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FULL-LENGTH RESEARCH ARTICLE

Field Assessment of a Plant Growth-Promoting *Pseudomonas* on Phytometric, Nutrient, and Yield Components of Maize in a Milpa Agrosystem

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Abstract The traditional milpa system, a polyculture originating in Mesoamerica, centers around maize (*Zea mays* L.), associated with pumpkin (*Cucurbita* sp.) and beans (*Phaseolus vulgaris* L.). The application of plant growth-promoting rhizobacteria (PGPR) under a milpa agrosystem has been little explored. In this study, a maize crop in a milpa system was fertilized with the PGPR *Pseudomonas fluorescens* UM270 during the 2021 and 2023 seasons, and various phytoparameters (plant height, root length, chlorophyll concentration, root dry weight and total plant dry weight), total production, and grain nutrition were evaluated. The results showed that UM270 improved chlorophyll concentration and increased plant height, root length, and dry weight in maize plants. Co-fertilization with UM270 and diammonium phosphate (DAP) significantly improved plant and corn cob weight compared to controls with single fertilizations in both the 2021 and 2023 seasons. Notably, corn production increased by more than 40% in the corn monoculture inoculated with UM270 compared to the uninoculated plants. The UM270 + DAP cofertilization in the monoculture was also increased by more than 50% in both cycles. When analyzing the nutritional content of the corn cob, nitrogen and phosphorus increased with the inoculation with UM270, while other elements, such as potassium and calcium, were higher in treatments co-inoculated with UM270 + DAP. Based on our research, this study is the first to report the milpa as a suitable model for bioinoculation with PGPR, demonstrating its potential to increase maize yield and benefit other associated crops.

Keywords Bioinoculation · PGPR · Rhizobacteria · *Phaseolus vulgaris*

Introduction

In the middle of the twentieth century, there was an increase in agricultural production and this exceeded the current increase in population. This transcendental change in agriculture was called "green revolution", and represented a boost in the world's most developed and later less developed nations [39, 48]. The main objective was to exponentially increase production through the use of hybrid seeds in large monocultures, planted with heavy machinery. To supply the nutrients required by the plants, different synthetic sources were applied as fertilizers [3, 35].

Maize has been one of the most impactful crops since the beginning of the Green Revolution, due to its nutritional value and high demand in the food, balanced feed, and pharmaceutical industries. In recent years, its use in bioethanol production has further cemented its status as one of the most important cereals worldwide [12]. However, its establishment as a monoculture has increased the presence and resistance of pests and diseases, and the soils are deteriorating and leading to increased soil toxicity due to the large amounts of agrochemicals that are supplied during the development of the crop [5, 26, 40, 50].

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Today it is necessary to implement different crop systems that include the use of technologies that are friendly to the environment and counteract the effects caused since the beginning of the green revolution [10, 46]. One of the systems that has regained importance in recent years is the traditional "milpa" system, one of its characteristics is that they apply minimum or zero tillage, do not need irrigation systems, and are based on the establishment of maize cultivation associated with other crops such as beans and pumpkin. [2, 11], where maize serves as a support for the entangling of beans through the production of nodules, increases nitrogen fixation that benefits maize and pumpkin, and the latter provides soil protection by reducing the growth of weeds, which retains moisture, and through the production of allelopathic compounds (cucurbits) released by the leaching of the rain, they keep insects away. It has been one of the most used systems over the years in Mexico, and its importance encompasses cultural, economic, social, biological, and environmental aspects [29, 44]. Additionally, the traditional milpa model system is key to conserving soil biological diversity. Research indicates that the plants in this system have co-evolved with microbial biodiversity, enhancing soil fertility and ecosystem health [13, 49].

The main objective of the milpa system is self-consumption. Due to changes in culture and environmental conditions, productivity has declined. To ensure food security, research into new production methods to increase milpa productivity is essential. One option is the use of microorganisms that promote plant growth, which in recent years has proven effective as biofertilizers, biopesticides, and biofungicides [6, 32].

The interaction between microorganisms and plants depends on the species and age of the plant, soil characteristics, and climate. Plant growth-promoting rhizobacteria (PGPR) are among the plant growth-promoting microorganisms [33, 47, 51]. Mechanisms by which PGPRs act as bioinoculants include phosphate solubilization, nitrogen fixation, phytohormone production, and iron reduction. PGPR protect crops by producing antibiotics, siderophores, lytic enzymes, and volatile organic compounds, and by triggering systemic resistance in plants [27, 38, 52]. However, the survival and proliferation of these non-native microorganisms in the soil are necessary for them to exert their mechanisms on plants [4].

Some of the most studied PGPR genera in the maize rhizosphere include *Burkholderia, Bacillus, Azotobacter, Streptomyces*, *Paenibacillus*, *Sphingobium*, and *Pseudomonas*. Pseudomonads stand out for their effectiveness as plant growth promoters in maize plants, fungicides against diseases such as *Rhizoctonia solani*, biostimulants that mitigate water stress, and bioremediators of copper toxicity in maize crops [9, 12, 41, 45]. *Pseudomonas fluorescens* strain UM270 has various PGP mechanisms, such as the production of siderophores, antibiotics, volatiles, ACC deaminase activity, biofilm formation, and phosphate solubilization. It has been proven that it is an excellent promoter of plant growth in vitro in plants, including *Solanum lycopersicum*, *Physalis ixocarpa*, *Medicago truncatula*, and antagonists of fungal pathogens such as *Botrytis cinerea* and *Fusarium oxysporum* [20–23]. However, its beneficial effects in the field are unknown and under a milpa model. Therefore, the objective of this work was to evaluate the effect of *P. fluorescens* UM270 inoculation on maize growth, plant nutrition, and production in a milpa agrosystem during two growth cycles (2021 and 2023).

Materials and Methods

Experimental Site

The experiment was conducted in Santa Clara del Cobre, located within the municipality of Salvador Escalante, Michoacán, México (Fig. 1), at 19° 24′ 23″ North and 101° 38′ 24″ West, with an elevation of 2239 m. The climate prevalent in this area is classified as humid subtropical (Köppen climate classification, Cwa). Maize production in this region follows a seasonal pattern, with cultivation of native varieties, including white, black, yellow, and pink maize. Soil analyses were conducted prior to the experiments to determine their physicochemical properties (such pH, textural class, organic matter, elements like P, K, N, Mg, among others), with samples sent to INIFAP-Celaya (Mexico) for processing.

Biological Material

Zea mays L., *Phaseolus vulgaris* L., and *Cucurbita* sp. seeds utilized in this experiment were sourced locally from the municipality of Salvador Escalante, Michoacán, México where the study took place and were sourced from local producers. The bioinoculant used was the UM270 strain, which has been previously isolated and characterized [20].

Chemical Fertilizer

Diammonium phosphate (DAP) 18–46-0 was applied. It was purchased from a local company. It is a granular inorganic fertilizer and an excellent source of phosphorus (P) and nitrogen (N), which is highly soluble and dissolves in the soil solution, developing an alkaline pH around the granule.

