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RESEARCH ARTICLE

# Efficiency of plant growth promoting rhizobacteria (PGPR) in the vegetative development of blackberries (*Rubus* spp.) in greenhouse

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## ABSTRACT

Plant growth promoting rhizobacteria (PGPR) play an important role in crop production by improving plant growth through various mechanisms that potentially increase sustainable agriculture. Blackberry (*Rubus* spp.) cultivation has acquired importance for its worldwide merchandising, including its high economic profitability, and for the health benefits in human consumption of the fruit. The quality of blackberry production depends on a good vegetative plant development; therefore, this research objective was to select strains of PGPR that stimulate vegetative growth of blackberry plants grown under greenhouse. The experiment was carried out using seedlings of the 'Tupy' blackberry, under a completely randomized design, using six rhizobacterial strains as treatments (A46, AC-35, P61, R44, BSP1.1, JLB4) and a control (no inoculated) with six replicates each. The plants were in the greenhouse for 78 d. At destructive sampling eight agronomical variables were determined. The results showed that strains A46 and P61 inoculated on blackberry plants stimulated height (45%), stem diameter (17%), leaf area (110%), and shoot DM (150%). Inoculation of strain A46 on blackberry plants increased nutrient extraction of N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn. *Pseudomonas tolaasii* strains P61 and A46 stimulated growth and nutrient extraction in blackberry plants, and these will be selected to inoculate the blackberry crop for production under greenhouse conditions.

**Key words:** Biomass, nutrient extraction, rhizobacteria, *Rubus* spp., vegetative development.

## INTRODUCTION

Blackberry (*Rubus* spp.) plants have a perennial life cycle; however, fructification is a biannual process. The primocane, or the vegetative sprout, developed during the annual growth season emerges, grows, and develops floral and vegetative sprouts. For these reasons, good control of vegetative development influences the quality and yield of polydrupes during the following growth season (Strik, 2017).

Due to the great productive demand of exportation and national markets, international regulations that emphasize sustainable growth with minimal use of agrochemicals have been presented, therefore; a production alternative is to implement the use of microorganisms involved, directly or indirectly, in the development and growth of plants, these microorganisms are known as plant growth promoting rhizobacteria (PGPR) (Syed and Prasad, 2019), these present a minimal environmental impact and more importantly, do not pose a threat to human health (Anuradha et al., 2019). These PGPR can act as a source and demand of minerals in soil and stimulate the production of vegetative hormones that have an influence in plant's biochemical, physiological, and morphological changes: Some PGPR are even capable of inhibiting the growth of pathogens transmitted through the soil (Gupta et al., 2021). The main phytohormones that produce or intervene in their availability of PGPR are cytokinins, auxins, gibberellins, ethylene and abscisic acid (Goswami et al., 2016), in addition to the production of phytohormones, the fixation of free N, and solubilization of phosphate (Prasad et al., 2019).

The PGPR are a vital component of the soil microbiome, various studies have been carried out in different crops on their capacities for plant promotion and improvement of crop yield, such as increasing the absorption of soil nutrients through phosphate solubilization and the synthesis of siderophores, which helps in the growth of habanero pepper (Castillo-Aguilar et al., 2017) and blackberry (Contreras et al., 2016). The use of different strains of *Pseudomonas* has increased the germination of corn and chili seeds (Marquina et al., 2018). Likewise, an increase in the production of plant hormones that benefited tomato growth (Kalam et al., 2020) and chili pepper (*Capsicum annuum* L. 'Cacique Gigante') (Marquina et al., 2018) has been reported.

The PGPRs increase the production of secondary metabolites in blackberry fruits (Martin-Rivilla et al., 2021). In addition to offering benefits on salt stress tolerance in lettuce cultivation (Fasciglione et al., 2015), in millet (*Panicum miliaceum*) and sorghum sprouts (*Sorghum vulgare*) (Sagar et al., 2020), and protection against biotic stress in blackberry (Contreras et al., 2016).

To achieve the expected benefits from PGPRs to plants, the selection of efficient strains for cultivation, which comprise complex interactions between plants, bacteria and environmental factors, is required, and requires understanding how to include PGPRs in soilless culture (Azizoglu et al., 2021). In addition, the available nutrient content and the quantity and quality of associated radical exudates are involved with the growth and establishment of rhizobacteria (Backer et al., 2018). Therefore, the application of PGPR in agricultural production is relevant for the possibility of significantly reducing the use of chemical fertilizers and pesticides; it is considered as an excellent ecological biotechnological approach (Prasad et al., 2019). Therefore, the objective of this study was to determine the efficiency of PGPR strains in the vegetative state of blackberry plants grown under greenhouse in hydroponics as a first stage of intensive production of this crop, under the hypothesis that PGPRs promote physiological changes that affect the productive quality of blackberry.

## MATERIALS AND METHODS

### Materials used

The experiment was set up during May and July 2021 in a greenhouse on the Plant Nutrition area of Colegio de Postgraduados of Campus Montecillo, Mexico. Blackberry (*Rubus* spp.) 'Tupy' seedlings were used, brought from a nursery in Uruapan, Michoacán, propagation was carried out using in vitro cloning technique in the laboratory, once the sprouts had roots and two true leaves, they were subsequently acclimatized in the nursery for development until reaching heights of 7-10 cm for transplant. Black polyethylene bags of 6 L capacity were used, filled with tezontle with particles smaller than 1 cm as a substrate, disinfected with chlorine (sodium hypochlorite) at a concentration of 200 mg $L^{-1}$ and washed with water, a blackberry seedling was later transplanted for each bag or pot, and a guide or tutor system with raffia for agricultural tutoring was installed.

