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RESEARCH ARTICLE

Effect of rhizobacteria (PGPR) and growing medium on the productive and nutraceutical quality of blackberry (*Rubus* spp.)

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ABSTRACT

The growing demand and consumption of blackberry (*Rubus* spp.) fruits is based on their nutraceutical quality. One option for achieving sustainable production is the use of plant growth-promoting rhizobacteria (PGPR). The productive and nutraceutical quality of blackberry is linked to its management and nutrition during its development. Therefore, the objective of this research was to evaluate the effect of PGPR strains of the genus *Pseudomonas* on different growing medium on the productive and nutraceutical quality of blackberry under greenhouse conditions. The experiment established rhizomes with roots of the 'Tupy' blackberry, under a completely randomized design with a factorial arrangement (2 × 3). The factors were the growing medium (tezontle, soil) and PGPR strains (without PGPR, P61, A46) with six replicates each. Defoliation was performed 192 d after transplantation. During fruiting, morphological and nutraceutical variables were determined in fruits, and foliar nutritional analysis. According to the results, strain A46 stimulated the length (4%) and fruit diameter (10%), foliar concentrations of S and Fe and the nutraceutical variables are exploratory for further research with this strain, while strain P61 increased foliar concentrations of N (58%) and Mo (51%). The use of tezontle improved foliar concentrations of S, and in soil, increased fruit size, and foliar concentrations of N, Fe and Mo. Therefore, the productive quality of blackberry under greenhouse conditions, could be optimized by inoculation the A46 strain in soil as a growing medium.

Key words: Beneficial microorganisms, hydroponics, morphological quality.

INTRODUCTION

The blackberry (*Rubus* spp.) is considered healthy due to their rich content of minerals, trace elements, phenolic compounds, anthocyanins, with antimicrobial, antioxidant, antitumor, and anti-inflammatory properties (Moraes et al., 2021; Chauhan and Chauhan, 2022). Furthermore, it has high profitability, labor demand, return on investment, and export market, making it one of the most important agricultural crops in México (Martínez et al., 2022).

Due to the parallel increase demographic and food demand of worldwide, chemical fertilizers are used to achieve productivity and food security. Intensive blackberry production involves excessive use of fertilizers, which deteriorates soil and groundwater quality with increased costs and harm to human and soil health (Daniel et al., 2022). To achieve food security, the preferential consumption of foods nutraceutical or containing bioactive compounds (biofortified) has been promoted for health care and well-being (Rico and Martín, 2023). Keep in mind that social conflicts, climate phenomena, inequalities sociality, and the high cost of food production prevail as significant obstacles for food security (Daniel et al., 2022). It is necessary to rely on ecological approaches, with complementary nutrition from crops in a sustainable way. Recently, plant growth-

promoting rhizobacteria (PGPR) have been used to improve crop productivity (İpek and Eşitken, 2022; Palacio-Rodríguez et al., 2022; Zapata-Sifuentes et al., 2022; Ameen et al., 2023; Çakmakçı et al., 2023; Dhiman et al., 2023), as well as to maintain long-term soil fertility, a fundamental basis to satisfy food demand level worldwide and to achieve food security (Ghosh et al., 2024).

For optimal crop development and fruit quality, essential nutrients are required, and PGPRs through solubilization, increase the bioavailability of the macronutrients P, K, and S, and micronutrients Fe and Mn (Bala, 2022; Ghosh et al., 2024), likewise, PGPRs synthesize phytohormones such as auxins, gibberellic acid, abscisic acid, cytokinins and ethylene (Stefen et al., 2022; Ghosh et al., 2024). Inoculation of *Pseudomonas fluorescens* N 21.4 in blackberry improved the concentration of flavonoids in fruits (18%), epicatechin (18%) and catechin (36%), compared to the control (Martin-Rivilla et al., 2021). While, in tomato (*Solanum lycopersicum* L.) under greenhouse, the inoculation of *Pseudomonas lini* increased the content of total soluble solids (7%), citric acid (7%), lycopene (49%), total sugars (27%) and ascorbic acid (17%), with respect to the non-inoculated treatment (González Rodríguez et al., 2018). On the other hand, in cucumber (*Cucumis sativus* L.) under greenhouse conditions, it was reported that the PGPR *Sinorhizobium meliloti*, *Acinetobacter radioresistens* and *Pseudomonas paralactis*, increased the content of phenols, flavonoids, antioxidant activity and ascorbic acid or vitamin C, with respect to the non-inoculated treatment (Zapata-Sifuentes et al., 2022). In general, PGPRs have been considered promising for the biofortification of crops for the benefit human health and recovery of infertile soils (Dhiman et al., 2023).

Therefore, the objective of this research was to evaluate the effect of PGPR strains of the genus *Pseudomonas* on different growing medium on the productive and nutraceutical quality of blackberry under greenhouse conditions, and with the hypothesis, that the use of PGPRs improves the productive and nutraceutical quality of blackberry fruits under greenhouse conditions.

MATERIALS AND METHODS

Materials used

The experiment was set up in a greenhouse in the Plant Nutrition Area of the Edaphology Program of the College Postgraduate, campus Montecillo, Mexico, from December 2022 to December 2023. Two side curtains were used for ventilation. The average minimum temperature was 8.8 °C and the maximum 29.4 °C, and relative humidity 35%. Blackberry (*Rubus* spp.) 'Tupy' plants were used. For establishment, plants were pruned at ground level, then the rhizome with roots was transplanted into new growing media, in 6 L black polyethylene bags and a support with trellis mesh was installed.