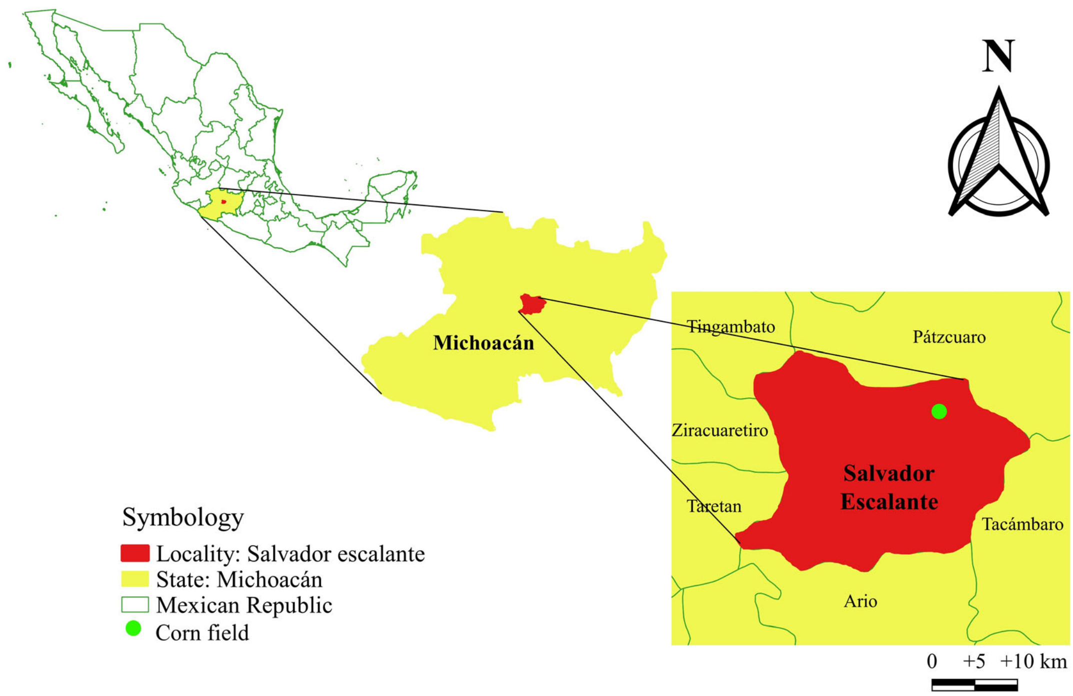

Fig. 1 Geographic location map of the experimental site for maize cultivation (Green dot) under the milpa model in Santa Clara del Corn cobre, Michoacán, Mexico

Inoculum Preparation and Seed Treatments

Inoculum preparation was carried out as follows. Briefly, *P. fluorescens* strain UM270 was activated by inoculating a bacterial loop into a flask containing 500 mL of Nutrient Broth (BD BIOXON). The flask was then placed on a shaker set to 120 rpm and incubated at 28 °C for 24 h until it reached an optical density at 560–600 nm of 1. Subsequently, the supernatant was separated from the bacterial pellet, and the pellet was resuspended in a solution containing 0.1 mM magnesium sulfate. Finally, colony-forming units (CFUs) per mL were determined.

Seed preparation consisted of a superficial disinfection process involving washing with 70% ethanol, 5% sodium hypochlorite, and sterile distilled water. The seeds used for the treatments in the presence of the bacterial strain were inoculated at a concentration of approximately $1 \times 10^3 - 1 \times 10^4$ CFU per seed.

Establishment of the Experiment in the Field

Maize planting was carried out in May during 2021 and 2023, with the entire cultivation stage ending in December of each year. Native maize seeds known as 'white maize' were used (Fig. 2). This variety is selected in the area for its characteristics of nixtamalization and tortilla flavor. After two weeks, guide beans and pumpkin were planted. One month after the maize planting, a second inoculation within the same treatment with the UM270 strain at a concentration of 1×10^8 UFC was carried out on the crops with the inoculated seeds, and after another month, a third inoculation was carried out at the same concentration.

The dose of DAP fertilizer applied to the selected crops was 200 kg/ha in the respective treatments. The maize crop was fertilized at the time of sowing; the second fertilization was carried out 1 month later by applying the same doses. Weed management was performed manually through weekly selective weeding, and vegetative development of the plants was monitored every 15 days. The maize harvest was carried out in December, and the bean and pumpkin were harvested when they reached physiological maturity and left to dry in the open air under shade.

The maize phenological scale in which the crop was evaluated included the following stages: emergence stage (VE), stages of development from the first to the nth leaf (V1 to V(n)), panicle stage (VT), reproductive stages that carry out the process from aqueous grain to hard grain (R1 to R5), and finally, the stage of physiological maturity (R6). The evaluated phytometric parameters were chlorophyll concentration, plant height, root length, plant dry weight, root dry weight, and maize ear weight.

Grain Yield and Chemical Composition Analysis

To determine grain yield, the number of maize ears per hectare was calculated by counting the number of ears in an

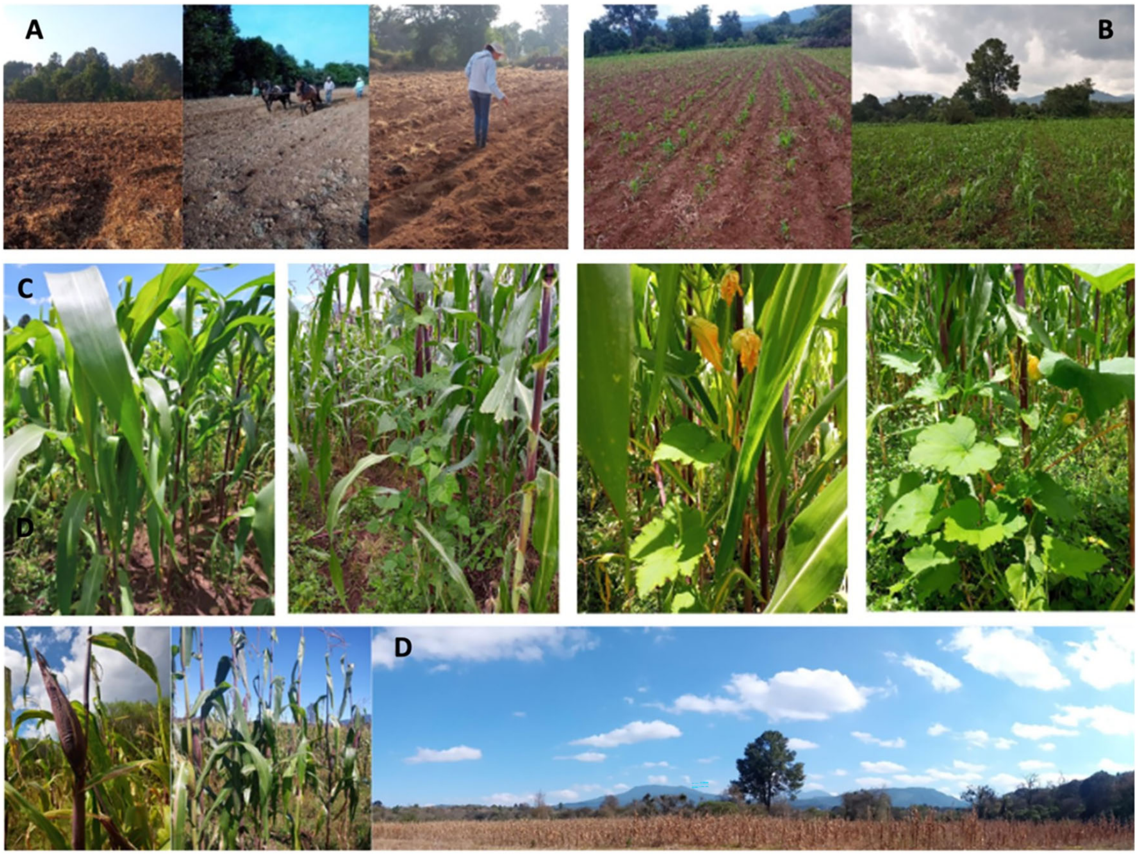

Fig. 2 Composite pictures of the sowing of maize in a milpa system. Panel A represents the process of preparing the land that consists of making the fallow, to later carry out the plowing and planting of corn with beans and pumpkin. The letter B represents the vegetative growth stage of plants. Panel C represents, from left to right, the planting of corn, corn co-cultivated with beans, corn with pumpkin, and corn with beans and pumpkin. Complete cycle of maize cultivation. Panel D represents the reproductive stage of the maize cycle (Representative photographs taken during the 2021/2023 seasons)

area of 10 m^2, and the number of grains per ear was determined by counting the number of rows in each ear and the number of grains per row. The final number of kernels per ear was calculated by multiplying the number of rows by the number of kernels in each row. Finally, the number of grains per hectare and the weight of a thousand grains were measured.