### Establishment and experimental design

The experiment was established under a completely randomized design (CRD), with a total of six treatments (six strains of rhizobacteria) and the control (without inoculation), repeating six times each. The experimental unit consisted of a blackberry pot or plant, which was irrigated with Universal Steiner nutrient solution at 35% (EC = 0.7 dS $m^{-1}$), maintaining the pH within the range of 5.5 to 6.5, considered the optimal range where phosphates and mostly all essential nutrients are available, when variations occurred, adjustments were made, using NaOH (1 N) or $H_2SO_4$ (98%) to raise or lower the pH respectively, until pH is within the indicated range. Irrigations were carried out with a 4 L $h^{-1}$ drip system, in six programmed irrigations every 2 h during the day with a duration of 2 min.

### Microbiological material

Six strains of plant growth-promoting rhizobacteria (PGPR) were used as inoculum: P61 (*Pseudomonas tolaasii*) isolated from the rhizosphere soil of *Solanum tuberosum* L., AC-35 (*Serratia liquefaciens*), A46 (*P. tolaasii*) isolated from the soil of the rhizosphere culture of *S. tuberosum* 'Alpha', R44 (*Bacillus pumilus*) isolated from the rhizosphere soil of *S. tuberosum* 'Rosita', BSP1.1 (*Paenibacillus* sp.) isolated from an Andisol soil, JLB4 (*Arthrobacter pokkalii*) isolated from the rhizosphere soil of *S. lycopersicum* L., and the control to which only sterile nutrient broth was applied. The Rhizobacteria belong to the microbial collection of the Soil Microbiology area of the Soil Science Program of the Postgraduate College, who isolated them and tested their rhizobacterial capacity. The inoculation of the strains or application of treatments was carried out at 4 and 35 d after transplanting, in the morning hours 5 mL inoculum was applied with a concentration greater than $1\times10^8$ $mL^{-1}$ cells at the base of each plant. Due to the age of the plant, the amount of inoculum was distributed in the rhizosphere of the plant and was uniform due to the programmed irrigations.

### Evaluated variables

Destructive sampling of the entire aerial part (leaves and stems) was performed 56 d after transplanting (dat) in the vegetative stage. The evaluated variables were: Stem diameter (mm), plant height (cm), SPAD readings, leaf area ($cm^2$) was determined by using the LI-COR equipment (LI-3100C, Lincoln, Nebraska, USA). The plants were later dried in oven at 65 °C for 72 h and weighed to obtain DM. With the values obtained, the growth index, specific leaf area (SLA) was calculated according to Hunt (2017):

$$\text{SLA (cm}^2\text{ g}^{-1}\text{)} = \text{Leaf area of plant/Leaf DM}$$

The dry material of the aerial part (leaves and stems) of the blackberry plants was ground to obtain particles < 2 mm, which were subsequently used to carry out the nutritional analyses, total N was determined and microKjeldahl method (Alcántar and Sandoval, 1999) was used for the quantification of P, K, Ca, Mg, S, Fe, Mn, B, Mo, Zn, and Cu. Wet digestion was carried out using the Alcántar and Sandoval (1999) methodology. The (filtered) extracts were read in an inductively coupled plasma (ICP) optical emission spectroscopy equipment (ICP-OES 725, Agilent Technologies Australia, Mulgrave, Victoria, Australia). Blanks were used in both methodologies in order to eliminate the error of the technique and in the handling of the samples, and subsequently the corresponding calculations were made to determine the nutritional extraction considering the total DM of the aerial part, in g $plant^{-1}$ for N, P, K, Ca, Mg, S, and in mg $plant^{-1}$ for Fe, Mn, B, Mo, Zn, and Cu.

### Statistical analysis

The data obtained from the variables evaluated were exposed to the Shapiro-Wilk normality test ($\alpha = 0.05$) and the Levene test for homogeneity of variance. The stem diameter, height and SLA variables met both tests and the ANOVA Tukey's mean comparison test ($\alpha = 0.05$) were applied. The variables SPAD readings, leaf area, DM of leaf, stem and total, which did not comply with the homogeneity of variance tests, were transformed to natural logarithm (ln) and both tests were repeated, and it was found that they did not meet homoscedasticity again, so the non-parametric Kruskal-Wallis analysis and the sum of Wilcoxon ranges were performed, according to the Siegel (2015) methodology. The nutrient analysis variables were subjected to ANOVA and Tukey's mean comparison test ($\alpha = 0.05$). For every case, the Statistical Analysis System (SAS) Version 9.4 (SAS Institute, Cary, North Carolina, USA) was used.

## RESULTS AND DISCUSSION

### Agronomic variables

The results of the ANOVA (Table 1) and the non-parametric analyses (Table 2) of the agronomic variables evaluated were highly significant ($P \leq 0.001$) among treatments and the reliability of the analyses is implied by the low values of the coefficient of variation.

**Table 1.** Statistical significance of treatments ($P < 0.05$) in agronomical variables and growth index in 'Tupy' blackberry plants inoculated with different strains of plant growth promoting rhizobacteria (PGPR). CV: Coefficient of variation. $^{**}$Significant ($P > 0.001$-$P \leq 0.05$). $^{***}$Highly significant ($P \leq 0.001$).

| Source of variation | Height | Stem diameter | Specific leaf area |
|---|---|---|---|
| Treatments | *** | ** | *** |
| CV, % | 36.16 | 10.62 | 3.64 |
| $R^2$ | 0.86 | 0.45 | 0.79 |
| Media | 84.95 | 6.64 | 107.12 |

**Table 2.** Average ranges (Wilcoxon) and de Kruskal-Wallis test of SPAD readings, foliar area, leaf DM, stem DM and total DM of 'Tupy' blackberry plants inoculated with different strains of plant growth promoting rhizobacteria (PGPR). signed-rank. Values in parenthesis equals the media expressed in g plant$^{-1}$. $^{*}$Significant ($P > 0.01$-$P \leq 0.05$). $^{**}$Highly significant at 5% ($P \leq 0.01$).

