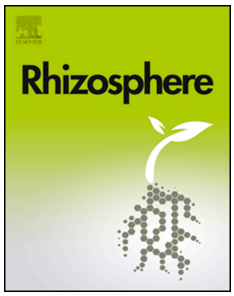

Native plant growth promoting rhizobacteria improve the growth of pepper seedlings and modify the phenolic compounds profile

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Native plant growth promoting rhizobacteria improve the growth of pepper seedlings and modify the phenolic compounds profile

Miguel Andrés Lobato-Ureche[a], María Micaela Pérez-Rodriguez[a], Emiliano Malovini[b], Patricia N. Piccoli[a], Romina P. Monasterio[a] and Ana Carmen Cohen[a,*]

[a]Instituto de Biología Agrícola de Mendoza (IBAM-CONICET), Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Almirante Brown 500 (5505) Chacras de Coria, Mendoza, Argentina.

[b]Tecnosiembra S.A. Nicolás Serpa 1808 Rodeo del Medio, (5529) Maipú, Mendoza, Argentina.

Corresponding author: acohen@fca.uncu.edu.ar

Abstract

In the establishment of a highly productive pepper crop, obtaining quality seedlings is a decisive step. An alternative to boost rapid plant growth has been the use of plant growth promoting rhizobacteria (PGPR). The study of PGPR and its effect on different plant species has made it possible to establish, among other physiological parameters, a direct correlation between total phenolic compounds and a positive systemic response induced in plants, which could act as growth regulators. The evaluation of the phenolic compound profile and its change in relation to PGPR-pepper seedlings interaction, using liquid chromatography, has scarcely been reported. The aim of the present study was to evaluate changes in the morphology, nitrogen (N) accumulation and the phenolic compounds profile produced by the inoculation of four native PGPR strains: *Pseudomonas* 42P4, *Cellulosimicrobium* 60I, *Enterobacter* 64S1, and *Ochrobactrum* 53F during the growth of Calahorra pepper seedlings (cv. Calafyuco INTA). Our results showed that all the PGPR tested can promote growth in pepper seedlings. However, *Pseudomonas* 42P4 and *Cellulosimicrobium* 60I1 were more effective in increasing N uptake, and improving the morphological, biochemical, and physiological parameters in pepper seedlings. Flavonoids, such as naringenin, naringin, and catechin, could favor growth in plants inoculated with *Pseudomonas* 42P4, whereas only catechin in *Cellulosimicrobium* 60I1. The combined effect of gallic acid, hydroxytyrosol, tyrosol, phloridzin, and the exacerbated production of (-)-epigallocatechin gallate may contribute synergistically to limiting the growth of Control seedlings. Finally, PGPR applied in this study could be used as

biofertilizers, thereby reducing the use of nitrogen fertilizers, cutting down on production time and cost, and improving the quality of seedlings for horticulturists and nurseries.

Keywords: PGPR, *Capsicum annuum*, Pseudomonas, Cellulosimicrobium, phenolic compound profiles

1. Introduction

Pepper (*Capsicum annuum*.), family *Solanaceae* is considered one of the most important horticultural crops in the world. It is estimated that the annual world production of peppers is around 42.3 million tons, in a cultivated area of 3.7 million ha (Mori et al., 2022). In the province of Mendoza, Argentina, it is estimated that around 1246 ha of peppers are grown for the packaging industry (FAOSTAT, 2021). The intensive production of pepper seedlings is an important factor to satisfy the high demand from horticulturists. Nevertheless, in the production of peppers, the use of agrochemicals that deteriorate the environment and affect human health is frequent (Xiao et al., 2020).

Bioinoculants are an alternative for increasing production while reducing adverse effects on the environment. Some bioinoculants are composed of plant growth promoting rhizobacteria (PGPR), which are characterized by fixing atmospheric N in the soil, producing siderophores to improve Fe uptake, and solubilizing insoluble phosphates which makes them available to plants. In addition, PGPR can produce a considerable number of plant growth regulators (Cohen et al., 2008; Glick, 2012; Mehmood et al., 2018).