Establishment and experimental design

The experiment was established under a completely randomized design (CRD) with a 2 × 3 factorial arrangement, where the factors were the type of growing medium; tezontle (particle size < 1 cm, apparent density 0.81 kg L^{-1}, total pore space 61.5%, aeration capacity 49.6%) and soil (from an agricultural field in the community of San Pablo Ixayoc, Texcoco, State of Mexico, Mexico, whose characteristics were: Clay = 20%, silt = 25%, sand = 55%), pH 6.5, electrical conductivity (EC) 0.26 dS m^{-1}, OM 5.4%, cation exchange capacity (CEC) 27.1 $cmol_{(+)}$ kg^{-1}, macronutrients (g kg^{-1}) N = 2, P = 0.061, K = 0.507, Ca = 2.94 and Mg = 0.348, micronutrients (mg kg^{-1}) Fe = 59, Cu = 1, Zn = 6, Mn = 18 and B = 1. The second factor was the inoculation of plant growth-promoting rhizobacteria (PGPR) strains (P61, A46 and without PGPR), with a total of six treatments, and with six replicates. The experimental unit consisted of a blackberry plant, which were irrigated with the Universal Steiner (Steiner, 1985) nutrient solution at 37.5% (EC = 0.75 dS m^{-1}), maintaining the pH within the range of 5.5-6.5. Sodium hydroxide (NaOH, 1 N) or H_2SO_4 (98%) was used to raise or lower the pH. Irrigation was carried out with a drip system (4 L h^{-1}), for 2 min in six scheduled irrigations during the day. At 192 d after transplant (dat), defoliation was carried out with $(NH_4)_2SO_4$ (20%) + $CuSO_4$ (3%) + agricultural oil (2%).

Microbiological material

Two PGPR strains were used as inoculants, previously tested for their effectiveness in cultivation (Cabanzo-Atilano et al., 2024), strains P61 and A46 (both *Pseudomonas tolaasii*), isolated from the rhizosphere soil of *Solanum tuberosum* L. and belonging to the microbial collection of Soil Microbiology in the Edaphology Program,

College Postgraduate, campus Montecillo, Mexico. These were cultured in nutrient broth with agitation for 3 d. Only sterile nutrient broth was applied to the control treatments (control treatment in tezontle = Tc, and control treatment in soil = Sc). The first inoculation was carried out at 21 dat with a concentration of 1.08×10^8 and 1.86×10^8 cells mL^{-1} (strains P61 and A46, respectively), the second inoculation was carried out 201 dat, with a concentration of 1.8×10^8 cells mL^{-1} for both strains; 5 mL inoculum were applied to the base of each stem.

Leaf nutritional analysis variables

Ten recently mature leaves were sampled from each plant. These were dried in a forced-air oven at 65 °C for 72 h. The dry leaf material was ground to obtain particles < 2 mm. Total N was determined with the microKjeldahl method (Alcántar and Sandoval, 1999), with some modifications, for digestion 2 mL mixture H_2SO_4 + $HClO_4$ (2:1) and 1 mL 30% H_2O_2 were added. For the quantification of P, K, Ca, Mg, S, Fe, Mn, B, Mo, Zn and Cu, wet digestion was performed following the methodology of Alcántar and Sandoval (1999), using 8 mL 2:1 mixture HNO_3 + $HClO_4$. The extracts (filtered) were read in an equipment of inductively coupled plasma-optical emission spectroscopy (ICP-OES 725, Agilent Technologies Australia, Mulgrave, Victoria, Australia). Blank samples (without leaf matter) were used in both methodologies to eliminate technical errors and sample handling. The values obtained for nutrient concentration were reported in g kg^{-1} for N, P, K, Ca, Mg, S, and in mg kg^{-1} for Fe, Mn, B, Mo, Cu, and Zn.

Morphological variables

During fruiting, ripe fruits were harvested to determine the variables, fruit length and fruit diameter, with a digital vernier caliper (CALDI-6MP, Truper, China) in order to calculate the fruit roundness index (FRI):

$$\text{FRI (mm)} = \frac{\text{Fruit length (mm)}}{\text{Fruit diameter (mm)}}$$

where values less than 1.0 were considered oval, values equal to 1.0 as round, and higher than 1.0 as elongated (González-Jiménez et al., 2020). Fruit biomass (FB) was determined in the first 15 fruits using a digital scale (Pocket Scale MH-Series, China) with a capacity of 200 g, reported in g per fruit.

Total soluble solids

Total soluble solids (TSS) were determined with a digital refractometer (PR-100 with a scale of 0%-32%; ATAGO, Tokyo, Japan), with two drops of juice on the measuring cell, the values were expressed in °Brix.

For nutraceutical analyses, the fruits were ground with liquid nitrogen and the samples were homogenized by treatment with four replicates for each.

Titratable acidity

Titratable acidity (TA) was performed in accordance to AOAC method 939.05 (AOAC, 1998), with some modifications, for this, 1 g ground sample was taken and dissolved in 10 mL distilled water. Three drops of 0.1% phenolphthalein were added as an indicator and titrated with 0.1 N NaOH. The TA was obtained using the equation:

$$\text{Citric acid (\%)} = \frac{\text{Vol.}_{\text{NaOH}}\ \text{(mL)} \times \text{N}_{\text{NaOH}}\ \text{(meq mL}^{-1}\text{)} \times \text{meq of citric acid (0.064 g meq}^{-1}\text{)}}{\text{Vol.}_{\text{sample}}\ \text{(mL)}} \times 100$$

Vitamin C

Determination of vitamin C (vit C) was performed using AOAC method 967.21 (AOAC, 1998). The concentration of ascorbic acid or vitamin C was obtained using the formula:

$$\text{Ascorbic acid (mg 100 g}^{-1}\text{)} = \left[\frac{\left[\begin{pmatrix}\text{Tillman solution expense (mL)} - \\ \text{Blank Tillman solution expense (mL)}\end{pmatrix} \times \right]}{\text{Sample aliquot} \times \text{Sample weight}}\right] \times 100$$

Total antioxidant activity

Total antioxidant activity (AA) was determined with the methodology described by Ibarra et al. (2011), with some modifications. To 100 mg material, 1.5 mL 60% ethanol was added and the mixture was left to stand for 24 h at 4 °C. Subsequently, it was centrifuged for 20 min at 15 000 rpm at 4 °C. To 400 µL supernatant, 600 µL 80% methanol were added. Then, 1 mL free radical 2,2-diphenyl-1-picrylhydracil (DPPH) was added and incubated for 60 min at room temperature in the dark. These were read in a spectrophotometer (Synergy 2 Microplate Reader; Biotek, Winooski, Vermont, USA) at 517 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard, the values were expressed as percentage (%) of inhibited DPPH.