| Treatment | SPAD readings | Leaf area | Leaf DM | Stem DM | Total DM |
|---|---|---|---|---|---|
| | Rank | | | | |
| Control | 18.88 | 12.00 | 11.25 (9.32) | 10.50 (4.11) | 10.75 (13.43) |
| P61 | 6.25 | 22.00 | 22.25 (18.69) | 24.00 (18.35) | 21.50 (37.04) |
| AC-35 | 23.75 | 4.00 | 4.25 (7.55) | 7.00 (2.76) | 4.50 (10.31) |
| A46 | 17.88 | 25.00 | 25.25 (24.89) | 24.50 (17.33) | 25.50 (42.22) |
| R44 | 15.25 | 13.25 | 10.00 (9.31) | 11.50 (3.66) | 9.50 (12.97) |
| BSP1.1 | 5.25 | 8.50 | 11.25 (10.43) | 6.75 (2.75) | 10.25 (13.18) |
| JLB4 | 14.25 | 16.75 | 17.25 (13.88) | 17.25 (8.11) | 16.50 (21.99) |
| $\chi^2$ | 15.989 | 19.249 | 19.485 | 20.047 | 18.598 |
| $Pr > \chi^2$ | 0.0138 | 0.0038 | 0.0034 | 0.0027 | 0.0049 |
| Significance | * | ** | ** | ** | ** |

### Plant height

The strains P61, A46 and JLB4 favored the growth of the plants, reaching a height of 175.8, 150.5 and 117.0 cm respectively, despite being significantly equal to the control plants (110.2 cm), an increase in height of 50% was observed in strain P61 (175.8 cm). Meanwhile, in the rest of the treatments the height was lower than the control's (Figure 1A). This is attributed to the ability of PGPRs genus *Pseudomonas* to synthesize phytohormones such as auxins with properties similar to indole-3-acetic acid (IAA) (Sah et al., 2021), since it is the main signaling metabolite that induces plant growth, by increasing cell division and differentiation of plant cells (Enders and Strader, 2015; Gupta et al., 2021; Sah et al., 2021; Zhuang et al., 2021). The

PGPRs *Pseudomonas* species are involved in plant development, through the production of siderophores, P solubilization, and secretion of antagonistic compounds for various plant pathogens (Zhuang et al., 2021).

Regarding the PGPRs genus *Bacillus* (strain R44), it has been reported that the promoter capacity of *Bacillus subtilis* (strain GIBI-200) and *B. pumilus* (strain GIBI-206), did not present significant differences with respect to blackberry plants with conventional management without inoculation (Robledo-Buriticá et al., 2018). The inoculation of the *Bacillus* strain in this experiment resulted in shorter plants (strain R44) than the control (Figure 2), which can due to the fact that these rhizobacteria could synthesize ethylene, which has negative effect on plant growth (Ruzzi and Aroca, 2015). Something similar was determined in tomato plants (*Solanum lycopersicum* L.), when inoculated with a strain *Bacillus megaterium* development decreased due to an overproduction of ethylene (Porcel et al., 2014). Plant height was affected by inoculated PGPR strains (Figure 2). Another notable variable in Figure 2 is the size of leaves; plants inoculated with strains R44 and BSP1.1 presented a lower growth, and visually their leaves had a greater surface compared to the leaves of the plants of greater heights.

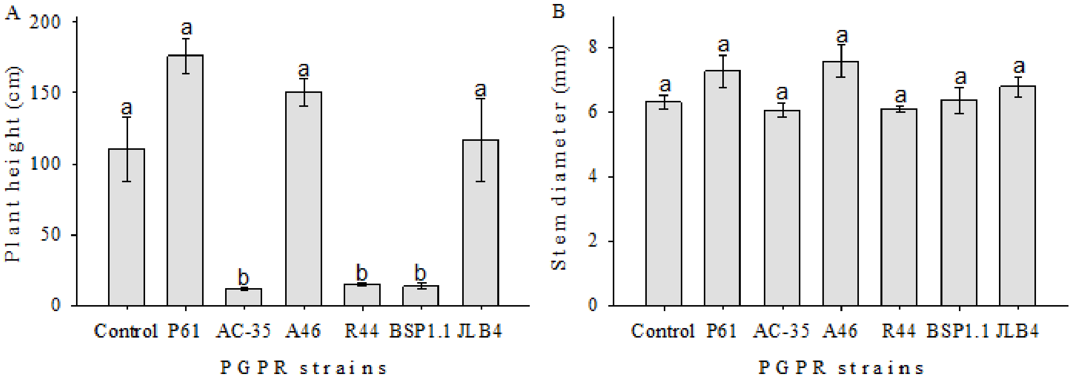


**Figure 1.** Plant height (A) and stem diameter (B) in 'Tupy' blackberry plants inoculated with different strain of plant growth promoting rhizobacteria (PGPR). Means with different letter are significantly different (Tukey, P < 0.05).