Previously, we isolated and characterized different native PGPR strains from Mendoza province. These strains promoted growth of tomato seedlings cultivated in the growth chamber (Pérez-Rodriguez et al., 2020a). *Pseudomonas* 42P4 and *Enterobacter* 64S1 strains alleviated the deleterious effects of salt stress by NaCl in tomato plants grown in a greenhouse (Pérez-Rodriguez et al., 2022). In addition, *Pseudomonas* 42P4, *Cellulosimicrobium* 60I, *Ochrobactrum* 53F and *Enterobacter*

64S1 reduced the time of germination and increased the percentage of germination, vigor index, length, and diameter of roots of pepper seeds. Furthermore, *Pseudomonas* 42P4 and *Cellulosimicrobium* 60I modified the profile of phenolic compounds and increased the phenolic acid in inoculated pepper seed suggesting an elicitation of phenylpropanoid pathways related to induced systemic response (IRS) (Lobato-Ureche et al., 2021; Lobato-Ureche et al., 2023).

Phenolic compounds are secondary metabolites of plants and they are synthesized through the shikimic acid and phenylpropanoid pathways (Alara et al., 2021). Some authors have suggested that phenolic compounds may have a role as plant growth regulators (Dare et al., 2013a). The synthesis of phenolic compounds in plants can occur in response to several biotic and abiotic factors (Riviere et al., 2012). Studies based on colorimetric techniques reported a positive correlation between improved growth and increased content of total phenolic compounds in plants inoculated with PGPR (Chiappero et al., 2019; Khanna et al., 2019). However, the use of separative analytical techniques, such as liquid chromatography (LC), can be important to observe changes in the profiles and elucidate the role of some phenolic compounds in plants inoculated with PGPR.

The aim of the present study was to evaluate the changes on morphology, nitrogen accumulation, photosynthetic, and photoprotective pigments observed in Calahorra pepper seedlings (cv. Calafyuco INTA) inoculated with four native PGPR strains: *Pseudomonas* 42P4, *Cellulosimicrobium* 60I, *Ochrobactrum* 53F, and *Enterobacter* 64S1. In addition, the phenolic compounds profile and the quantification of different families of them were studied.

2. Material and methods

2.1. Plant materials

Seeds of *Capsicum annuum* cv. Calafyuco INTA were kindly supplied by Dr. C. Galmarini (National Institute of Agricultural Technology, INTA-EEA, La Consulta, Mendoza, Argentina).

2.2. Bacterial cultures

The strains used were *Pseudomonas* 42P4 (42P4), *Cellulosimicrobium* 60I1 (60I1), *Enterobacter* 64S1 (64S1), and *Ochrobactrum* 53F (53F). These strains belong to the Plant Physiology and Microbiology Lab (IBAM-FCA, CONICET-UNCuyo, Mendoza, Argentina) and the partial sequences amplified of 16S ribosomal RNA gene have been deposited in the GenBank: MT045993.1, MT047266.1, MT047267.1, and MT047264.1, respectively. These strains were isolated from the rhizosphere and roots of tomato plants from a productive farm in Mendoza, Argentina. They were characterized as PGPR considering their effectiveness of fixing nitrogen, solubilizing phosphate, producing siderophores, and indole acetic acid (Pérez-Rodriguez et al., 2020a).

The pre-inoculum was prepared by growing strains 42P4, 60I1, 64S1, and 53F on a volume of 10 mL of rich medium of LB (Luria Broth, Sigma Chem. Co.) 24 h at 28°C and 120 rpm until reaching an OD_{530} = 1.2. Then, to prepare the inoculum, 500 µL of pre-inoculum were grown in an erlenmeyer flask with 50 mL of LB for 24 h at 28°C and 120 rpm until reaching 10^8 CFU mL^{-1}. This concentration was previously selected as adequate to increase pepper growth (Lobato Ureche et al.,

2023). The seedlings were inoculated with 1000 µL of each culture as detailed below.