The chemical composition of the maize corn cob was analyzed after the harvest of the crop in December, and the samples obtained were sent to INIFAP-Celaya, Mexico for processing. The parameters evaluated were concentration of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), and sodium (Na). The beans were harvested in September, as soon as they reached physiological maturity and were left to dry under shade in open air until they reached 14% humidity, after which they were weighed. Pumpkins were harvested between July and August as necessary. This crop was harvested twice a week in the form of flowers and green fruits until plant senescence. Afterward, the fruit was weighed, and the number of flowers was counted.

Experimental Design

The experiment was implemented over an area of 5600 m^2. The experimental design was completely randomized with 10 treatments, where the three crops were planted at various densities. According to recommendations from the producers in the region, eight Maize plants m^2 were planted. The composition of the polycultures was calculated as follows: planting a Maize plant is equivalent to 0.75 bean plants and 0.25 pumpkin plants.

The treatments evaluated were:

(1) *Zea mays* L. (M)
(2) *Zea mays* L. + *Phaseolus vulgaris* L. (M + P)
(3) *Zea mays* L. + *Cucurbita* sp. (M + C)
(4) *Zea mays* L., *Phaseolus vulgaris* L., *Cucurbita sp.* (TM)
(5) *Zea mays* L. + diammonium phosphate (M + DAP)

(6) *Zea mays* L. + UM270 (M + UM270)
(7) *Zea mays* L. + UM270 + *Phaseolus vulgaris* L. (M + P + UM270)
(8) *Zea mays* L. + UM270 + *Cucurbita* sp. (M + C + UM270)
(9) *Zea mays* L. + UM270 + *Phaseolus vulgaris* L. + *Cucurbita* sp. (TM + UM270)
(10) *Zea mays* L. + UM270 + diammonium phosphate (M + DAP + UM270)

Statistical Analysis

The data obtained were analyzed by analysis of variance, and the variables showing significant differences were further analyzed using Tukey's test ($p < 0.05$) with STATISTICA 12 software. Additionally, correlation analysis and a heat map were performed using the METABOANALYST 6.0 platform. The data were subjected to *t*-test ANOVA with autoscale samples and a Pearson distance measure with a complete clustering method.

Results

Physicochemical Traits of the Soil

The physicochemical characteristics of the soil are presented in Table 1, where it can be observed that the pH levels are 5.42 and 6.2, organic matter was measured at 7.18 and 7.9 respectively, and phosphorus levels were low. Additionally, calcium, manganese, copper, magnesium, and zinc levels were found to be in a moderately low range. K and Fe levels were within the medium range. However, for the establishment of corn, soils with a pH range of 5.5–7.8 were required. Beyond these values, the crop may exhibit symptoms of excess micronutrient toxicity. Corn's adaptability to various soil types contributed to successful cultivation in the conditions described in this study (cycles 2021 and 2023).

Table 1 Physicochemical characteristics of the experimental soil and minimal changes during the two cycles evaluated

Physical soil propieties	Season 2021	Season 2023
Textural class	Clay	Clay
Base saturation percent	62.2%	53.2
Field capacity	48.9%	46.9
pH	5.42	6.2
Organic matter	7.18%	7.9
Fertility		
N-Inorg	42.08 ppm	41
Phosphorous (P)	1.96 ppm	1.83
Potassium (K)	258 ppm	248
Calcium (Ca)	709.16 ppm	689.13
Magnesium (Mg)	119.54 ppm	125.34
Sodium (Na)	12.87 ppm	11.87
Iron (Fe	9.54 ppm	9.1
Zinc (Zn)	0.72 ppm	0.82
Manganese (Mn)	2.24 ppm	2.48
Copper (Cu)	0.46 ppm	0.42
Boron (B)	N. D	N. D

Maize Growth Promotion by Biofertilization with Strain UM270

During both corn crop cycles, different parameters were evaluated, such as plant height, root length, chlorophyll concentration (SDAP units), root dry weight, total plant dry weight and corn cob weight (Suppl. Table 1 and Figs. 3 and 4). In general, all treatments under the different milpa systems, with and without inoculum, showed an increase in chlorophyll concentration compared to the corn monoculture control treatment (without inoculum). The monoculture treatments fertilized with DAP, the Mesoamerican triad model (TM), and the corn-squash coculture increased the chlorophyll concentration by more than 50% during both cycles (Fig. 3A-A2), where significant differences were found between treatments ($p < 0.05$).

The height of the plants for both cycles increased in the treatments inoculated with the UM270 strain, highlighting the treatments of monoculture fertilized with DAP, TM, and corn-squash co-culture, which increased by more than 26% and by up to 56% during both cycles (Fig. 3B-B2), and significant differences were found between the treatments ($p < 0.05$). Similarly, root length increased in treatments inoculated with strain UM270, with an increase of more than 27% in each cycle (Fig. 3C-C2).

The dry weight of the plant with respect to the TM increased by more than 100% in the treatments inoculated with the UM270 strain, including the corn-squash co-culture, TM, and corn monoculture fertilized with DAP. for both cycles (Fig. 4D-D2). However, regardless of the type of milpa model with or without inoculum or DAP fertilization, there were significant differences ($p < 0.05$), and the dry weight of the plant increased with respect to the corn control treatment for both cycles.

There were no significant differences in the root dry weight after inoculation with strain UM270 (Fig. 4E-E2). Curiously, the dry weight of the corn cob presented significantly different ($p < 0.05$), highlighting the treatments inoculated with UM270, which included corn monoculture, corn-bean co-culture, and fertilized corn monoculture were found. with DAP, which increased their weight by more than 40% during the 2021 cycle. In the 2023 cycle,