Total soluble sugars

Total soluble sugars were determined using the anthrone method described by Witham et al. (1971), with some modifications. To 1 g ground material was added 50 mL 80% ethanol and four boiling beads. It was boiled until less than 20 mL was obtained. The sample was allowed to cool and filtered with cotton, then made up to 20 mL with 80% ethanol and left to stand at -20 °C for 24 h. Then, 1 mL sample was taken and evaporated in a water bath; 20 mL distilled water were added to the dehydrated solution and stirred, from which taken 1 and 2 mL distilled water and 6 mL anthrone with sulfuric acid (0.4 g anthrone diluted in 100 mL H_2SO_4) were added. Samples were placed in a water bath in boiling water for 3 min, then the tube was placed in cold water to stop the reaction. The samples were read in a spectrophotometer at 600 nm. Sucrose was used as a standard. Values were expressed in g 100 g^{-1} plant material.

Total phenols

Extraction of total phenols was performed in accordance to the Folin and Ciocalteu method described by Waterman and Mole (1994). Readings were taken in a spectrophotometer at 760 nm. Gallic acid was used as a standard. The results were expressed as mg gallic acid per 100 g biomass.

Total flavonoids

Total flavonoids concentrations were evaluated following the methodologies described by Nanyonga et al. (2013) with some modifications. To 75 mg material 1.5 mL 80% methanol were added and incubated for 1 h at 70 °C. It was then centrifuged for 20 min at 15 000 rpm at 4 °C; 200 µL supernatant were taken, and 600 µL 80% methanol, 40 µL 10% aluminum chloride, 40 µL 1 M potassium acetate, and 1120 µL distilled water were added. The mixture was left to stand for 40 min at room temperature in the dark. The samples were read in the spectrophotometer at 415 nm. Quercetin was used as a standard. The results were expressed in mg quercetin equivalents per 100 g biomass (mg EQ 100 g^{-1} biomass).

Total anthocyanins

The determination of total anthocyanins was performed in accordance to the AOAC differential pH method 2005.02 (AOAC, 2006) with some modifications. Ground material (0.5 g) was taken, 1.5 mL 80% ethanol was added, and then centrifuged for 20 min at 15 000 rpm at 4 °C. Next, a 1:4 dilution (40 µL each sample per 160 µL each buffer) was performed in 96 cavity microplates for spectrophotometer readings. The absorbance was read in spectrophotometer at 520 and 700 nm for both buffers (pH 1.0 and 4.5). The measurement at 700 nm was taken to correct for turbidity. The concentration of anthocyanins in cyanidin-3-glucoside equivalents (cyd-3-glu) was calculated with the following equation:

$$\text{Cyd} - 3 - \text{glu equivalents (mg 100 g}^{-1}) = \left(\frac{\text{A} \times \text{MW} \times \text{DF} \times 1000}{\varepsilon \times \text{l}}\right) / 10 \times \text{AD}$$

where A is absorbance = pH 1.0 (A_{520nm} - A_{700nm}) - pH 4.5 (A_{520nm} - A_{700nm}); MW is molecular weight = 449.2 g·mol^{-1} for cyd-3-glu; DF is dilution factor (1); 1000 is conversion factor from g to mg; ε is 26 900, molar extinction coefficient in L mol^{-1} cm^{-1} for cyd-3-glu; l is 0.38 as linear length in cm; AD is apparent density in g cm^{-3}.

Statistical analysis

The data obtained from the evaluated variables were subjected to the Shapiro-Wilk normality test (α = 0.05) and homogeneity of variance of Bartlett's test. The variables that complied to the initial tests were subjected to ANOVA, Tukey HSD test, and factorial ANOVA, with a significance level of α = 0.05. The variables that did not

complied to the initial tests were analyzed using the Kruskal-Wallis nonparametric one-way test and the Wilcoxon rank sum test with the original data, in accordance to the methodology of Siegel (2015). In each case, the SAS Version 9.4 (SAS Institute, Cary, North Carolina, USA) was used.

RESULTS AND DISCUSSION

Leaf nutrient analysis variables

The Kruskal-Wallis nonparametric test showed a highly significant difference between treatments ($p < 0.01$) for N concentration but not for Mo (Table 1). The results of the parametric analysis (ANOVA) of leaf concentrations of macronutrients (Table 2) and micronutrients (Table 3), significant differences between treatments were only detected for S and Fe, while the leaf concentrations of the remaining macronutrients (P, K, Ca, and Mg) and micronutrients (Mn, B, Cu, and Zn) did not register significant differences.

Table 1. Average ranks (Wilcoxon) and Kruskal-Wallis test of leaf N and Mo concentration in blackberry inoculated with plant growth promoting rhizobacteria (PGPR) on different growing media. Values in parentheses equal mean ± standard error. ***Highly significant ($P < 0.01$), ^{ns}nonsignificant ($P \leq 0.05$).