2.3. Seed germination

Seeds were surface disinfected with 20% sodium hypochlorite for 1 min and then washed three times with sterile distilled water. The seeds were sown in sterile trays containing the sterilized Kekkilä DSM 1 W growth medium (Kekkilä professional). The medium contained 70% brown and 30% dark *Sphagnum fuscum* dominant peat ($N\text{-}P_2O_5\text{-}K_2O$ 15-12-29 and microelements 0.6 kg m^{-3}, pH 5.9, electrical conductivity 0.2 dS m^{-1}). A completely randomized design of six treatments was established, with three replicates of 10 seeds each. Thirty days after sowing, the seedlings with two fully expanded leaves were inoculated with 1000 µL of PGPR containing 10^8 CFU mL^{-1} of the corresponding bacterial culture. Thus, the treatments were seedlings treated with: 1) Hakaphos® 18-18-18 (N-P-K), Fertilized treatment ; 2) inoculated with 42P4 strain; 3) inoculated with 60I1 strain; 4) inoculated with 64S1 strain; 5) inoculated with 53F strain; 6) inoculated with LB medium, Control. Then, the seedlings were located in a growth chamber at 24±1°C with a 12/12 h photoperiod (100 µmol m^{-2} s^{-1}) and a relative humidity ~50%.

Finally, all growth parameters were measured at the end of the assay (after 55 days) and data was collected to evaluate the morphological aspects including: leaf area (measured using the Micrometrics SE premium software), and the aerial and root dry weights were determined after drying the samples for 7 days in the stove at 60ºC. The workflow is presented in Fig. 1.

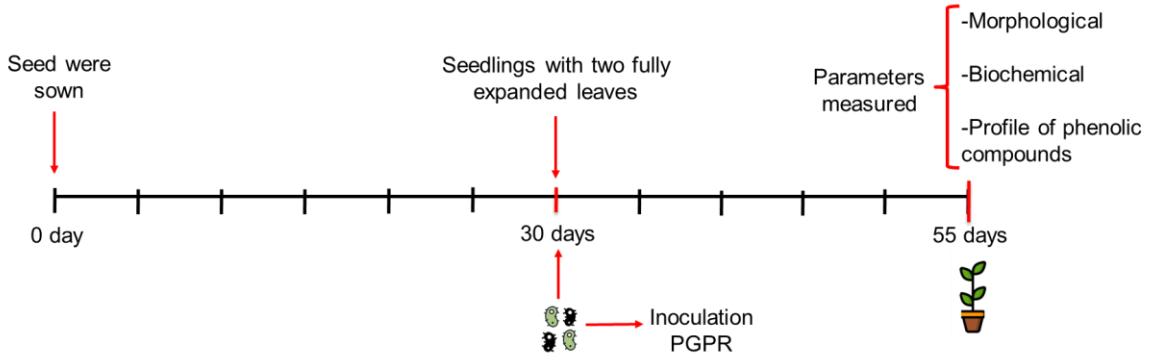


Figure 1. Experimental design and timeline of parameters studied.

2.4. Nitrogen determination

Leaves of 55-day-old plants were dried at 60°C on the stove. Later, they were ground and the nitrogen content was determined by the Micro-Kjeldahl method as described by Guebel et al. (1991).

2.5. Photosynthetic and photoprotective pigments

Determinations were performed spectrophotometrically as described by Chapelle et al. (1992), with modifications of Cohen et al. (2015), using leaf samples. Chlorophyll a, b, and total (Chl a, Chl b and total = Chl a + Chl b), carotenoid and anthocyanin levels were measured from 1 cm^2 diameter disc samples and expressed in mg^{-1} of leaves.

2.6. Extraction of phenolic compounds

The phenolic compounds were isolated by using a solid-liquid extraction according to a previously reported procedure (Moussi et al., 2015), which can be briefly described as follows: a portion of 0.5 g of lyophilized material (leaves) was weighed in a conical centrifuge tube and mixed with 5 mL of ethanol. Then, the

tube was left in an ultrasonic bath for 10 min and the supernatant obtained by centrifugation (2500 *g* for 10 min) was evaporated to dryness using a rotary evaporator at 40°C. The residue was redissolved in 1 mL of 0.1% (v/v) formic acid.

