatment with *Bacillus* sp. (T3) really shone with a significant increase in amino acids and carotenoids, indicating a boost in N_2 metabolism and the production of antioxidant compounds. Lastly, the combined treatment (T4: *T. asperellum* + *Bacillus* sp.) delivered the most comprehensive results, achieving the highest readings in SPAD, total chlorophyll, and nitrate reductase activity.

Discussion

The interactions between plants and microbes have been widely studied due to the current importance of promoting sustainable agriculture, which leads to environmental conservation. The role of microorganisms that can promote plant growth in crop cultivation is particularly important, as they offer eco-friendly alternatives that can minimize or replace chemical fertilizers. These microbes can be drilled into the seeds or sprayed on the soil near the root system to improve plant growth through nutrient enhancement, plant resistance to abiotic and biotic stress and modulation of phytohormones (Varma et al. 2019; Khoshru et al. 2020; Khan et al. 2020). PGPM, also contribute to enhanced foliage and leaf expansion, increased chlorophyll levels, improved photosynthetic efficiency, accelerated seed germination, greater seedling vigor, taller plants, more developed root systems, and higher biomass yield (Lopes et al. 2021). In this research the chlorophyll index (SPAD value) was higher with the microbial inoculation alone and in combination. Chlorophyll index is the fast, non-invasive method for chlorophyll content determination in plants and for the estimation of their nutritional status (Shrestha et al. 2012). In native blue corn, this index increased with the inoculation of the tested microorganisms, a result that can be attributed to their beneficial actions applied to the plants. (plant hormones production, solubilization of phosphorus (P) in the soil, fix atmospheric N_2 , and strengthening the plant's defence against diseases). When these microorganisms are used together, they can enhance and boost each other's effects, resulting in improved photosynthetic activity. These results align with several earlier studies on different corn varieties and crops. For instance, Dutta et al. (2024) in onions (*Allium cepa* L.), where using *T. asperellum* and *T. longibrachiatum* led to increases in the SPAD index and other growth metrics such as plant height and relative water content. Similarly, Jarecki (2023) reported higher chlorophyll content in corn leaves treated with microbial biofertilizers, which also resulted in better grain yield and protein levels. In another study in corn, Cuenca-Sedamano et al. (2022) also demonstrated that various *Trichoderma* species enhance physiological indicators, including the number of leaves per plant and cob diameter, further highlighting the potential of these strains as effective biostimulants. Regarding *Bacillus* spp. Ijaz et al. (2021) found that inoculating of maize plants with several strains notably enhanced the

SPAD index, linking this improvement to better solubilization of vital nutrients like iron and manganese, as well as increased chlorophyll synthesis in plants.

Photosynthetic pigments like chlorophyll (Chl) and carotenoids (Car) are vital players in the photosynthesis game for maize. They soak up sunlight and convert it into chemical energy (Gitelson et al. 2006). The results obtained in this research indicate that it is possible to reduce the use of chemical fertilizers without harming photosynthetic process, which opens the door to the use of microorganisms as a more sustainable option. The levels of chlorophyll and carotenoids can give insights into photosynthetic capacity (Cha-Um and Kirdmanee 2009), disease presence (Chen et al. 2015), nutritional health, plant development (Schlemmer et al. 2013), and soil moisture—all of which play a role in quality and crop yield. So, variations in the level of these pigment are important plant health indicators (Darvishzadeh et al. 2008). In a comparable study on hybrid maize (BM207), de Lima Gonilha et al. (2024) reported reduced photosynthetic pigment levels when chemical fertilization was fully replaced with *B. subtilis*, *T. harzianum*, or their combination—whereas the highest pigment levels occurred at 50% fertilization with individual inoculants—while in this study with blue maize, no significant differences in pigment content were found between full (100%) and reduced (50%) fertilization combined with microbial inoculation, highlighting the potential to minimize chemical inputs without compromising photosynthetic performance. Similar evidence from another research demonstrated that applying *T. harzianum* T-22 at a concentration of 1×10^8 CFU, along with a 50% reduction in mineral (NPK) fertilization and supplemental organic manure, not only improved photosynthetic pigment levels in *Z. mays* under drought stress, but also significantly enhanced stomatal conductance, transpiration rates, and overall yield performance (Radzikowska-Kujawska et al. 2025). Moreover, research like that of Tahir et al. (2017) has shown that volatile compounds released by *B. subtilis* not only boost root growth but also enhance chlorophyll accumulation and net photosynthesis, which is clearly reflected in higher SPAD values. This backs up the idea that microorganisms can influence plants through both direct methods (like producing phytohormones and solubilizing nutrients) and indirect methods (like helping plants tolerate stress). The findings presented here not only align with what has been observed in other species and environments, but they also shed light on the application of these biotechnologies in native corn varieties, an area that hasn't been thoroughly explored. This knowledge is incredibly valuable for achieving more sustainable production systems, particularly in regions where access to chemical fertilizers is limited or where there is a push to minimize their use.