Treatments	Nitrogen	Molybdenum
	g kg^{-1}	mg kg^{-1}
Tezontle + without PGPR (Tc)	13.63 (4.73 ± 0.44)	9.67 (4.03 ± 1.15)
Tezontle + P61 (TP61)	8.00 (3.68 ± 0.34)	9.33 (3.89 ± 0.79)
Tezontle + A46 (TA46)	3.50 (2.80 ± 0.29)	11.00 (4.58 ± 0.66)
Soil + without PGPR (Sc)	12.75 (4.55 ± 0.45)	5.00 (2.34 ± 1.21)
Soil + P61 (SP61)	21.38 (11.03 ± 4.96)	13.00 (5.53 ± 1.61)
Soil + A46 (SA46)	15.75 (7.53 ± 2.89)	11.00 (4.70 ± 1.64)
χ^2	15.6867	3.7474
Pr > χ^2	0.0078	0.5863
Significance	***	ns

Table 2. Leaf concentration of macronutrients in blackberry inoculated with plant growth promoting rhizobacteria (PGPR) on different growing media. Mean ± standard error. Columns with the same letter are statistically equal to each other according to Tukey HSD test ($P \leq 0.05$). Tc: Control in tezontle; Sc: control in soil; CV: coefficient of variation in percentage; HMSD: honest minimum significant difference.

Treatments	Growing medium	PGPR	P	K	Ca	Mg	S
			g kg^{-1}				
Tc	Tezontle	Without PGPR	6.54 ± 1.58^{a}	6.94 ± 0.32^{a}	38.92 ± 2.84^{a}	6.86 ± 0.37^{a}	6.10 ± 0.25^{b}
TP61	Tezontle	P61	7.62 ± 1.41^{a}	6.81 ± 0.44^{a}	43.03 ± 5.79^{a}	8.72 ± 1.25^{a}	8.50 ± 0.98^{ab}
TA46	Tezontle	A46	3.60 ± 0.28^{a}	6.00 ± 0.24^{a}	44.39 ± 3.26^{a}	9.38 ± 1.24^{a}	10.60 ± 0.85^{a}
Sc	Soil	Without PGPR	4.75 ± 1.15^{a}	7.26 ± 0.61^{a}	32.80 ± 2.21^{a}	6.80 ± 0.53^{a}	6.71 ± 0.28^{b}
SP61	Soil	P61	5.77 ± 0.64^{a}	7.64 ± 0.43^{a}	47.88 ± 4.27^{a}	6.39 ± 0.72^{a}	7.43 ± 0.67^{b}
SA46	Soil	A46	4.37 ± 0.58^{a}	7.30 ± 0.54^{a}	39.75 ± 4.21^{a}	7.93 ± 0.59^{a}	8.45 ± 0.71^{ab}
Pr > F			0.1269	0.2087	0.1737	0.1339	0.0029
R^2			0.358	0.309	0.327	0.353	0.606
CV			38.608	12.773	19.162	22.200	17.091
HMSD			4.721	2.007	17.710	3.831	3.059

Table 3. Leaf concentration of micronutrients in blackberry inoculated with plant growth promoting rhizobacteria (PGPR) on different growing media. Mean ± standard error. Columns with the same letter are statistically equal to each other according to Tukey HSD test ($P \leq 0.05$). Tc: Control in tezontle; Sc: control in soil; CV: coefficient of variation in percentage; HMSD: honest minimum significant difference.

Treatments	Growing medium	PGPR	Fe	Mn	B	Cu	Zn
			mg kg^{-1}				
Tc	Tezontle	Without PGPR	708.6 ± 40.9^{ab}	2585.7 ± 373.1^{a}	570.4 ± 24.5^{a}	12.2 ± 1.5^{a}	411.0 ± 58.7^{a}
TP61	Tezontle	P61	535.5 ± 71.9^{b}	1600.5 ± 72.1^{a}	525.6 ± 37.2^{a}	16.3 ± 1.1^{a}	314.9 ± 73.9^{a}
TA46	Tezontle	A46	798.9 ± 89.5^{ab}	1727.3 ± 535.1^{a}	607.7 ± 64.0^{a}	12.2 ± 1.6^{a}	195.5 ± 28.5^{a}
Sc	Soil	Without PGPR	903.4 ± 121.4^{ab}	1425.2 ± 203.1^{a}	437.6 ± 44.8^{a}	11.6 ± 1.7^{a}	320.3 ± 55.2^{a}
SP61	Soil	P61	677.2 ± 39.1^{ab}	1646.2 ± 170.0^{a}	525.0 ± 74.9^{a}	11.9 ± 1.0^{a}	267.1 ± 18.6^{a}
SA46	Soil	A46	1059.6 ± 116.8^{a}	1383.8 ± 256.8^{a}	439.8 ± 38.9^{a}	11.7 ± 0.5^{a}	271.2 ± 39.6^{a}
Pr > F			0.0075	0.1196	0.1534	0.1335	0.1121
R^2			0.558	0.363	0.340	0.353	0.369
CV			22.128	35.572	19.451	20.493	33.343
HMSD			388.1	1381.4	226.3	5.8	222.3

According to the Wilcoxon average ranks, the treatment SP61 (21.38) obtained the highest value (Table 1), and with a mean of 11.03 ± 4.96 g kg^{-1}, it exceeded the mean N of the controls, control treatment in tezontle (Tc) and control treatment in soil (Sc), by 133% and 142%, respectively. Similarly, SP61 was up to 37% and 136% higher in Mo leaf concentration compared to the controls Tc and Sc (in that order). The N is a constituent of amino acids, proteins, and chlorophyll; therefore, it is considered the most demanding macronutrient in productive crop development (Taiz et al., 2015). A N deficiency can cause proteolysis in leaves, chloroplast degradation, and low chlorophyll content (Taiz et al., 2015; Bala, 2022). In this experiment, no limitation in the availability of this macronutrient was registered, mainly in the soil as growing medium, this could be due to its organic matter content.