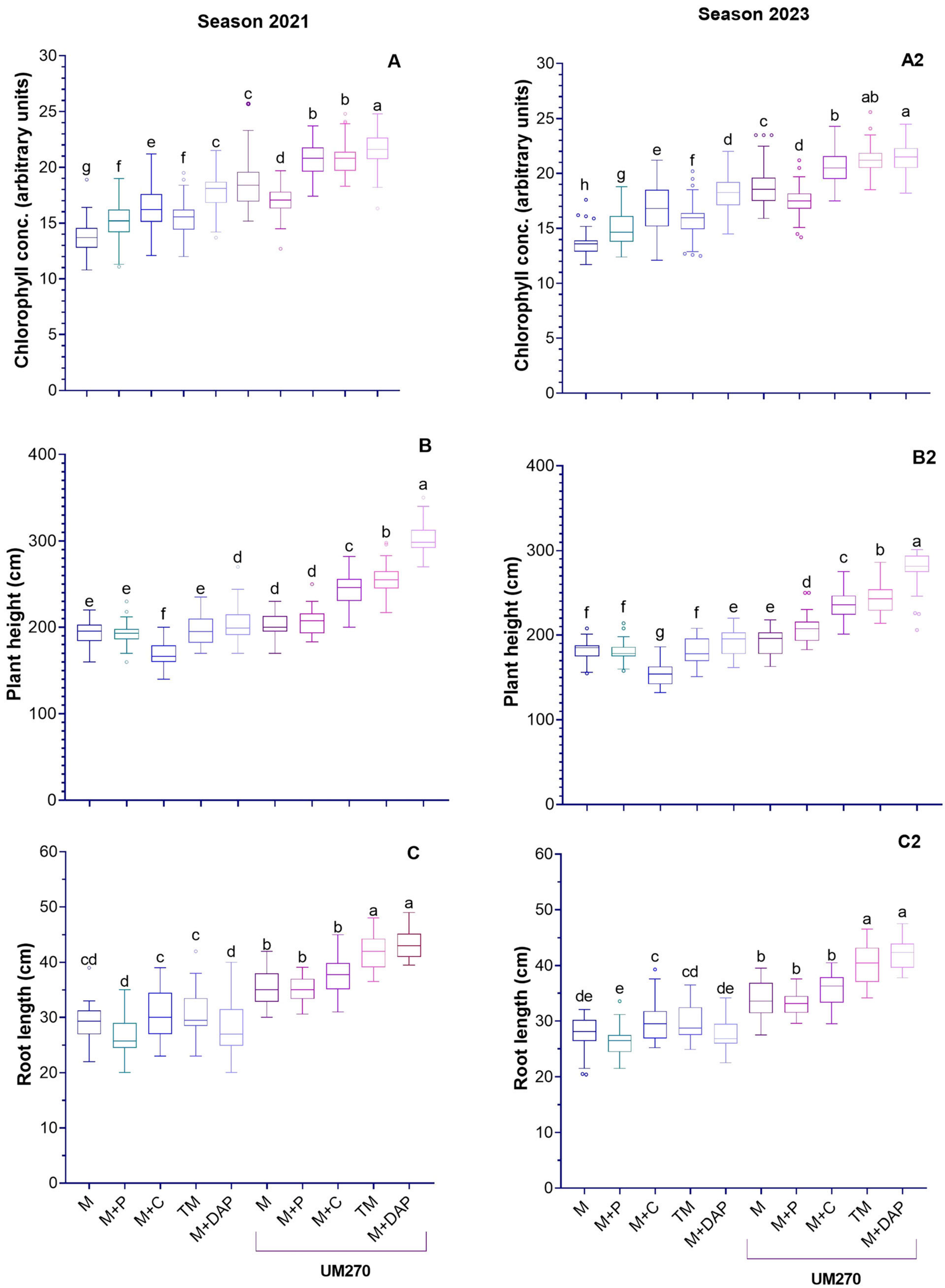

Fig. 3 Effect of biofertilization of maize plants by *fluoresces* UM270 under the milpa model. Different letters indicate significant difference calculated by a Tukey test ($p < 0.05$) $n = 100$

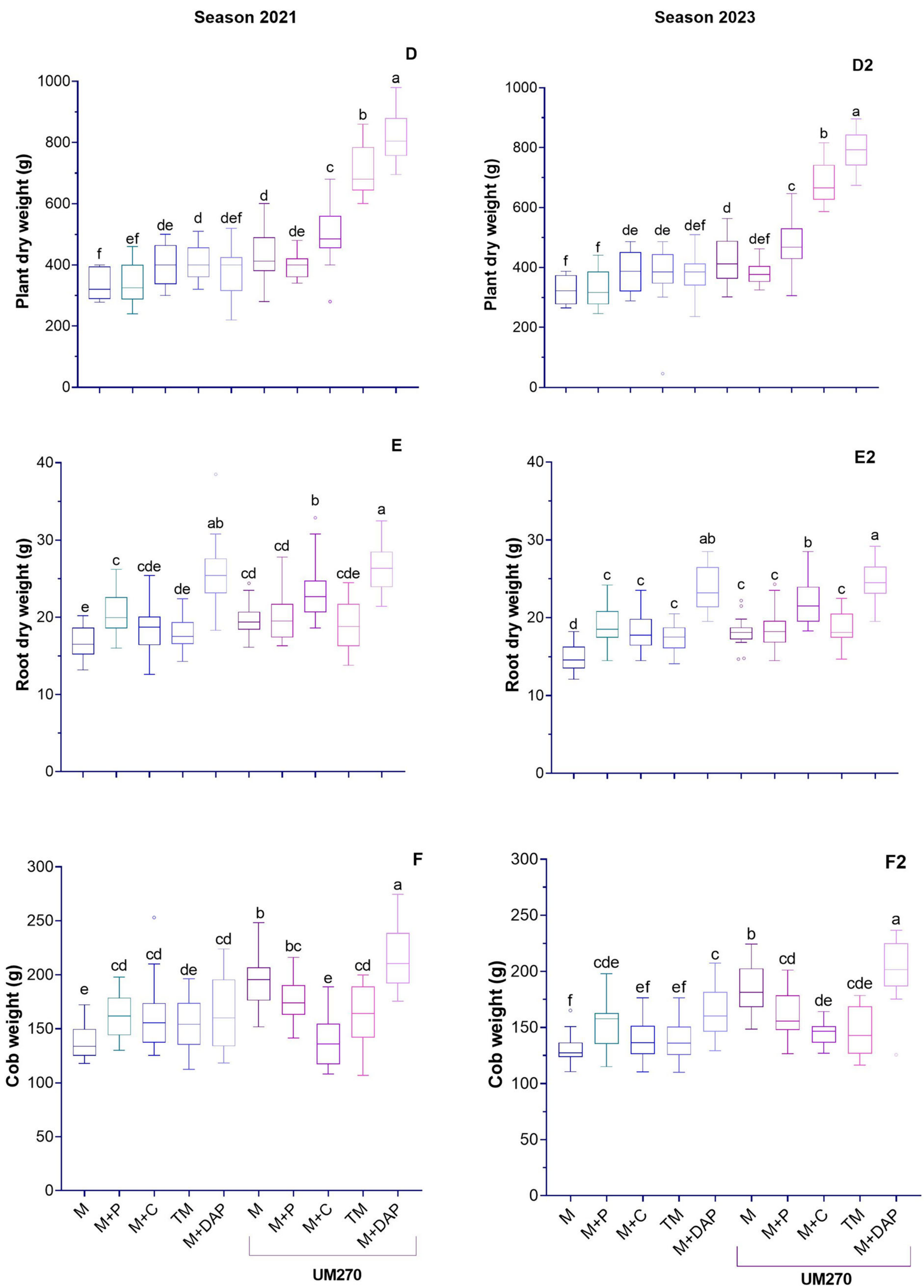

Fig. 4 Effect of biofertilization of maize plants by *P. fluoresces* UM270 under the milpa model. Different letters indicate significant difference calculated by a Tukey test ($p < 0.05$) $n = 100$

treatments inoculated with UM270 included corn monocultures with and without DAP; on the other hand, the corn monoculture fertilized with DAP without inoculum increased by 56%, 42%, and 25%, respectively (Fig. 4F-F2).

Based on the correlation analysis between treatments, it can be determined that during the 2021 cycle, the corn-bean co-culture and the corn monoculture, both inoculated with UM270, were positively correlated. In the same way the corn monoculture fertilized with DAP and the TM, both inoculated with UM270, were positively correlated (Fig. 5).

During the 2023 cycle, the TM, corn monoculture, and fertilized DAP were positively correlated, and both treatments were inoculated with UM270 (Fig. 5).