2.7. Phenolic compound analysis

For phenolic compound quantification, high-performance liquid chromatography coupled with diode array and fluorescence detectors (LC-DAD-FLD) Dionex UltiMate 3000 HPLC system (California, USA) was used. Chromatographic separations were carried out in a reversed-phase Kinetex C_{18} column (3.0 mm × 100 mm, 2.6 µm) Phenomenex (Torrance, CA, USA) at 35°C. The mobile phases were ultrapure water with 0.1% (v/v) formic acid (phase A) and acetonitrile (phase B). Separation of the analytes was performed using the following gradient: 0–1.7 min, 5% B; 1.7–10 min, 30% B; 10–13.5 min, 95% B; 13.5–15 min, 95% B; 15–16 min, 5% B; 16–19, 5% B. The flow rate was set constant at 0.8 mL min^{-1} during the whole process, and the injection volume was 5 µL as was described by Ferreyra et al., (2021).

The identification and quantification of the target phenolic compounds in the extracts was based on the comparison of the retention times and maximum absorbance value of detected peaks in samples of interest with those obtained by the injection of pure standards. The working wavelengths for the different families of analytes for DAD were 254 nm, 280 nm, 320 nm, and 370 nm, while an excitation wavelength (Ex) of 290 nm and monitored emission (Em) responses of 315 and 400 nm were used depending on the targeted analytes for FLD, as was described by Ferreyra et al. (2021). The Chromeleon 7.1 software was used to

131 control all the acquisition parameters of the LC-DAD-FLD system and also to
132 process the obtained data.

133 *2.8 Statistical analysis*

134 Data were processed by analysis of variance followed by a Duncan test to
135 discriminate between the means by the least difference with a significance level of
136 *P-value* ≤ 0.0001. The InfoStat statistical software (InfoStat version 2020v. Grupo
137 InfoStat, Argentina) was used (Di Rienzo et al., 2020).

138 ***3.* Results**

139 *3.1. Effects of inoculation on the growth of pepper seedlings*

140 Inoculation with all rhizobacteria significantly improved the aerial and root dry
141 weight of the pepper seedlings with respect to the Control treatment (Fig. 2).

142 

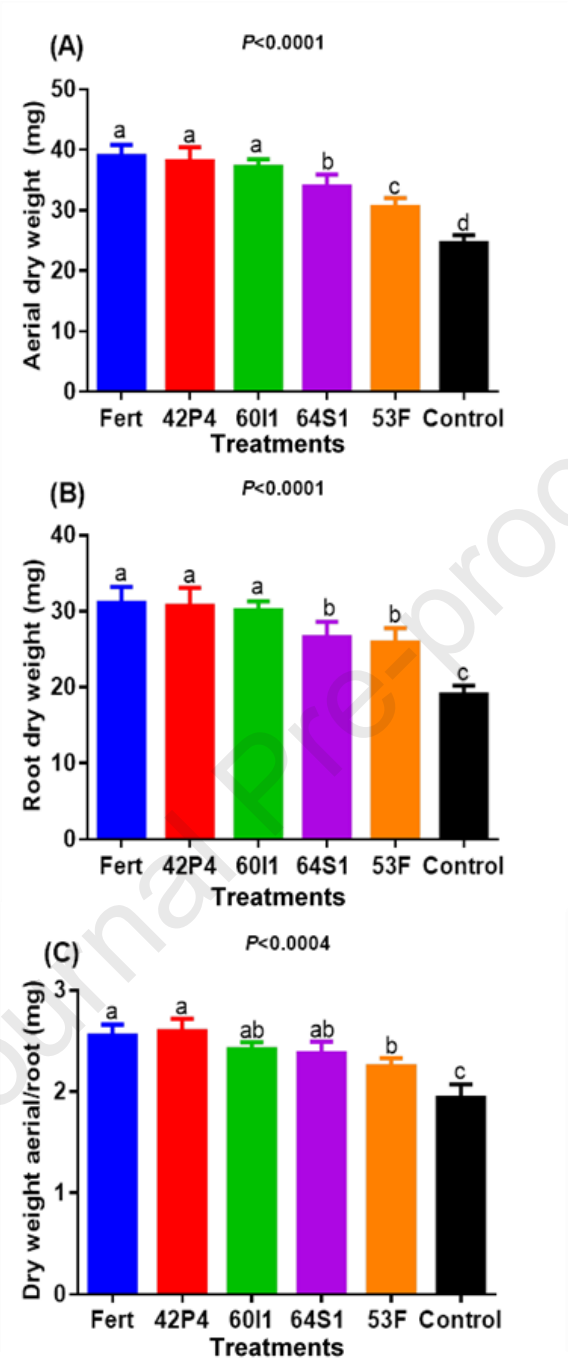
143 **Figure 2.** Pepper seedlings after 25 days after treatment with: Fertilized (a),
144 *Pseudomonas* 42P4 (b), *Cellulosimicrobium* 60I1 (c), *Enterobacter* 64S1 (d),
145 *Ochrobactrum* 53F (e), and, Control (without bacteria) (f).