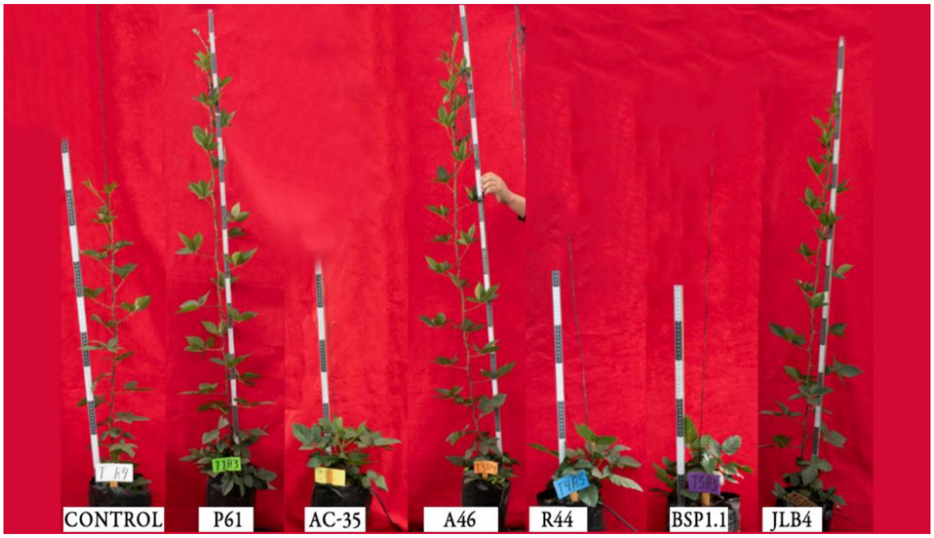


**Figure 2.** Vegetative development of 'Tupy' blackberry plants, inoculated with strains of plant growth promoting rhizobacteria (PGPR).

## Stem diameter

ANOVA showed significant differences among treatments (Table 1). The Tukey means comparison (Figure 1B) determined nonsignificant differences among treatments for stem diameter. The plants inoculated with PGPR reached stem diameters of 6.0 to 7.5 mm, without significant differences among themselves neither with control plants (not inoculated), the latter registered a stem diameter of 6.3 mm.

In tomato crop, an increase of 8.5% in stem diameter was reached when inoculation was performed with PGPR *Pseudomonas* (strain FA-56) in relation to non-inoculated plants (Chiquito-Contreras et al., 2020); in another study where PGPR genus *Bacillus* were inoculated, an increase in the diameter of corn (*Zea mays* L.) stem of 9% (strain RC9), and up to 22% (EAM5 strain) was achieved in tomato in regard to treatment without inoculation (Rojas-Badía et al., 2020).

## SPAD readings

This tool is used to quantify the greenness of leaves in any period of growth, in this study the inoculation of the PGPR strain AC-35 in blackberry plants registered the higher average Wilcoxon range 23.75 (Table 2), which corresponds to an average reading of 63.2 (Figure 3A). The leaves of the blackberry plants in this treatment presented greater intensity of green color, exceeding the average recorded by the control (61.5) by 3%. On the other hand, the inoculation of the BSP1.1 strain in the plants registered the lowest SPAD value, up to 11% lower than the control, while the rest of the treatments behaved significantly similar to the control plants (Figure 3A).

The SPAD readings of this experiment differed, perhaps because of differences in the ability of PGPR strains to solubilize or promote nutrient uptake (Backer et al., 2018). *Bacillus* strains inoculated in rice (*Oryza sativa* L.) increased by 21% in SPAD reading compared to non-inoculated plants (Naher et al., 2018).

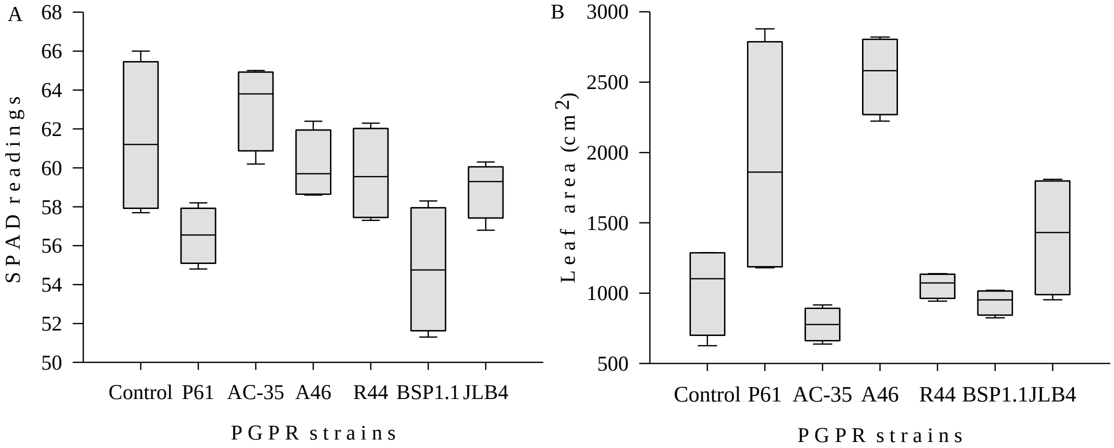


**Figure 3.** SPAD readings (A) and leaf area (B) of 'Tupy' blackberry plants, inoculated with strains of plant growth promoting rhizobacteria (PGPR).

## Leaf area

The plants inoculated with the A46 strain registered the highest average Wilcoxon range of 25.0 (Table 2) and a mean leaf area of 2551.8 cm$^2$ (Figure 3B), followed by plants inoculated with strain P61 with an area of 1944.9 cm$^2$, 147% and 88% higher respectively than control plants (1029.9 cm$^2$). Meanwhile, plants inoculated with strains AC-35 (777.35 cm$^2$) and BSP1.1 (937.37 cm$^2$) registered 25% and 9% respectively; less than the leaf area obtained with the control treatment plants (1029.9 cm$^2$).

Leaf area is one of the most important variables related to plant growth, because leaves are the fundamental organs of the foliage area, which are important for photosynthesis and transpiration, and these processes are necessary for the exchange of water, nutrients and energy in the soil-plant-atmosphere system (Taiz et al., 2015). The inoculation of *Pseudomonas* presents good results in terms of the leaf area, as well as when combined with other rhizobacteria. In the case of inoculated *Tabebuia donnell-smithii* Rose plants with *Azospirillum brasilense* + *Pseudomonas fluorescens* + *Rhizophagus intraradices*, they exceeded the control plants (without inoculation) by 45% and 55% at 74 and 103 dat (Aguirre-Medina et al., 2017). On the other hand, the inoculation of BSP1.1 and R44 strains in habanero pepper plants (*Capsicum chinense* Jacq.), induced greater leaf area and thus exceed up to 28% and 21% respectively, compared to plants without inoculation (Castillo-Aguilar et al., 2017).