It was reported that plant growth-promoting rhizobacteria (PGPR) inoculation benefits leaf nutrient concentration of N, as the N-fixing and P-solubilizing strain RC521, increased the N content in oregano leaves (*Origanum onites*) by up to 94% regarding to the control (Çakmakçı et al., 2023). On the other hand, it was documented that inoculation of P61 and A46 strains in pak choi crop (*Brassica rapa* subsp. *chinensis*) did not register significant differences between treatments in N concentration (Cazares-Esquivel et al., 2023). Mo affects the structure and function of soil microorganisms by participating in the synthesis and metabolism of several Mo-containing enzymes (Sun et al., 2013), such as nitrate reductase, which promotes nitrate reduction in plants and at adequate concentration can improve fruit quality (Taiz et al., 2015), as well as the nitrogenase enzyme, fundamental in N-fixing, and a structural part of the enzyme that generates abscisic acid (Sun et al., 2013; Taiz et al., 2015). In this work, the positive response of the PGPRs could be due to their mechanisms to favor the availability of micronutrients to the plant (Ghosh et al., 2024). However, there are no reports of the effects of PGPR on the Mo leaf concentration in plants or crops.

Although Tukey HSD test did not detect significant differences between treatments in leaf macronutrient concentrations (Table 2), treatment TP61 (7.62 ± 1.41 g kg^{-1}) registered a nominally higher value in leaf concentration of P, up to 16% and 60%, regarding to Tc and Sc, respectively. While, TA46 was superior in S concentration, exceeding the controls of both substrates (Tc and Sc, respectively) by 74% and 58%.

According to the macronutrient factorial analysis (Table 4), the growing medium factor had significant effects on K concentration (P = 0.0383), instead on Mg concentration (P = 0.0831) there were effects at 10%. With the PGPR factor and Growing medium × PGPR interaction, no registered effects on aforementioned concentrations. As for PGPR factor, it showed a factorial effect at 10% on the leaf concentrations of P and Ca, while, a highly significant effect was detected on leaf concentration of S (P = 0.0009). No effects were detected for growing medium or factorial interaction (growing medium × PGPR) in any of these variables. With the means for each factor, only those that favored leaf nutrient concentration in ANOVA are highlighted. In PGPR, P61 strain was 18% higher on P and A46 strain 49% higher on S regarding to plants not inoculated with PGPR. On the other hand, the growing medium factor showed differences in K, where the soil exceeded tezontle by 12%.

Table 4. *P* values in factorial ANOVA of leaf nutrient concentration in blackberry inoculated with plant growth promoting rhizobacteria (PGPR) on different growing media.

	Factorial analysis of variance		
Variable	Growing medium	PGPR	Growing medium × PGPR
Macronutrients			
P	0.2785	0.0572	0.3819
K	0.0383	0.4197	0.5548
Ca	0.5482	0.0722	0.3413
Mg	0.0831	0.1276	0.4225
S	0.1338	0.0009	0.1522
Micronutrients			
Fe	0.0113	0.0053	0.7906
Mn	0.0686	0.3114	0.1636
B	0.0251	0.8954	0.2427
Cu	0.0986	0.1844	0.2435
Zn	0.6103	0.0485	0.2442

For optimal plant development, the availability of macronutrients is required due to their main functions, such as P is fundamental in nucleic acids and cellular energy as ATP, Mg as the central structure of chlorophyll, the Ca as structure in cell wall and S as SO_4 is part of sulfolipids and heteropolysaccharides and, in reduced form, a reduction that takes place mainly in chloroplasts (Taiz et al., 2015). It was reported that PGPRs stimulate the nutrition of blackberry plants in greenhouses. Inoculation with the SY-43 strain (*Pantoea agglomerans*) increased the leaf concentration of N (37%), SK-39 strain (*Herbaspirillum huttiense*) increased N (32%) and P (63%), the SK-4 strain (*Herbaspirillum huttiense*) increased K (44%), and the SK-50 strain (*Achromobacter xylosoxidans denitrificans*) increased the concentration of P (79%), Ca (60%) and S (77%), all regarding to the control (Ameen et al., 2023). Similarly, in pak choi cultivation, inoculation with P61 strain increased P concentration by 10.8% and S concentration by 12.3% with respect to the control. However, no differences in K concentration were recorded with strains P61 and A46 (Cazares-Esquivel et al., 2023). The presence of PGPR improves root development and, consequently, the efficient absorption of soil nutrients (Ghosh et al., 2024), although soils are often more deficient in N than in any other nutrient, it is not surprising that N, along with P and K, are the main key macronutrients in mineral nutrition (Taiz et al., 2015), and *Pseudomonas* PGPR have the capacity to synthesize auxins with properties similar to indole-3-acetic acid (IAA), gibberellins, and solubilize nutrients (Daniel et al., 2022). In this experiment, the increase in leaf P concentration could be attributed to the ability of the inoculated strains to solubilize P. Furthermore, the availability of K in soil is due to the exchangeable and non-exchangeable forms of K, which pass into the soil solution for absorption by plant roots, and is also very mobile within plant tissue (Sardans and Peñuelas, 2015).