146

147 The Fertilized, 42P4, and 60I1 treatments increased the aerial biomass (59%, 55%
148 and 52%, respectively), with respect to the Control (Fig. 3A). 64S1 and 53F strains
149 produced a minor stimulation of the aerial part of the seedlings with respect to the
150 Fertilized treatment. However, they differed from the Control treatment.

151 The treatments of inoculations with 42P4 and 60I1 increased root dry weight (62%
152 and 59%, respectively), with respect to the Control and these values were similar
153 to the Fertilized treatment (64%) (Fig. 3B). Similar behavior was observed between
154 the 64S1 and 53F strains, increasing root dry weight by more than 30% with
155 respect to the Control.

156

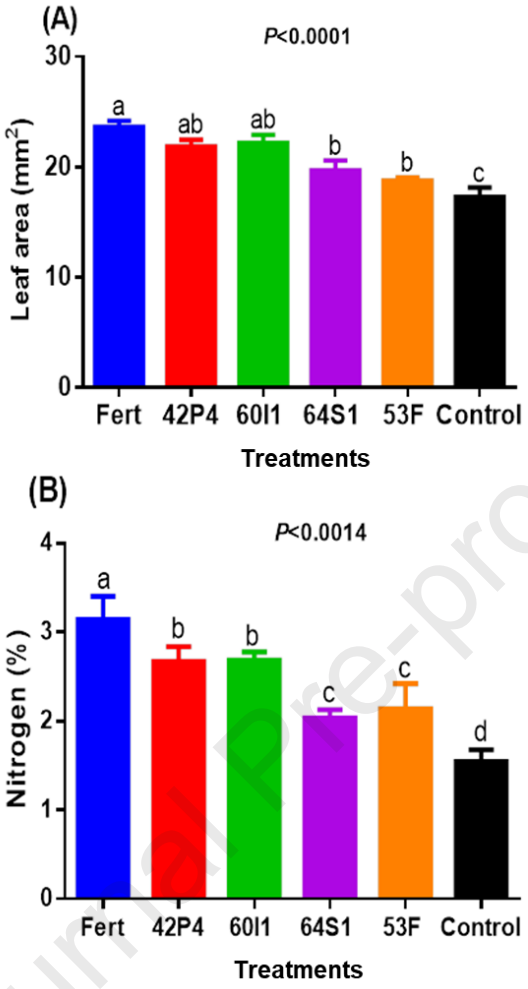


157

158 **Figure 3.** Morphological parameters studied on pepper seedlings, aerial dry weight
159 (A), root dry weight (B), and relation aerial and root dry weight (C) treated with Fert:
160 Fertilized; 42P4: *Pseudomonas* 42P4; 60I1: *Cellulosimicrobium* 60I1; 64S1:
161 *Enterobacter* 64S1; 53F: *Ochrobactrum* 53F, and Control (without bacteria).

The Inoculated and Fertilized treatments had a similar ratio between aerial and root dry weight, and they were different from the Control (Fig. 3C). The leaf area increased in the treatments inoculated with the bacterial strains with respect to the Control. The 60I1 and 42P4 strains were the most effective, increasing the leaf area, (28% and 27%, respectively) over the Control without inoculation (Fig. 4A).

The inoculated and Fertilized treatments had greater nitrogen (N) content with respect to the Control (Fig. 4B). 60I1 and 42P4 inoculations increased the N content (74% and 73%, respectively), with respect to the Control. Similar behavior was observed between the 64S1 and 53F strains, increasing the N content by more than 38% with respect to the Control.