The results of the heat map that considers the six phytometric parameters show that the treatment of the corn monoculture inoculated with UM270 and fertilized with DAP presented the highest values of all the parameters in both cycles. The treatments where UM270 biofertilization was applied increased the values of the parameters depending on the type of model evaluated (Fig. 6). Based on the results of the principal component analysis, it can be determined that the first axis of the PCA explains 30.5 and 30% of the variation and the second 16.6 and 16.2% for the 2021 and 2023 cycles, respectively. During the first cycle, they grouped the height of the plant, root growth, and chlorophyll concentration during the second cycle, only the dry weight of the plant was not grouped with the other phytometric parameters (Fig. 7).

Maize Yield

The increase in maize yield was evaluated at the end of the harvest in both cycles. During the first cycle, the treatments that showed an increase in maize yield were those inoculated with UM270, maize in co-culture with bean plants, and maize fertilized with DAP by 41.96%, 28.28%, and 58.13%, respectively, in comparison with the maize plants controls (uninoculated) (Table 2). During the second cycle, the corn monocultures inoculated with the UM270 strain and the co-fertilized one (UM270-DAP) were the ones that presented the greatest increase in production by 42.03% and 56.59%, respectively (Table 2). This result indicates that biofertilization with *P. fluorescens* UM270 has great potential to increase maize crop yield.

Phaseolus vulgaris L. and *Cucurbita* sp. Yield

Bean yield was determined under the milpa model; the interaction, given in the corn-bean co-culture inoculated with the UM270 strain increased by 12.5% and 13.32% in the 2021 and 2023 cycles, respectively, compared to the corn-bean co-culture without inoculum (Table 3). Biofertilization with the UM270 strain increased pumpkin yield, indicating the treatment of the TM with an increase of 30.27% and 20.90% in the 2021 and 2023 cycles, respectively, compared to the corn-squash co-culture without inoculum (Table 4).

Nutritional Composition of the Maize Corn Cob

The nutritional composition of maize grains is presented as the mean of the two seasons (Table 5). One of the elements analyzed was the concentration of total N, which presented significant differences ($p > 0.05$) between treatments. Notably, maize co-cultured with pumpkin and inoculated with the UM270 strain showed an 18.8% increase in nitrogen concentration compared to the maize control treatment and the traditional TM system. The same behavior was observed, but with an increase of 14.87%.

P is another of the treatments that was evaluated, and in this case the treatments of the TM, that of maize fertilized with DAP, and that of maize in co-culture with beans and inoculated with the bacterial strain, stood out for presenting the highest concentration, increasing by 52.94%, 43.38%, and 45.58% compared to the maize control treatment. In this case, we determined that even without inoculation with the bacterial strain, the TM system could increase P concentration.

K increased in maize treatments inoculated with strain UM270 and in maize inoculated and fertilized with DAP, showing an increase of 20.47% and 16.5%, respectively, compared to the control treatment of maize alone. Ca presented its highest concentration in the maize treatment inoculated with the strain and added with DAP fertilizer, increasing by 56% compared to the maize control treatment. Mg, Zn, Mg, Cu, and Na did not show significant differences between the treatments. S had the highest concentration in the TM treatment, increasing by 41.09%. Fe presented its highest concentration in the maize treatment co-cultivated with beans, where it increased by 509.48% compared with the maize control treatment.

The correlation analysis between the different milpa models in the nutritional content of the corn cob, the treatments without TM inoculum, corn, corn-bean co-culture, and corn monoculture fertilized with DAP were positively correlated, whereas the treatments inoculated with the TM, strain UM270, and corn-bean co-culture were positively correlated (Fig. 8A). In the heat map based on the elements in the corn cob, it was observed that Fe had the highest values in the control treatment of the corn monoculture, even though there was no increase in the values given by the inoculation of the UM270 strain, which can be determined depending on the type of system, and

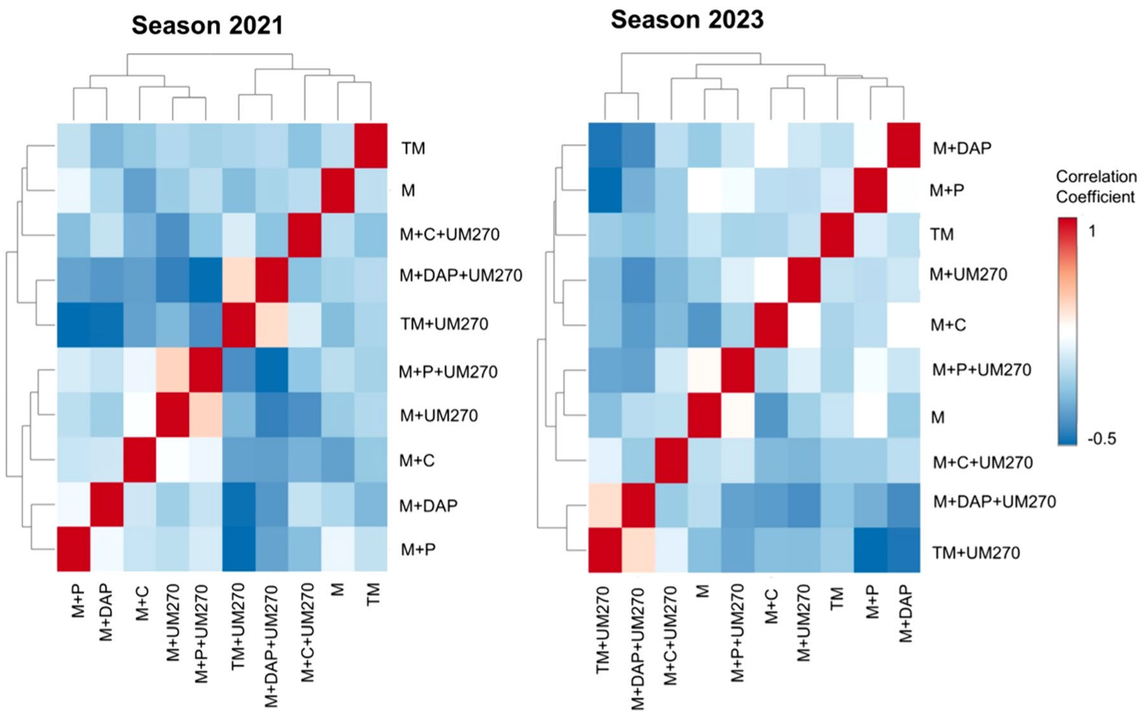

Fig. 5 Pearson correlation matrix between treatments of different cornfield models biofertilized with *P. fluorescens* UM270, based on the phytometric parameters of plant growth promotion. Correlations are shown in blue (negative) and red (positive)

the different parameters evaluated increased in different ways (Fig. 8B).

Discussion

Corn is one of the most important cereals worldwide, and its nutritional value makes it key to food security including its cultivation via the Milpa model, which is key to generating a variety of agricultural products in a small space and stimulating soil biodiversity [18, 19, 44]. No agrochemicals were applied in the milpa; therefore, its production was highly eco-friendly. However, the threat of pathogens and their cultivation in nutrient-poor soils can reduce their efficient production. Therefore, it is necessary to use and apply biological fertilizers and fungicides to naturally restore agroecosystems [16, 17, 42].

The results of this study demonstrate that the application of a biofertilizer based on the PGPR *P. fluorescens* UM270 under the Milpa model (TM or Mesoamerican Triad), among other treatments, including corn-pumpkin co-inoculation, managed to increase all analyzed parameters (e.g., concentration of chlorophyll, biomass, root length, corn plant height, and corn cob weight) compared to the control treatment of corn monoculture without bacterial inoculum. Similar beneficial effects have been observed in corn crops under a monoculture system with the application of biocapsules formulated with chitosan and PS2 and PS10 (*Bacillus* spp.) [7]. The effect of *P. fluorescens* UM270 on maize plants also increased the chlorophyll concentration, regardless of the type of Milpa system treatment established in the field.