According to Wilcoxon's average ranges (Table 2), inoculation with the strains A46 (25.25) and P61 (22.25) in blackberry plants were higher in leaf DM compared to the control plants (11.25), with up to 24.89 and 18.69 g plant$^{-1}$ respectively (Figure 4A), that represents 166% and 100% more than the control plants (9.32 g plant$^{-1}$). The same trend was obtained in stem DM (Figure 4B), where inoculation with strains P61 (18.35 g plant$^{-1}$) and A46 (17.33 g plant$^{-1}$) succeeded by 347% and 322% respectively compared to the control plants, and likewise in the sum of both variables in the total aerial part DM. The average range of Wilcoxon (Table 2) showed higher values for plants inoculated with strains A46 (25.5) and P61 (21.5), there were 214% and 129% higher than the control plants (Figure 4C), meanwhile plants inoculated with strains AC-35 and R44 according to Wilcoxon's average ranges, 4.5 and 9.5 respectively, affected the development of blackberry plants as they registered 24% and 5% less total DM than the control plant (13.43 g plant$^{-1}$).

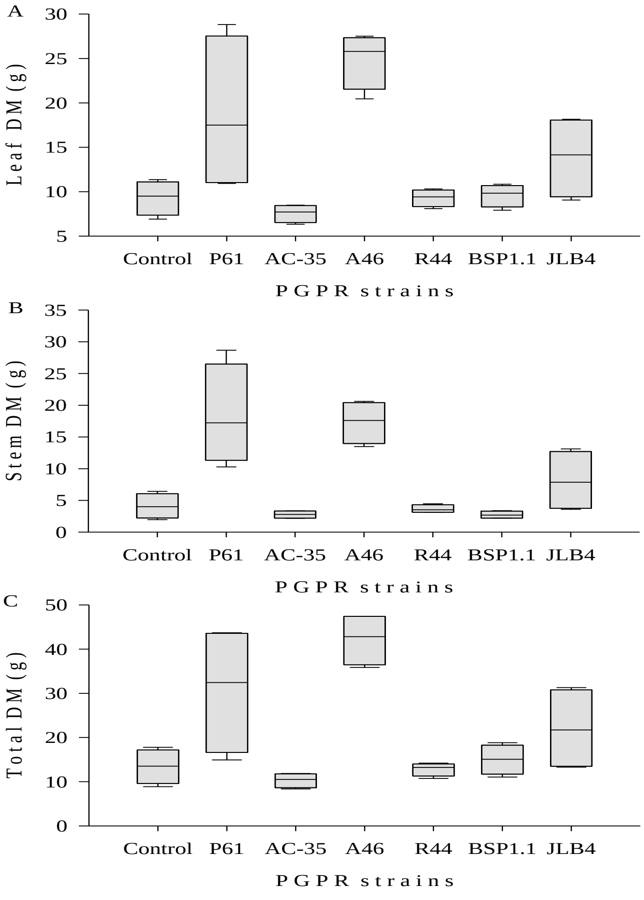


**Figure 4.** Dry matter of leaves (A), stem DM (B) and total DM from the aerial part (C) of 'Tupy' blackberry plants, inoculated with different strains of plant growth-promoting rhizobacteria (PGPR).

*Pseudomonas* strains have demonstrated a capacity for promoting DM in tomato plants produced in greenhouses; when inoculating *P. putida* the strains FA-8 and FA-56 exceeded uninoculated plants by 18% and 34% (Chiquito-Contreras et al., 2020). In habanero pepper plants the inoculation of strains P61 and A46 did not register any significant differences in aerial DM compared to the control plants, meanwhile the inoculation of strain BSP1.1 increased DM of the aerial part by 30% compared to non-inoculated plants (Castillo-Aguilar et al., 2017).

The promotion capacity of the inoculated *Pseudomonas* PGPRs in this experiment, showed positive effects compared to the control plants and with other strains, which is attributed to the fact that this genus has the capacity of N fixation, production of siderophores, solubilization of minerals and production of phytohormones that lead to a greater development of the plant (Sah et al., 2021).

The growth recorded by the plants in this experiment can be explained by the ability of PGPR to synthesize phytohormones, such as auxins, which are involved in cell division and elongation, and induce higher and rapid growth in plants, mainly IAA in the meristematic region of shoots, buds and the root tip of plants (Enders and Strader, 2015; Casanova-Sáez et al., 2021), while gibberellins can accelerate the shoot growth and higher elongation of organs such as stem and internode (Mukherjee et al., 2022). Due to the greater number of roots, they can better assimilate nutrients, in addition to the fact that the organic acids produced by the PGPR manage to solubilize and chelate nutrients such as P, K, Zn and Fe (Etesami and Adl, 2020), which induced the growth of the blackberry plants.

## Nutritional extraction of blackberry plants

The results of the ANOVA (Table 3) of the nutritional extraction by the aerial part of the plants were highly significant ($P \leq 0.001$) between treatments, and the reliability of the analyses is given by the low values of the coefficient of variation.