172

173 **Figure 4.** Leaf area (A) and nitrogen content in leaves (B) of pepper seedlings
174 treated with: Fert: Fertilized; 42P4: *Pseudomonas* 42P4; 60I1: *Cellulosimicrobium*
175 60I1; 64S1: *Enterobacter* 64S1; 53F: *Ochrobactrum* 53F and Control (without
176 bacteria).

177

178 *3.2. Effect of inoculation on the accumulation of photosynthetic and photoprotective*
179 *pigments*

The effects of inoculation with PGPR on photosynthetic content and photoprotective pigments are shown in Table 1. PGPR inoculation increased the chlorophyll content (a, b, and total) similar to the Fertilized treatment, except for the 53F strain that did not differ from the Control. 64S1 and 42P4 inoculation increased total chlorophyll (25% and 25%, respectively) with respect to the Control. No significant differences in carotenoids and anthocyanin content were observed.

3.3. Effect of inoculation on the profile of phenolic compounds in leaves of Calafyuco pepper seedlings

The profiles of the phenolic compounds in the non-inoculated pepper seedlings (Control), inoculated with 42P4 and 60I1 strains, and Fertilized seedlings are shown in Table 2. A total of 24 phenolic compounds were identified and quantified, which are grouped into four families based on their chemical structure: phenolic alcohols, flavonoids, phenolic acids and stilbenoids. This study revealed that the sum of the phenolic compounds was higher in the Fertilized treatment, followed by the treatments inoculated with 42P4 strain, Control, and finally inoculated with 60I1 strain.

As phenolic alcohols, we identified hydroxytyrosol and tyrosol. The concentration of both compounds was highest in the Control treatment compared to the inoculated and Fertilized treatments, which were similar. The Control and 60I1 treatments had a similar concentration of hydroxytyrosol (1.37 and 1.11 mg g^{-1}, respectively), whereas in seedlings inoculated with 42P4 and Fertilized the levels were a third part or less (0.27 and 0.41 mg g^{-1}, respectively). The Control had the highest

concentration of tyrosol (0.55 mg g^{-1}) followed by 42P4 (0.41 mg g^{-1}), Fertilized treatment (0.38 mg g^{-1}) and 60I1 (0.30 mg g^{-1}).

According to the flavonoid family, the following compounds were identified and quantified: (+)-catechin, (+)-epicatechin, rutin, (-)-gallocatechin gallate, (-)-epigallocatechin, (-)-epigallocatechin gallate, quercetin, astilbin, naringin, naringenin, myricetin, phloridzin and procyanidin B1. The 42P4 showed the highest concentration of total free flavonoids (76.90 mg g^{-1}) followed by the Fertilized treatment (72.61 mg g^{-1}), Control (30.05 mg g^{-1}), and 60I1 (29.49 mg g^{-1}). The 42P4 showed the highest concentration of naringin (25.65 mg g^{-1}) followed by Fertilized (18.92 mg g^{-1}) and Control (2.01 mg g^{-1}). However, it was not detected in 60I1.

The concentration of (+)-catechin was highest in the 42P4 treatment (0.47 mg g^{-1}) followed by the Fertilized and 60I1 treatments (0.31 and 0.26 mg g^{-1}, respectively), whereas it was the lowest in the Control treatment (0.10 mg g^{-1}). With respect to (-)-epigallocatechin gallate, the Control treatment had the highest concentrations (8.12 mg g^{-1}) followed by 60I1, Fertilized and 42P4 treatments (4.15, 2.95 and 2.64 mg g^{-1}, respectively).

The Control presented the highest concentration of (-)-epigallocatechin (3.71 mg g^{-1}), followed by 42P4 and Fertilized treatments which showed similar levels (2.55 and 2.51 mg g^{-1}, respectively). However, the (-)-gallocatechin gallate was inverse, in the Control was the lowest concentration (7.89 mg g^{-1}), and the inoculated (42P4 and 60I1) and Fertilized treatments had triple that concentration (23.71, 21.41 and 23.30 mg g^{-1}, respectively).