Plant height growth increased only in the treatments in which the UM270 strain was inoculated. Similarly, root growth increased only in the treatments inoculated with the UM270 strain, which indicated that the plant-microorganism interaction promoted the growth of maize plants roots, regardless of which Milpa model was established. The dry weight of the plant increased only in the treatments inoculated with the UM270 strain in corn-squash, TM and corn monoculture fertilized with DAP, with the results of this parameter it can be determined that depending on the established Milpa system, the weight increases of plant height by the end of the cycle. Likewise, previous studies have shown that in maize crops under a monoculture system by inoculating consortia of plant growth-promoting bacterial strains such as *Bacillus* and *Pseudomonas*, they increase the growth of maize plants compared to the treatments where only an inoculant was applied [37]. Strains like *Pseudomonas geniculata*, *Pseudomonas psychrotolerans*, *Bacillus circulans, Pseudomonas putida*, and *Pseudomonas pseudoalcaligenes*, increase the growth of corn plants through various mechanisms under abiotic conditions both in vitro and in the field; however, it is worth mentioning that the majority of crops where bioinoculants are applied are under the establishment of

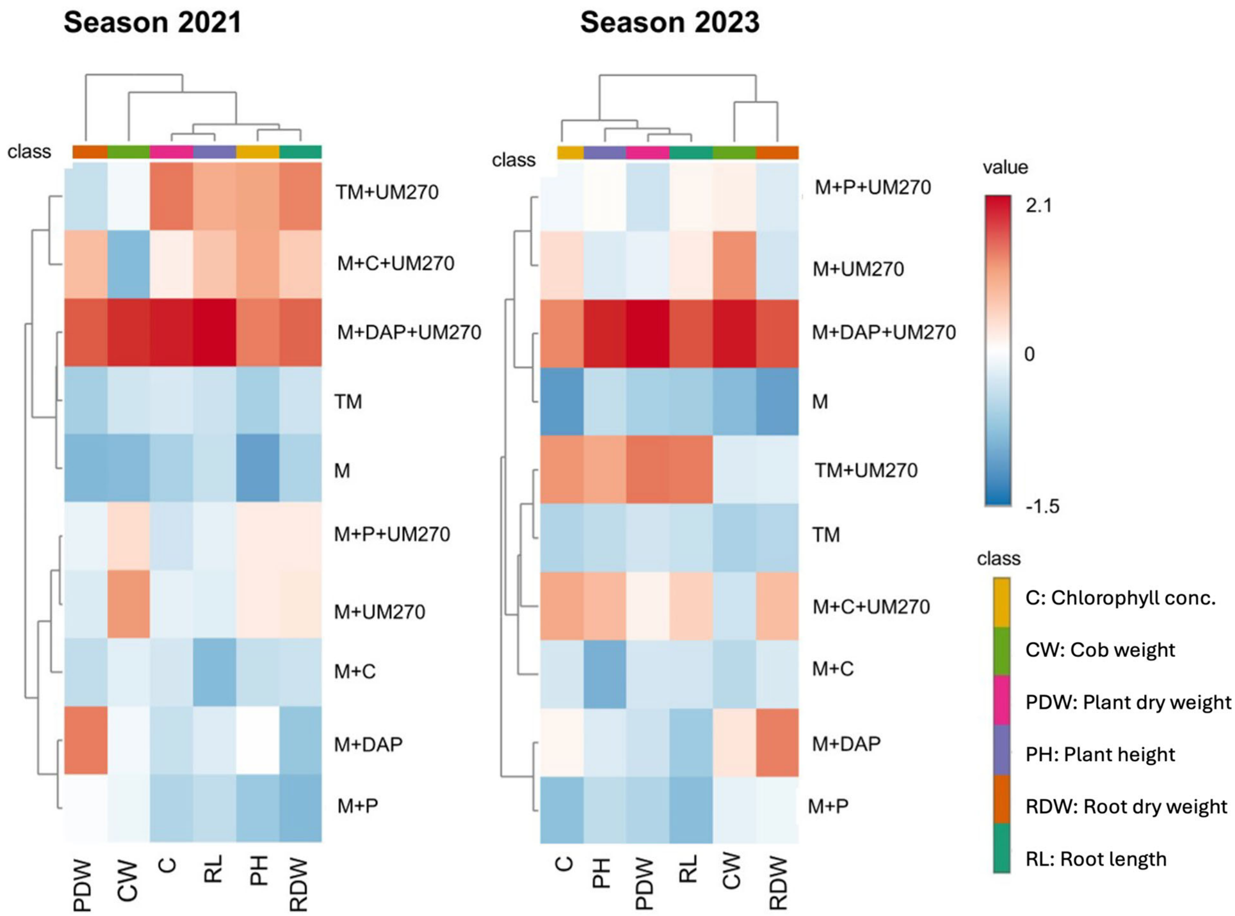

Fig. 6 Heat map diagram that represents the effect of biofertilization of *P. fluorescens* UM270 on corn under different milpa models and its phytometric parameters (chlorophyll concentration, plant height and root growth, plant dry weight and root and corn cob weight

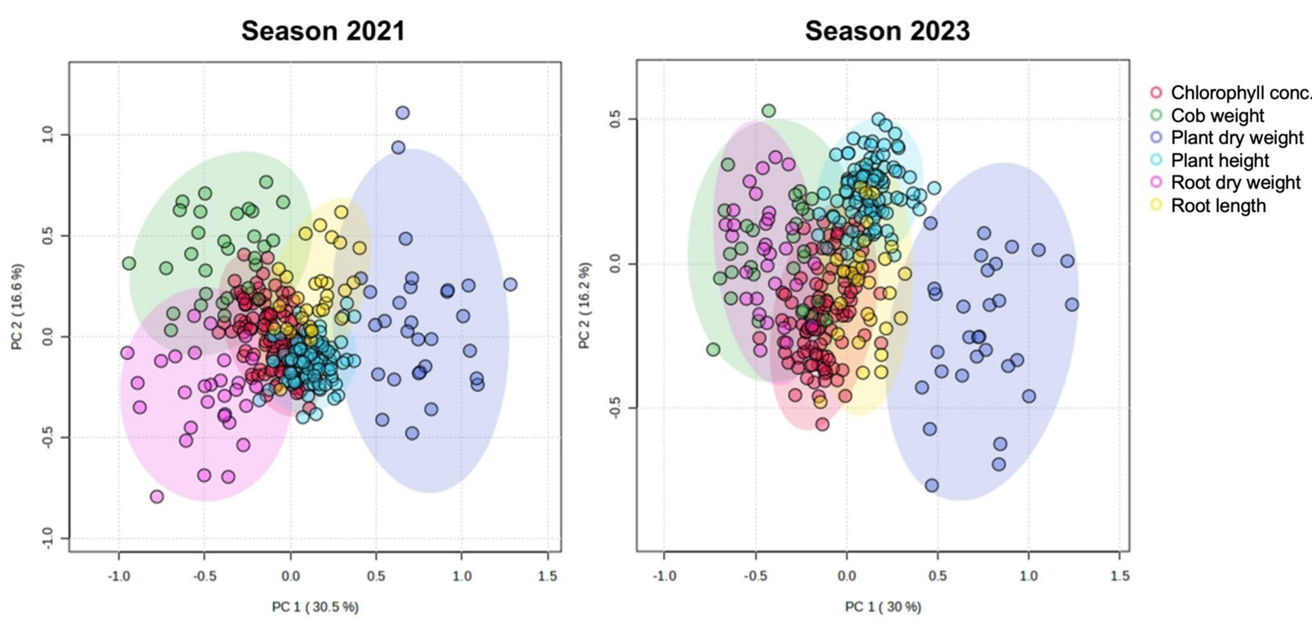

Fig. 7 Principal component analysis for the phytometric parameters of plant growth promotion among treatments of different milpa models biofertilized with *P. fluorescens* UM270