**Table 3.** Statistical significance of treatments ($P < 0.05$) in the nutritional extraction of 'Tupy' blackberry plants inoculated with different strains of plant growth promoting rhizobacteria (PGPR). SV: Source of variation; CV: coefficient of variation. $^{***}$Highly significant ($P \leq 0.001$). From N to S the media is expressed in g plant$^{-1}$ and from B to Zn in mg plant$^{-1}$.

| SV | N | P | K | Ca | Mg | S | B | Cu | Fe | Mn | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| CV, % | 37.59 | 42.15 | 39.48 | 33.87 | 37.72 | 39.63 | 34.91 | 45.77 | 66.94 | 55.26 | 36.15 |
| $R^2$ | 0.68 | 0.62 | 0.70 | 0.70 | 0.66 | 0.67 | 0.71 | 0.61 | 0.45 | 0.56 | 0.69 |
| Media | 0.493 | 0.026 | 0.197 | 0.124 | 0.058 | 0.026 | 1.41 | 0.11 | 5.15 | 4.00 | 0.47 |

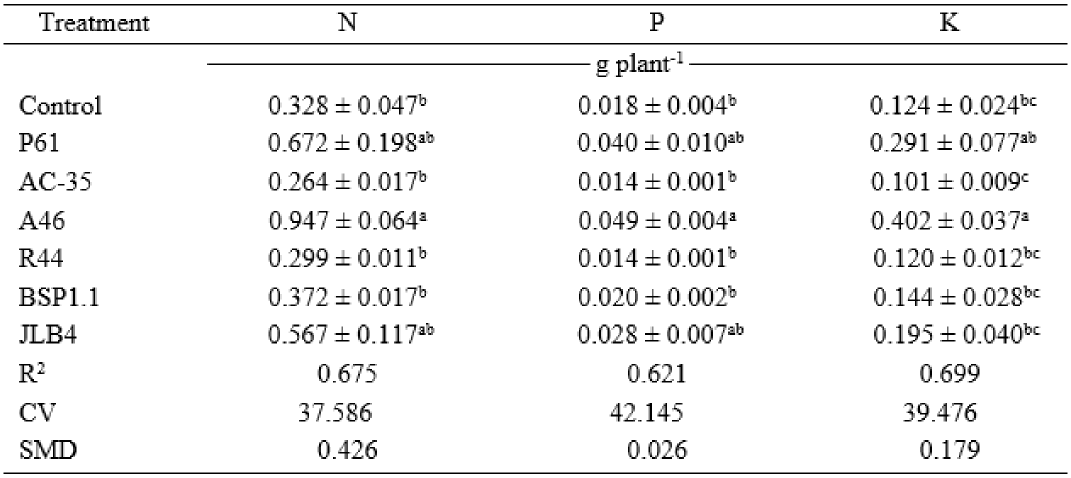
The nutritional extraction of blackberry plants is regulated by radical activity and its interaction with the microbial community, and in this research the highest extraction of macronutrients was recorded in blackberry plants inoculated with strain A46 (Table 3).

For the extraction of N, the plants inoculated with the A46 strain reached an average of 0.947 ± 0.064 g plant$^{-1}$ exceeding the control plants by188% (0.328 ± 0.047 g plant$^{-1}$), followed by the treatments inoculated with the strains P61 and JLB4 (0.672 ± 0.198 and 0.567 ± 0.117 g plant$^{-1}$ respectively), meanwhile the inoculation of the AC-35, R44 and BSP1.1 strains recorded extractions of N significantly equal to the control plants (0.264 ± 0.017, 0.299 ± 0.011 and 0.372 ± 0.017 g plant$^{-1}$, respectively).

In habanero pepper plants 8 wk after emergence, nonsignificant differences were found in terms of N extraction when the A46, P61 and R44 strains were inoculated, regarding the non-inoculated plants (Castillo-Aguilar et al., 2017). The importance of N lies in the fact that it is a constituent of amino acids, proteins, purine rings and pyrimidine of nucleic acids, chlorophyll, and enzymes, which are important in the metabolism and growth of plants and for these reasons, sufficient supply and absorption is required (Mitra, 2017).

Phosphorus is considered a limiting nutrient of plant growth, it is essential for cellular energy (ATP) and cellular structures (DNA, RNA and phospholipids) necessary in plant life (Mitra, 2017). With the inoculation of PGPR in this experiment, the highest P extraction was obtained with the A46 strain, with an average of 0.049 ± 0.004 g plant$^{-1}$ (Table 4), which represents 160% higher than the control treatment, meanwhile the inoculation of strain AC-35, R44 and BSP1.1 registered the lowest P extraction and behaved significantly equal to the control (0.018 ± 0.004 g plant$^{-1}$). The efficiency of the A46 strain can be attributed to the phosphate solubilization capacity of the PGPR genus *Pseudomonas* (Sah et al., 2021). Similar results were reported by Castillo-Aguilar et al. (2017) in habanero pepper plants with the inoculation of A46 strain, which induced an extraction 21% higher than the control.

**Table 4.** Extraction of N, P, and K in 'Tupy' blackberry plants inoculated with different strains of plant growth promoting rhizobacteria (PGPR). Media ± standard error. Columns with different letter, are significantly different from each other (Tukey, P < 0.05). CV: Coefficient of variation; SMD: significant minimum difference.



| Treatment | N | P | K |
|---|---|---|---|
| | g plant$^{-1}$ | | |
| Control | 0.328 ± 0.047$^{b}$ | 0.018 ± 0.004$^{b}$ | 0.124 ± 0.024$^{bc}$ |
| P61 | 0.672 ± 0.198$^{ab}$ | 0.040 ± 0.010$^{ab}$ | 0.291 ± 0.077$^{ab}$ |
| AC-35 | 0.264 ± 0.017$^{b}$ | 0.014 ± 0.001$^{b}$ | 0.101 ± 0.009$^{c}$ |
| A46 | 0.947 ± 0.064$^{a}$ | 0.049 ± 0.004$^{a}$ | 0.402 ± 0.037$^{a}$ |
| R44 | 0.299 ± 0.011$^{b}$ | 0.014 ± 0.001$^{b}$ | 0.120 ± 0.012$^{bc}$ |
| BSP1.1 | 0.372 ± 0.017$^{b}$ | 0.020 ± 0.002$^{b}$ | 0.144 ± 0.028$^{bc}$ |
| JLB4 | 0.567 ± 0.117$^{ab}$ | 0.028 ± 0.007$^{ab}$ | 0.195 ± 0.040$^{bc}$ |
| $R^2$ | 0.675 | 0.621 | 0.699 |
| CV | 37.586 | 42.145 | 39.476 |
| SMD | 0.426 | 0.026 | 0.179 |