One effective way to gauge the efficiency of N_2 fertilization is by assessing the activity of the NR enzyme, which is crucial for the N_2 assimilation process. This enzyme is influenced by a variety of internal (endogenous) and external (exogenous) factors, all of which can directly impact crop productivity (Hernández-Cruz et al. 2015). The enzyme ac-

tivity increased in the treatment where both microorganisms were used together (T4), while a decline was noted in the treatment with *T. asperellum* (T2). This pattern might suggest that *T. asperellum* has a more indirect role in enhancing N_2 availability, possibly by boosting microbial activity in the rhizosphere and encouraging root development, which in turn facilitates better nutrient absorption. Recent studies in this field have indicated that different *Trichoderma* species possess varying abilities to solubilize nutrients like P and potassium (K). While they aren't direct N_2 fixers like some bacteria commonly found in the rhizosphere, these fungi can still help improve N_2 availability in the soil. For example, Vinale et al. (2008) showed that *Trichoderma* can solubilize nutrients in the soil, leading to increased uptake by plant roots. Also, Halifu et al. (2019) hypothesized that the levels of N_2 and P in soil were enhanced following the inoculation of *Trichoderma* because the fungus could degrade nutrient macromolecules into forms that plants could more easily absorb. Therefore, what was observed in treatment T2 may really be due to the enhancement of soil conditions by *T. asperellum* instead of a better ability to modulate the NR enzyme. In the treatment where the microorganisms were combined (T4), the enzyme activity increased, which can be explained by a synergistic interaction between both microorganisms that must increase intracellular growth or colonization of bacteria in the fungal hypha, and with this the growth of roots facilitating the absorption of nutrients and therefore N_2 (Warmink et al. 2011; Araújo et al. 2022). The synergistic effect that enhances nutrient assimilation has been confirmed by various studies, such as Silva et al. (2023) reported that corn plants (*Z. mays*) exhibited improvements in growth-related parameters—like plant height, leaf area, shoot dry matter, and root dry matter—when inoculated with beneficial microorganisms and supplemented with rock powder, even under a reduced chemical fertilization regime (50% of the standard dose) compared to control treatments. Their findings highlighted the potential of combining *B. subtilis* and *T. harzianum* inoculation with rock dust to enhance both soil health and plant fertility. Similarly, other studies have attributed the growth-promoting effects of *Bacillus* spp. and *Trichoderma* spp. to improved nutrient absorption, modulation of phytohormones, and elevated carbohydrate metabolism and photosynthetic activity (Fiorini et al. 2016; Backer et al. 2018; Meena et al. 2020).