Table 2 Corn yield under a milpa system inoculated with the PGPR *Pseudomonas fluorescens* strain UM270

Treatments	Corn yield (Kg/ha) Season 2021	Increased percentage in corn yield	Corn yield (Kg/ha) Season 2023	Increased percentage in corn yield
Zea mays L. (M)	2817.11e		2598.07f	
Zea mays L. + *Phaseolus vulgaris* L. (M + P)	3329.88 cd	18.20	3022.13cde	16.35
Zea mays L. + *Cucurbita* spp. (M + C)	3270.50 cd	16.09	2818.02ef	8.46
Zea mays L. + *Phaseolus vulgaris* L. + *Cucurbita* spp. (M + P + C)	3171de	12.56	2751.86ef	5.91
Zea mays L. + DAP (M + DAP)	3104.52 cd	10.20	**3263.93c**	**25.62**
Zea mays L. + UM270 (M + UM270)	**3999.27b**	**41.96**	**3690.20b**	**42.03**
Zea mays L. + *Phaseolus vulgaris* L. + UM270 (M + P + UM270)	**3614.01bc**	**28.28**	3210.40 cd	23.56
Zea mays L. + UM270 + *Cucurbita* spp. (M + C + UM270)	2795.92e	− 0.75	2909.45de	11.98
Zea mays L. + *Phaseolus vulgaris* L. + *Cucurbita* spp. + UM270 (M + C + UM270)	2939.83 cd	4.35	2977.42cde	14.60
Zea mays L. + UM270 + DAP (M + DAP + UM270)	**4454.92**[a]	**58.13**	**4068.54a**	**56.59**

To have a comparative evaluation of the beneficial effect of the bioinoculant based on UM270, several treatments were fertilized with diammonium phosphate (DAP). Different letters indicate significant difference calculated by a Tukey test ($p < 0.05$) $n = 3$. See text for further details

Table 3 Common bean yield under a milpa system inoculated with the PGPR *Pseudomonas fluorescens* strain UM270

Treatments	Bean yield (Kg/ha) Season 2021	Bean yield (Kg/ha) Season 2023
Zea mays L. + *Phaseolus vulgaris* L. (M + P)	1000 b	985 b
Zea mays L. + *Phaseolus vulgaris* L. + *Cucurbita* spp. (TM)	1065.50 ab	1002.30 b
Zea mays L. + *Phaseolus vulgaris* L + UM270 (M + P + UM270)	**1125 a**	**1116.3 a**
Zea mays L. + *Phaseolus vulgaris* L. + *Cucurbita* spp. + UM270 (TM + UM270)	937.5 b	875.4 c

Different letters indicate significant difference calculated by a Tukey test ($p < 0.05$) $n = 3$. See text for further details

Table 4 Pumpkin yield under a milpa system inoculated with the PGPR *Pseudomonas fluorescens* strain UM270

Treatments	Pumpkin yield (Kg/ha) Season 2021	Pumpkin yield (Kg/ha) Season 2023
Zea mays L. + *Cucurbita* spp. (M + C)	15,300 b	14,325 bc
Zea mays L. + *Phaseolus vulgaris* L. + *Cucurbita* spp. (TM)	13,855 bc	13,266 c
Zea mays L. + *Cucurbita* spp. + UM270 (M + C + UM270)	17,850 b	16,585 b
Zea mays L. + *Phaseolus vulgaris* L. + *Cucurbita* spp. + UM270 (TM + UM270)	**19,932 a**	**17,320 a**

Different letters indicate significant difference calculated by a Tukey test ($p < 0.05$) $n = 3$. See text for further details

hybrid seeds in monoculture systems and through irrigation systems, which contrasts with the system established in this work, where native seeds were used in a traditional and agro-sustainable system [8, 14, 28, 43, 45, 54].

The correlations between treatments during both cycles allowed to determine that the treatments with the UM270 bioinoculum correlate with each other, as is the case of the corn monoculture with the corn-bean co-culture. On the other hand, the monoculture fertilized with DAP was

Table 5 Chemical composition of maize cob analyzed under a milpa system

Treatment	Element										
	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)	Na (ppm)
M	1.6^{abc}	0.136^{b}	0.503^{b}	0.05^{b}	0.143^{a}	0.073^{ab}	49.14^{d}	29.02^{a}	4.46^{a}	3.89^{a}	0.01^{a}
	SE = 0.072	SE = 0.016	SE = 0.028	SE = 0.005	SE = 0.014	SE = 0.008	SE = 4.03	SE = 1.17	SE = 0.64	SE = 0.27	SE = 0
M + P	1.486^{bc}	0.16^{ab}	0.523^{ab}	0.048^{b}	0.14^{a}	0.076^{ab}	**299.5^{a}**	31.40^{a}	4.66^{a}	5.14^{a}	0.01^{a}
	SE = 0.08	SE = 0.015	SE = 0.029	SE = 0.007	SE = 0.005	SE = 0.023	SE = 15.80	SE = 2.81	SE = 0.56	SE = 0.22	SE = 0
M + C	1.683^{abc}	0.155^{ab}	0.55^{ab}	0.05ab	0.15^{a}	0.091^{ab}	47.83^{d}	30.01^{a}	5.24^{a}	4.62^{a}	0.01^{a}
	SE = 0.057	SE = 0.012	SE = 0.02	SE = 0.006	SE = 0.01	SE = 0.014	SE = 4.8	SE = 2.46	SE = 0.83	SE = 0.24	SE = 0
TM	1.613^{abc}	**0.208^{a}**	0.523^{ab}	0.053^{b}	0.153^{a}	**0.103^{a}**	92.28^{bc}	29.68^{a}	3.63^{a}	4.34a	0.01^{a}
	SE = 0.071	SE = 0.021	SE = 0.031	SE = 0.003	SE = 0.003	SE = 0.008	SE = 10.95	SE = 1.25	SE = 0.25	SE = 0.29	SE = 0
M + DAP	1.578^{bc}	**0.195^{a}**	0.52^{b}	0.053^{b}	0.143^{a}	0.051^{b}	57.46^{cd}	29.21^{a}	3.74^{a}	4.03^{a}	0.01^{a}
	SE = 0.093	SE = 0.023	SE = 0.015	SE = 0.003	SE = 0.008	SE = 0.013	SE = 5.26	SE = 2.8	SE = 0.38	SE = 0.114	SE = 0
M + UM270	1.423^{c}	0.163^{ab}	**0.606^{a}**	0.063^{ab}	0.146^{a}	0.076^{ab}	77.62^{bc}	30.55^{a}	4.88^{a}	4.9^{a}	0.01^{a}
	SE = 0.06	SE = 0.014	SE = 0.017	SE = 0.008	SE = 0.003	SE = 0.006	SE = 8.5	SE = 2.84	SE = 0.39	SE = 0.53	SE = 0
M + P +UM270	1.7^{ab}	**0.198^{a}**	0.568^{ab}	0.056^{ab}	0.163^{a}	0.083^{ab}	109.17^{b}	29.73^{a}	4.8^{a}	4.63^{a}	0.01^{a}
	SE = 0.129	SE = 0.016	SE = 0.022	SE = 0.003	SE = 0.016	SE = 0.02	SE = 24.54	SE = 2.97	SE = 1.0	SE = 0.82	SE = 0
M + C + UM270	**1.853^{a}**	0.16^{ab}	0.54^{ab}	0.046^{b}	0.153^{a}	0.083^{ab}	57.26^{cd}	27.90^{a}	4.73^{a}	4.17^{a}	0.01^{a}
	SE = 0.066	SE = 0.015	SE = 0.015	SE = 0.003	SE = 0.014	SE = 0.006	SE = 4.83	SE = 1.13	SE = 0.42	SE = 0.39	SE = 0
TM + UM270	1.7^{ab}	0.185^{ab}	0.543^{ab}	0.046^{b}	0.13^{a}	0.075^{ab}	72.72^{cd}	28.24^{a}	5.09^{a}	4.00^{a}	0.01^{a}
	SE = 0.06	SE = 0.017	SE = 0.026	SE = 0.003	SE = 0.005	SE = 0.018	SE = 2.70	SE = 1.85	SE = 0.70	SE = 0.28	SE = 0
M + DAP + UM270	1.616^{abc}	0.171^{ab}	**0.586^{a}**	**0.078^{a}**	0.15^{a}	0.066^{ab}	54.45^{d}	30.98^{a}	5.49^{a}	4.89^{a}	0.01^{a}
	SE = 0.059	SE = 0.016	SE = 0.035	SE = 0.017	SE = 0.01	SE = 0.012	SE = 3.91	SE = 1.68	SE = 0.64	SE = 0.34	SE = 0