For the means of K extraction, the inoculation of strain A46 allowed the blackberry plants to obtain a better extraction of the nutrient (Table 4) by reaching an average of 0.402 ± 0.037 g plant$^{-1}$, meanwhile the plants inoculated with strain AC-35 recorded the least K extraction with an average of 0.101 ± 0.009 g plant$^{-1}$; 19% less than the control treatment. Similar results were obtained in Turkish oregano plants (*Origanum onites* L.), when inoculated with PGPR *P. putida* and *P. fluorescens*, the extraction of K increased by 37% and 45% respectively, compared to the non-inoculated plants (0.228 g plant$^{-1}$) (Kutlu et al., 2019).

The extraction of Ca and Mg in blackberry plants (Table 5) with the inoculation of the A46 strain obtained the highest average, exceeding 254% and 169% respectively, to the control (0.245 ± 0.018 and 0.112 ± 0.006 g plant$^{-1}$ de Ca and Mg, respectively). The rest of the treatments did not surpass the control plants. The highest extraction of S was recorded in the inoculation of strain A46 (Table 5), which exceeded the control treatment by 176%. On the other hand, blackberry plants inoculated with strains AC-35 and R44 recorded the lowest extraction of S, being 24% and 16% lower than the average extraction obtained with the control (0.018 ± 0.003 g plant$^{-1}$).

**Table 5.** Extraction of Ca, Mg, and S in 'Tupy' blackberry plants inoculated with different strains of plant growth promoting rhizobacteria (PGPR). Media ± standard error. Columns with different letter are significantly different from each other (Tukey, $P < 0.05$). CV: Coefficient of variation; SMD: significant minimum difference.

| Treatment | Ca | Mg | S |
|---|---|---|---|
| | g plant$^{-1}$ | | |
| Control | 0.069 ± 0.016$^{b}$ | 0.041 ± 0.008$^{b}$ | 0.018 ± 0.003$^{bc}$ |
| P61 | 0.149 ± 0.036$^{ab}$ | 0.083 ± 0.022$^{ab}$ | 0.040 ± 0.011$^{ab}$ |
| AC-35 | 0.083 ± 0.007$^{b}$ | 0.033 ± 0.002$^{b}$ | 0.013 ± 0.001$^{c}$ |
| A46 | 0.245 ± 0.018$^{a}$ | 0.112 ± 0.006$^{a}$ | 0.050 ± 0.003$^{a}$ |
| R44 | 0.089 ± 0.009$^{b}$ | 0.037 ± 0.004$^{b}$ | 0.015 ± 0.001$^{c}$ |
| BSP1.1 | 0.097 ± 0.010$^{b}$ | 0.044 ± 0.004$^{b}$ | 0.018 ± 0.001$^{bc}$ |
| JLB4 | 0.132 ± 0.030$^{b}$ | 0.058 ± 0.014$^{b}$ | 0.027 ± 0.006$^{abc}$ |
| $R^2$ | 0.704 | 0.662 | 0.671 |
| CV | 33.868 | 37.723 | 39.628 |
| SMD | 0.097 | 0.051 | 0.024 |

An increase in nutrient extraction is attributed to the greater radical interception with nutrients available in the soil, and because PGPRs induce greater absorption of macronutrients, as they are directly related to increased root development, due to PGPR promotion mechanisms involved in root elongation, such as the production of IAA phytohormones and 1-aminocyclopropane-1-carboxylate deaminase (ACCD) (Etesami and Adl, 2020).

The application of stimulants on plants such as in this study has favorable effects. Similar results were reported in research where dandelion extract was applied to blackberry plants, which allowed the concentration of N, P and K to increase by 2%, 30% and 2% respectively, in blackberry leaves, compared to the control plants (Misimovic et al., 2020). And as in this research, the values indicate that the primary nutrients N, P and K are required and extracted by blackberry plants in large quantities for the processes of metabolism and growth (Mitra, 2017).

The blackberry plants inoculated with the A46 strain obtained the highest extraction of B, Cu, Fe, Mn, and Zn (Table 6), higher up to 201%, 153%, 259%, 282%, and 245%, respectively, to the plants without inoculation.

On the other hand, the plants inoculated with the AC-35 strain registered the lowest extraction of B, 11% lower than the control plants, while the rest of the treatments were significantly equal to the control. The extraction of Cu by blackberry plants inoculated with AC-35 and R44 strains recorded the lowest extraction: 35% and 23% lower than control plants. For the extraction of Fe, Mn and Zn in blackberry plants the inoculation of strains P61, AC-35, R44, BSP1.1, and JLB4, were significantly equal to the control plants.

Micronutrients are required in small quantities for plants, but they are essential for their development, so it is recommended that they are always present in optimal quantities to prevent lower growth and deficiency symptoms (Mitra, 2017).

When interacting directly with plants, PGPRs increase the availability of essential macronutrients and micronutrients, and this correlate with the nutritional extraction of plants (Oleńska et al., 2020). The PGPRs *Bacillus* and *Pseudomonas* sp. have low costs and simple application methods, so they are frequently used in sustainable agriculture to improve the nutritional status of crops of interest that affects profitability (Prasad et al., 2019). In addition, *Pseudomonas* PGPRs increase the absorption capacity of micronutrients available and incorporated through different nutritional sources, with the capacity to promote the production of organic acids and chelating agents (Etesami and Adl, 2020).