Plant micronutrient requirements are small but essential for their development, since they are enzymatic constituents, that is why their availability and absorption are important (Taiz et al., 2015; Daniel et al., 2022), their concentration in tissues is less than 0.1%, and their homeostasis is regulated by the availability of micronutrients in the rhizosphere, translocation and transport in the xylem, utilization and storage in the leaf, and remobilization via phloem (Taiz et al., 2015). According to Tukey HSD test, the leaf concentration of Fe registered significant differences between treatments (Table 3), in which treatment SA46 outperformed the controls by up to 49% in Tc and 17% in Sc. When performing the factorial ANOVA on micronutrients (Table 4), a significant effect was detected on the leaf concentration of Fe with the growing medium factor ($P = 0.0113$) and a highly significant effect with PGPR ($P = 0.0053$), while no effect was recorded in the interaction of both factors (Growing medium × PGPR). Similarly, the growing medium factor showed effects at 10% on the leaf concentrations of Mn and Cu, however, there was a significant effect on the leaf concentration of B ($P = 0.0251$), and no effects were noted with PGPR and the Growing medium × PGPR interaction, at the leaf concentrations mentioned. In turn, a significant effect was recorded on the leaf concentration of Zn with the PGPR factor, and

no effect with growing medium and factorial interaction. By highlighting the significant effects with the factors means on leaf nutrient concentrations, it was detected that, in Fe, the soil exceeded tezontle by 29%, and PGPR, inoculation with strain P61 was better up to 45% higher than strain A46, and 15% higher than without inoculation Meanwhile, for leaf concentration of B, tezontle exceeded soil by 20%. In contrast, in the concentration of Zn, the inoculation of strains showed negative effects up to -39% in relation to plants without PGPR inoculation.

Despite being present in large quantities in the soil, Fe has low availability for plants, and since it is required for redox processes and enzymes, it is important for plant development, a stable and abundant form of Fe is that which accumulates in chloroplasts, about 80% of Fe in leaves is located in these organelles as phytoferritin (Taiz et al., 2015). The mechanism of *Pseudomonas* PGPRs for synthesize siderophores helps chelate soil Fe and solubilize micronutrients such as Mn and Zn to increase their availability to plants (Ghosh et al., 2024), which could be the mechanism used by the A46 strain in this experiment. The positive soil response increased, possibly due to the high organic matter content (5.4%), which enhanced the micronutrient availability and improved the activity of microorganisms. Similar results were reported in blackberry plants when inoculated *A. xylosoxidans denitrificans* (SK-50), which increased the leaf concentration of Fe by 15%, while the inoculation of *H. huttiense* (SK-4) improved Mn (10%) and Cu (77%), and *H. huttiense* (K-39) increased the leaf concentration of Zn by 76%, all strains with respect to the control (Ameen et al., 2023).

Fruit morphological variables

The results of ANOVA of fruit morphological variables, recorded significant differences between treatments in length and diameter of fruit, as well as in total soluble solids (Table 5), while, fruit roundness index (FRI) and fruit biomass, did not register differences.

Table 5. Fruit morphological variables of blackberry inoculated with plant growth promoting rhizobacteria (PGPR) on different growing media. Mean ± standard error. Columns with the same letter are statistically equal to each other according to Tukey HSD test ($P \leq 0.05$). CV: Coefficient of variation in percentage; HMSD: honest minimum significant difference.

Treatments	Growing medium	PGPR	Fruit length	Fruit diameter	Total soluble solids
			mm	mm	°Brix
Tc	Tezontle	Without PGPR	26.07 ± 1.72^{ab}	21.85 ± 1.09^{ab}	7.36 ± 0.13^{a}
TP61	Tezontle	P61	22.95 ± 0.13^{b}	20.48 ± 0.24^{b}	7.65 ± 0.20^{a}
TA46	Tezontle	A46	26.89 ± 0.83^{ab}	24.23 ± 0.75^{a}	6.48 ± 0.22^{b}
Sc	Soil	Without PGPR	27.55 ± 1.35^{ab}	21.95 ± 0.74^{ab}	7.58 ± 0.19^{a}
SP61	Soil	P61	27.72 ± 0.24^{ab}	23.50 ± 0.54^{ab}	6.37 ± 0.09^{b}
SA46	Soil	A46	28.82 ± 1.50^{a}	24.12 ± 0.95^{a}	7.46 ± 0.25^{a}
Pr > F			0.0197	0.0088	< 0.0001
R^2			0.348	0.388	0.606
CV			10.458	8.297	6.445
HMSD			4.897	3.305	0.809

The fruit length was higher with treatment SA46 (28.82 ± 1.50 mm), which increased 10.6% and 4.6% with respect to the control fruits Tc and Sc, respectively, of both growing media (Table 5). Likewise, treatments TA46 (24.23 ± 0.75 mm) and SA46 (24.12 ± 0.95 mm) recorded the highest fruit diameter, regarding to the uninoculated treatments Tc (10.8%) and Sc (9.9%), in the same order. In contrast, no differences were registered in FRI according to Tukey HSD, because the fruits of all treatments registered indices higher than 1.0, which meant that all were classified as elongated fruits, despite that, the treatment TA46 registered an FRI of 1.21 ± 0.03, which slightly exceeded its control (Tc) by 1.3%.

In the factorial ANOVA of morphological variables (Table 6), a highly significant effect was detected on fruit length (P = 0.0064) and a 10% effect in fruit biomass with the growing medium factor. Meanwhile, in fruit diameter showed a highly significant effect with PGPR (P = 0.0084), and a 10% effect with the interaction of both factors. When highlighting the effects with the means of the factors, it was recorded that fruit length in soil was 11% higher than in tezontle. However, in fruit diameter with A46 strain increased by 10% compared to P61 strain and without PGPR inoculation.

Table 6. *p* values in factorial ANOVA of fruit morphological variables of blackberry inoculated with plant growth promoting rhizobacteria (PGPR) on different growing media.