Letters in bold mean significant differences regarding control treatments. Different letters indicate significant difference calculated by a Tukey test (p <0.05) n = 3. See text for further details

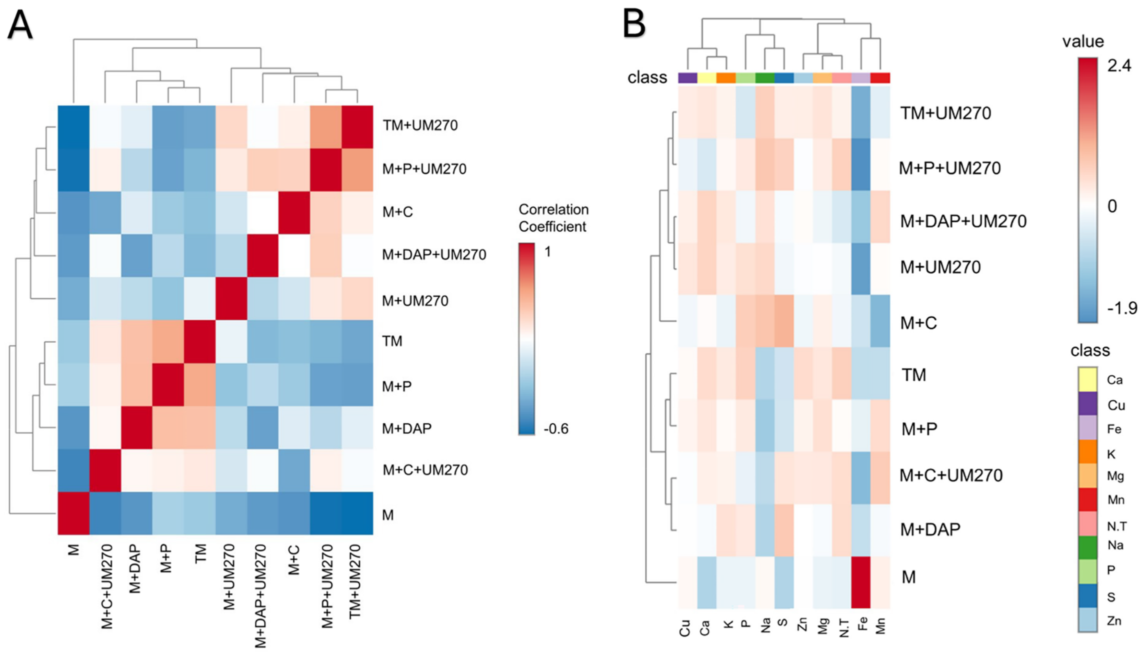

Fig. 8 Pearson correlation matrix between treatments of different cornfield models biofertilized with *P. fluorescens* UM270, based on the concentration of the elements of the corn cob (Panel A). Correlations are shown in blue (negative) and red (positive). Heat map diagram representing the effect of *P. fluorescens* UM270 biofertilization on the nutrient content of corn corn cob under different milpa models (Panel B)

correlated with the TM. The heat map shows that the highest parameters during both cycles are presented in the same way in the treatments with the inoculum. This indicated that the strain's effect on corn growth depends on the interaction between plants, and plants with microorganisms.

One of the most important parameters for field crops is the dry weight of the cob, which directly influences grain yield. In the TM, corn production increased by 12.56 and 5.91% compared with the control treatment during the 2021 and 2023 cycles, respectively. By adding the UM270 inoculum to the corn monoculture, the production increased by 41.96 and 42.03 for 2021 and 2023 cycles, respectively. respectively. Similarly, in the co-cultivation of corn and beans with the UM270 inoculum, it was determined that there was an increase in production of 28.28 and 23.56 during both cycles.

Previous reports have shown that inoculation with PGPR such as *Sinorhizobium* sp. A15, *Bacillus* sp. A28 and *Sphingomonas* spp. A55, isolated from the maize rhizosphere in the same area and inoculated separately, increased maize growth and grain yield between 22 and 29% [8]. The increase in corn yield is determined due to abiotic and biotic factors, but it has been proven that through the application of PGPR, corn yield increases even under stress conditions in the field [15, 34, 41]. In addition, promoting corn yield through the establishment of multi-species cropping increases productivity per unit area and, in turn, reduces the number of weeds and pathogens present [36].

Regarding bean production, it can be observed that the corn-bean interaction and the UM270 bioinoculant increased bean production by 13.32%, compared to the corn-bean co-culturepl. For its part, the pumpkin yield increased in the biofertilized treatments by 30.29% (2021 cycle) and 20.90% (2023 cycle). In previous field studies, the effect of plant promotion in wheat crops was determined due to the effect of bioinoculation of the *B. subtilis* strain [25]. Furthermore, under controlled conditions, the benefits of plant–plant interactions have been observed in intercrops such as corn-bean, where nodulation in broad bean was stimulated. In *Cajanus cajan-Zea mays* co-culture, an increase in the production of corn proteins and in the corn-bean co-culture, the induction of genes for nodulation in bean plants was determined, and in the case of maize genes for the degradation of mucilage and feluric acid, among other compounds [1, 30, 53].

The chemical composition of the grain provides a basic parameter for determining its nutritional quality [31]. Calcium, phosphorus, potassium, and nitrogen are among the elements with the greatest nutritional importance. Here, an increase was observed in certain elements, such as N and P, whereas K and Ca improved in treatments with UM270 + DAP. In a recently published study, Pereira et al. (2020) observed an increase in N and P in maize plants when inoculating with two PGPR, *Cupriavidus* *necator* 1C2 and *P. fluorescens* S3X. Although the nutritional grade of the corn grain was not analyzed, only that of the plants under conditions of water deficit was analyzed. In our study, it is possible that the UM270 strain, which is a phosphate solubilizer, increases its nutrient use efficiency, shoot biomass, and concentration in the grain cob. The nutritional value of seeds enhanced by bacterial inoculation has also been tested in other crops, such as faba beans [55] and wheat [24], among others.

Conclusions

Incorporating biofertilizers, such as the *P. fluorescens* strain UM270, into Milpa models not only enhances plant growth promoting parameters and improves grain nutrition by providing specific elements (e.g. K and P), but also boosts overall crop yield. This approach offers significant economic and agroecological benefits to local farmers by providing an alternative to reducing dependency on synthetic fertilizers and leading to more sustainable agricultural practices.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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