Amino acids and proteins represent the primary compounds derived from the N_2 assimilation process in plants, playing a fundamental role in both nutritional value and various metabolic functions (Gutiérrez-Ruelas et al. 2021; Agüero-Esparza et al. 2022). The increase in soluble amino acids obtained in native corn plants when inoculated with *Bacillus* sp. could be due to its bioactive metabolites such as gibberellins, IAA and high concentrations of free phosphate (Kang et al. 2015a). Colonization by the bacteria promotes atmospheric N_2 fixation and strongly influences the uptake of N_2 and amino acids by amino acid transporters in plants (Jone et al. 2005; Kang et al. 2015b). In another study on mustard plants (*Sinapis alba* L.), Kang et al. (2014) obtained increases in the contents of chloro-

phyll, fructose, glucose, sucrose and amino acids in plants inoculated with the *B. megaterium* strain mj1212, compared to non-inoculated plants. These results reinforce the hypothesis that *Bacillus* sp. has a multifunctional biostimulant effect on plant metabolism. Furthermore, a recent study by López-Ordaz et al. (2022) in corn and soybeans found that the inoculation with bacteria from the genus *Bacillus* not only improved crop growth and yield parameters but also significantly increased N_2 levels and microbial activity in the soil, suggesting a synergy between soil improvement and internal plant metabolism. These findings support the idea that *Bacillus* sp. acts as a versatile biostimulant, positively influencing plant metabolism.

The strong positive relationship between SPAD values and total chlorophyll content ($r = 0.71$, $p < 0.01$), along with soluble amino acids ($r = 0.72$, $p < 0.01$), indicates that these microorganisms directly influence chlorophyll production and N_2 uptake. This is in accordance with the results from Salman et al. (2025), who classified *Bacillus* spp. alongside others as phosphate-solubilizing endophytic bacteria that drastically increased the biomass and antioxidant activity in maize when yielding a drought-stressed environment. In the same manner, Mohamed and Abd-Elsalam (2024) remarked that *Trichoderma* spp., the growth-promoting fungi, increased nutrient uptake and resistance to stress which translated into better photosynthesis. Besides, Hussain et al. (2024) also communicated that through the exclusive functional role of chlorophyll and carotenoid increment; the fungi, *Aspergillus niger* can certainly contribute to better plants which is a sign of similar physiological pathways of different beneficial microorganisms.

Research has shown that beneficial fungi and bacteria work together more effectively than single inoculants by enhancing traits like nutrient mobilization, phytohormone production, and stress resilience (Zhou et al. 2021). The authors point out that these bacteria help in synthesizing amino acids and proteins by promoting N_2 fixation and activating biochemical pathways related to plant nutrition and defence. Overall, these findings align with the research where *Bacillus* sp. positively influenced the accumulation of soluble amino acids during the vegetative stage of blue native corn, ultimately enhancing its physiological efficiency and yield potential even with reduced fertilization.

Conclusions

The results of this study suggest that the use of beneficial microorganisms such as *T. asperellum* and *Bacillus* sp. can positively influence photosynthetic efficiency in blue native

corn during the vegetative stage with a reduction of chemical fertilization (50%). The increase in SPAD values and nitrate reductase activity, as well as the enhanced accumulation of soluble amino acids, indicate improved N_2 assimilation and chlorophyll-related functions—key components of efficient photosynthesis. Although the levels of photosynthetic pigments did not show significant differences, the ability to maintain these parameters while reducing chemical fertilizer input by 50% highlights the potential of microbial inoculants to sustain photosynthetic performance under lower-input conditions. These findings support the integration of microbial biofertilizers into sustainable crop management practices aimed at improving nutrient use efficiency and plant physiological performance.

Funding statement

The author has no funding to report.

Author contributions

Daniela Cabezas-Pérez: Conceptualization, Investigation; Sandra Pérez-Álvarez: Conceptualization, Validation, Investigation, Writing – original draft preparation and Supervision; Luisa Patricia Uranga-Valencia: Validation, Visualization Esteban Sánchez-Chávez: Methodology, Resources, Supervision; Erick H. Ochoa-Chaparro: Formal analysis, Writing – review and editing; Joel Rascón-Solano: Validation; Data curation; Marco Antonio Magallanes Tapia: Data curation, Writing – review and editing; Rodolfo Contreras-Martínez: Writing – review and editing. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Not applicable.

Conflicts of interest

The authors declare no conflicts of interest to report regarding the present study.

Acknowledgments

The authors have no support to report.

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