**Table 6.** Micronutrient extraction in 'Tupy' blackberry plants inoculated with different strains of plant growth-promoting rhizobacteria (PGPR). Media ± standard error. Columns with different letter are significantly different from each other (Tukey, $P < 0.05$). CV: Coefficient of variation; SMD: significant minimum difference.

| Treatment | B | Cu | Fe | Mn | Zn |
|---|---|---|---|---|---|
| | mg plant$^{-1}$ | | | | |
| Control | $0.90 \pm 0.19^{c}$ | $0.08 \pm 0.01^{bc}$ | $3.04 \pm 0.52^{b}$ | $2.16 \pm 0.58^{b}$ | $0.26 \pm 0.06^{b}$ |
| P61 | $2.00 \pm 0.51^{b}$ | $0.18 \pm 0.04^{ab}$ | $6.91 \pm 1.91^{b}$ | $5.08 \pm 1.77^{ab}$ | $0.64 \pm 0.12^{ab}$ |
| AC-35 | $0.80 \pm 0.05^{c}$ | $0.05 \pm 0.00^{c}$ | $2.92 \pm 0.55^{b}$ | $2.39 \pm 0.47^{b}$ | $0.26 \pm 0.04^{b}$ |
| A46 | $2.74 \pm 0.15^{a}$ | $0.20 \pm 0.03^{c}$ | $10.95 \pm 3.78^{a}$ | $8.29 \pm 0.69^{a}$ | $0.90 \pm 0.06^{a}$ |
| R44 | $0.89 \pm 0.05^{c}$ | $0.06 \pm 0.00^{c}$ | $3.26 \pm 0.30^{ab}$ | $2.60 \pm 0.29^{b}$ | $0.30 \pm 0.02^{b}$ |
| BSP1.1 | $0.99 \pm 0.07^{c}$ | $0.09 \pm 0.01^{abc}$ | $4.27 \pm 0.75^{ab}$ | $2.19 \pm 0.35^{b}$ | $0.40 \pm 0.04^{b}$ |
| JLB4 | $1.53 \pm 0.29^{c}$ | $0.09 \pm 0.02^{abc}$ | $4.67 \pm 1.23^{ab}$ | $5.29 \pm 2.03^{ab}$ | $0.52 \pm 0.14^{ab}$ |
| $R^2$ | 0.714 | 0.609 | 0.448 | 0.558 | 0.685 |
| CV | 34.915 | 45.769 | 66.940 | 55.258 | 36.146 |
| SMD | 1.13 | 0.12 | 7.92 | 5.08 | 0.39 |

## Specific leaf area

Regarding the SLA (Figure 5), the inoculation of the BSP1.1 strain in blackberry plants increased up to 122.6 cm$^2$ g$^{-1}$ dry leaf, which indicates greater leaf thickness (Hunt, 2017), which was significantly higher than the index obtained by the control plants by 14%, while the rest of the PGPR inoculated plants behaved significantly equal to the control plants, with a range from 101.4 to 107.9 cm$^2$ g$^{-1}$.

The importance of this variable is because SLA is considered an essential functional trait and indicator to estimate the behavior of plants in the face of environmental changes (Zhou et al., 2020), is directly influenced by leaf area, thickness, shape, and age, and is closely related to the plant's strategy for water usage or survival (Boucher et al., 2017).

When plants develop under a water scarcity, low SLA occurs, increasing the thickness of leaves, causing thicker cell walls and narrower connections to develop to avoid water loss by evaporation (Zhou et al., 2020). When plants develop in favorable, resource-rich environments, the SLA is larger to improve the photosynthetic capacity and productivity (Yao et al., 2016), and they adapt the smaller SLA strategy to improve stress resilience and competitive capacity in poor environments. The SLA decreases according to the plant's growth stage (Zhou et al., 2020).

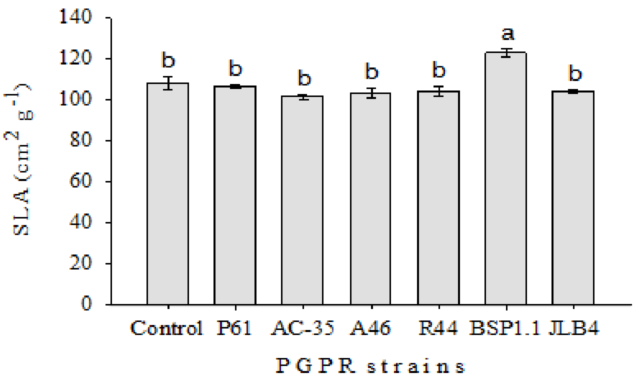


**Figure 5.** Specific leaf area (SLA) of 'Tupy' blackberry plants, inoculated with different strain of plant growth promoting rhizobacteria (PGPR). Means with different fonts are significantly different from each other (Tukey, $P < 0.05$).

# CONCLUSIONS

The plant growth promoting rhizobacteria (PGPR) strains P61 and A46 of *Pseudomonas tolaasii* species presented positive effects on the variables of height, stem diameter, leaf area and shoot DM when inoculated in blackberry plants, these strains can be inoculated in the cultivation of blackberry to improve their vegetative development under greenhouse conditions. The inoculation of the A46 strain promoted the blackberry plants to obtain a greater nutritional extraction of macronutrients and micronutrients.

**Author contribution**

Conceptualization: I.C-A., M.S-V. Methodology: I.C-A., M.S-V. Validation: J.J.A-S., M.S-V., 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: J.J.A-S., M.S-V., J.L.G-C., M.E.P-S. Project administration: M.S-V. Funding acquisition: M.S-V. All authors reviewed the final version and approved the manuscript before submission.

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