	Factorial ANOVA		
Variable	Growing medium	PGPR	Growing medium × PGPR
Fruit length	0.0064	0.1011	0.3095
Fruit diameter	0.1205	0.0084	0.0917
Fruit roundness index (FRI)	0.1804	0.1928	0.7461
Total soluble solids (TSS)	0.8568	0.0218	< 0.0001
Fruit biomass (FB)	0.0763	0.6579	0.3200

The FRI is important for quality, since longer fruits are considered to contain more pulp or edible tissue (González-Jiménez et al., 2020). Given that, 'Tupy' produces larger fruits, this makes them more attractive and with higher commercial value (Da Silva and Biasi, 2020). The initial development of fruit depends on nutritional input and climatic conditions, such as low relative humidity, especially when accompanied by high temperatures (Taiz et al., 2015). Furthermore, in most fruits, cell expansion (regulated by auxins) determines the final fruit size (Ljung, 2013; Taiz et al., 2015). The effect of PGPR could be attributed to the ability of the A46 strain (*Pseudomonas tolaasii*) to synthesize auxins (Stefen et al., 2022), since, this phytohormone allows for cell wall plasticity and, consequently, cell elongation from the beginning of the development of flower bud, fruit growth and development (Ljung, 2013). In addition, *Pseudomonas* have the ability to synthesize gibberellins, which influence cell expansion and therefore, fruit size. Likewise, the *Pseudomonas* PGPRs intervene with mechanisms for the production of siderophores, solubilization of phosphates and micronutrients (Ghosh et al., 2024). It was documented that in cucumber (*Cucumis sativus* L.) under greenhouse conditions, the strains *Pseudomonas paralactis* (KBendo6p7), *Sinorhizobium meliloti* (KBecto9p6) and *Acinetobacter radioresistens* (KBendo3p1), increased fruit length (25%, 25% and 33%, respectively) and fruit diameter (13%, 18% and 23%, in the given order) respect to the control (Zapata-Sifuentes et al., 2022) and the KBEcto4 strain (*Pseudomonas lini*), increased the fruit diameter of tomato (*Solanum lycopersicum* L.) by 17% in a shade house, with respect to the control (Palacio-Rodríguez et al., 2022).

In the total soluble solids (TSS) content, according to Tukey HSD test (Table 5), the TP61 and SA46 treatments behaved statistically similarly to the control treatments (Tc and Sc). Despite that, TP61 was slightly higher (4%) than its control (Tc). A significant factorial effect was observed in TSS (Table 6) with PGPR (P = 0.0218), and a highly significant effect with the interaction of factors (Growing medium × PGPR, P < 0.0001). When comparing the factor means, the PGPR inoculation was negative (-7%). However, in the factorial interaction, the P61 strain showed a higher effect in tezontle compared to soil, instead, the A46 strain showed a higher effect in tezontle, compared to in soil. The TSS are one of the quality parameters used to indicate the sweetness of fresh fruits and vegetables, as they include sugars, acids, vitamins, proteins, phenols and dissolved minerals (Chauhan and Chauhan, 2022). The fact that nonsignificant differences were registered in the concentration of TSS in this experiment could be related to the availability of nutrients (salts) in the rhizosphere when the nutrient solution was supplied equally in both growing media, even though the soil had a high cation exchange capacity and organic matter, contrary to what was reported by González Rodríguez et al. (2018), that the salt content in organic materials increases the TSS content in the fruits. Similar results to this research were reported in tomato cultivation, to inoculated with PGPR, *Pseudomonas fluorescens*, and *Azospirillum brasilense*, no differences in TSS content in fruit were registered regarding to the control (Pérez-Rodriguez et al., 2020). On the other hand, the TSS content in tomato fruit increased with inoculation of *Bacillus* sp. (14%), *Aeromonas* sp. (5%), and *P. lini* (7%), with respect to no inoculated plants (González Rodríguez et al., 2018).

In the factorial ANOVA of fruit biomass (FB) (Table 6), an effect of 10% was detected with growing medium, and when highlighting the means, the increase of FB was 25% higher in soil respect to tezontle. A higher production and translocation of photosynthates to the fruit stimulates higher fruit biomass (Taiz et al., 2015). The fruit is a sink for DM, but variations in fresh weight are faster than those of DM accumulation (Taiz et al., 2015; Pérez-Rodriguez et al., 2020). The higher FB obtained in this experiment in soil may be due to the amount of nutrients available to the plants and the organic matter content in the soil used as growing medium, which are useful for carbohydrate production and also beneficial for the colonization and activity of PGPR (Ghosh et al., 2024). In blackberry in alkaline soil, it was reported that the use of PGPR 637Ca (*Alcaligenes faecalis*) and SK63 (*Kocuria rhizophila*) increased the FB up to 25% with respect to the control (İpek and Eşitken, 2022).

Fruit nutraceutical variables

Nonsignificant differences were detected between treatments for titratable acidity according to the Kruskal-Wallis test ($P = 0.1034$), so the data were not shown. Similarly, through ANOVA, the remaining fruit nutraceutical variables, antioxidant activity (AA), sugars, phenols, flavonoids, anthocyanins, and vitamin C (Table 7), did not registered significant differences. This may be due to the low number of repetitions and unforeseen environmental factors, such as low temperatures and heat. Although there was a high variability or a high coefficient of variation between treatments in anthocyanins, it was not sufficient to detect differences with Tukey HSD test.

Table 7. Fruit nutraceutical variables of blackberry inoculated with plant growth promoting rhizobacteria (PGPR) on different growing media. Mean ± standard error. Columns with the same letter are statistically equal to each other according to Tukey HSD test ($P \leq 0.05$). DPPH: 2,2-Diphenyl-1-picrylhydracil; CV: coefficient of variation in percentage; HMSD: honest minimum significant difference.

Treatments	Growing medium	PGPR	Antioxidant activity	Sugars	Phenols	Flavonoids	Anthocyanins	Vitamin C
			% Inhibited DPPH	g 100 g^{-1}	mg 100 g^{-1}			
Tc	Tezontle	Without PGPR	18.96 ± 0.72[a]	9.57 ± 2.16[a]	7.81 ± 1.59[a]	1.46 ± 0.23[a]	1.39 ± 0.22[a]	0.091 ± 0.00[a]
TP61	Tezontle	P61	19.84 ± 0.57[a]	15.10 ± 2.55[a]	8.20 ± 2.11[a]	1.32 ± 0.26[a]	1.19 ± 0.45[a]	0.096 ± 0.02[a]
TA46	Tezontle	A46	19.60 ± 0.58[a]	13.21 ± 2.38[a]	9.84 ± 0.86[a]	1.36 ± 0.18[a]	1.41 ± 0.48[a]	0.121 ± 0.02[a]
Sc	Soil	Without PGPR	16.64 ± 2.37[a]	13.04 ± 2.47[a]	9.82 ± 1.14[a]	1.10 ± 0.09[a]	1.94 ± 0.41[a]	0.078 ± 0.01[a]
SP61	Soil	P61	18.36 ± 0.46[a]	13.05 ± 1.47[a]	8.65 ± 0.77[a]	1.11 ± 0.15[a]	1.31 ± 0.56[a]	0.099 ± 0.01[a]
SA46	Soil	A46	18.53 ± 0.74[a]	13.17 ± 2.27[a]	11.13 ± 1.32[a]	1.74 ± 0.08[a]	2.20 ± 0.61[a]	0.121 ± 0.01[a]
Pr > F			0.4265	0.6709	0.5527	0.1742	0.6254	0.2632
R^2			0.224	0.151	0.185	0.327	0.164	0.283
CV			12.034	34.896	29.791	26.713	60.272	28.096
HMSD			5.045	10.081	6.187	0.809	2.136	0.064

The total soluble sugars in blackberry fruits are mainly glucose, fructose, and sucrose. Phenols and flavonoids are considered physiologically active molecules without nutrients and the anthocyanins can be found in the cell in locations known as anthocyanoplasts, which are vacuole sites and are responsible for the red, purple, and black pigments of fruits, although the colors depend on pH, light, and temperature, together with vitamin C act as antioxidants with the ability to protect human cells against free radicals and reduce the risks of chronic diseases (Ayvaz et al., 2022; Chauhan and Chauhan, 2022; Dhiman et al., 2023; Rico and Martín, 2023).

Although nonsignificant differences were registered in fruit nutraceutical variables (Table 7), the TP61 treatment registered a nominally high value in AA to 4.6% and 19.2%, and total sugar content to 57.8% and 15.8%, respect to the controls Tc and Sc, in the same order. Meanwhile, the treatment SA46 registered a nominally high trend in the concentration of total phenols (42.5% and 13.4%), total flavonoids (42.5% and 13.4%), anthocyanins (58.2% and 13.3%), and vitamin C or ascorbic acid (32.3% and 55.2%), with respect to Tc and Sc, respectively. The data from the factorial ANOVA are not shown, given that, not detect any significant differences at $p \leq 0.05$, but it stood out a significant effect at 10% in AA with growing medium ($P = 0.0943$), and in vitamin C with PGPR ($P = 0.0589$). When considering the factor means, AA increased by 9% in tezontle compared to soil, and with PGPR, vitamin C increased by 15% with P61 strain and 46% with A46, respect to without PGPR.

In cucumber cultivation under greenhouse, it was published that inoculation with *S. meliloti* (KBecto9p6) increased the content of phenols (73%), flavonoids (126%), and AA (47%), while with *P. paralactis* (KBendo6p7) increased AA (40%), phenols (35%), flavonoids (95%), and ascorbic acid (97%), with respect to the control (Zapata-Sifuentes et al., 2022). In blackberry, inoculation with *P. fluorescens* (N 21.4) increased in mature fruits the AA (17%), total phenols content (30%), and total flavonoids (33%), respect to the control (Martin-Rivilla et al., 2021).

The biosynthesis of secondary metabolites is conditioned by the stage of development of the plant and specialized cells, and the presence of biotic and abiotic stress, generally constitute the world of chemical signals for the plant-environment relationship, and their accumulation determined the development of the fruits (Taiz et al., 2015; Moraes et al., 2021; Dhiman et al., 2023). The improvement of the nutraceutical quality of blackberry fruits It is possibly due to the ability of the PGPRs to synthesize and release organic acids (gluconic acid, carboxylic acid, ferulic acid, coumaric acid, oxalate, citrate, among others) and protons, which are used to acidify the rhizosphere soil and improve the absorption of nutrients and water, especially the availability of P that is required by plants to produce secondary metabolites (Daniel et al., 2022; Ghosh et al., 2024).

CONCLUSIONS

Based on the results, the *Pseudomonas* A46 strain optimized leaf concentration of S and Fe and greater size of 'Tupy' blackberry fruits, with possible exploratory interest in nutraceutical quality, while the P61 strain improved leaf concentration of N and Mo. Although, inoculation in soil as growing medium resulted in greater size, and as an exploratory issue in biomass and secondary metabolite content in fruit. Therefore, the productive quality of blackberry under greenhouse conditions, could be optimized by inoculation the A46 strain in soil as a growing medium.

Author contribution

Conceptualization: I.C-A., M.S-V. Methodology: I.C-A., M.S-V. Validation: M.S-V., J.J.A-S., M.G.P-S., J.L.G-C., M.E.P-S. Formal analysis: I.C-A., M.S-V. Investigation: I.C-A. Resources: M.S-V. Plant mineral analysis: I.C-A., M.G.P-S. Data curation: I.C-A., J.L.G-C. Writing-original draft: I.C-A., M.S-V. Writing-review & editing: I.C-A., M.S-V. Supervision: M.S-V., J.J.A-S., M.G.P-S., J.L.G-C., M.E.P-S. Project administration: M.S-V. Funding acquisition: M.S-V. All co-authors reviewed the final version and approved the manuscript before submission.

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