In the phenolic acids group, the Control had the highest total level (71.82 mg g^{-1}), followed by Fertilized (35.94 mg g^{-1}) and 42P4 treatment (22.03 mg g^{-1}) without any significant differences between them, whereas the lowest concentration was found in the 60I1 treatment (6.12 mg g^{-1}). The Control had the highest concentration of gallic acid (48.87 mg g^{-1}), followed by Fertilized, in which less was found (4.98 mg g^{-1}). However, in the inoculated treatments it was not detected. The cinnamic acid was the most abundant compound in the Fertilized and inoculated treatments, while it was in lower concentrations in the Control treatment.

The stilbenoid group, consisting of polydatin, trans-resveratrol, ε-viniferin, and pterostilbene, were quantified. The Control had the highest total concentration of stilbenoid (25.93 mg g^{-1}), followed by the Fertilized treatment (13.41 mg g^{-1}), whereas the 60I1 and 42P4 treatments had similar concentrations (4.23 and 3.85 mg g^{-1}, respectively).

The concentration of polydatin was highest in the Control treatment (21.02 mg g^{-1}), but in the Fertilized and Inoculated treatments (42P4 and 60I1), it did not exceed 22% in respect to the Control. The abundance of polydatin in the Control contrasts with the low concentration of trans-resveratrol (3.10 mg g^{-1}).

4. Discussion

In our study the inoculated seedlings showed similar behavior to the Fertilized treatment exhibiting a high root dry weight with respect to the Control seedlings. The Control plant distributed more matter to the aerial part than the roots. However, the inoculated and Fertilized plants distributed more matter to the roots.

These changes in the root system increase the capacity to absorb nutrients and water, increasing the growth rate of the seedlings. This favors greater exploration of the soil and increases the area of influence of root exudates that mediate the interaction with beneficial microorganisms in the rhizosphere. The production of quality seedlings in less time reduces the time of occupation in the greenhouse and gives an advantage to nursery horticulture by decreasing the cost of seedling production. The greater radical volume improves the anchorage of the transplants in the field, mitigating the incidence of the overturning of seedlings. It is known that inoculation with various PGPR has increased root dry weight and nutrient content of seedlings of *Cucumis sativus,* and *Solanum licopersicum* (Li et al., 2020; Pérez-Rodriguez et al., 2020a, 2020b).

We found that the positive effect on the growth of the inoculated seedlings is correlated with greater nitrogen absorption, which increases the production of chlorophyll, improves the photosynthetic rate, and consequently increases the production of photoassimilates. Similar results have been reported in other plant species inoculated with PGPR (Bal et al., 2013; Abbasi et al., 2013; Ding et al., 2019; Khan et al., 2018).

Despite evidence associated with increased total phenolic compounds in different plant species inoculated with different PGPR, at present, there is no information on the profile of phenolic compounds in pepper plants treated with rhizobacteria. Taking account of that context, the modifications in the profile of phenolic compounds by LC-DAD-FLD in the physiology and growth of pepper seedlings

inoculated with the most effective native PGPR strains (*Pseudomonas* 42P4 and *Cellulosimicrobium* 60I1) in promoting growth were explored.

In this study, the Control treatment had the highest concentrations of phenolic alcohols (hydroxytyrosol and tyrosol). Date et al. (2021) demonstrated that the presence of hydroxytyrosol could act as a root growth inhibitor in several species of agricultural interest. While tyrosol can act as a powerful antioxidant, protecting the seeds and, consequently, increasing the germination percentage (Macedo et al., 2018). However, when it was added in high doses, this compound can restrict the growth of seedlings (Silva et al., 2021).

We found that the higher concentration of endogenous hydroxytyrosol and tyrosol in the Control treatment could have limited the radical growth of the pepper seedlings. The lower growth of roots in the Control treatment could limit the nitrogen absorption, causing a decrease in the chlorophyll content in leaves, restricting the photosynthesis process and thus the production of photoassimilates, which decreased the growth of the aerial part.

The 42P4 and Fertilized treatments had similar levels of flavonoids, while the same behavior was observed between the Control and 60I1 treatments. Naringenin is considered an important precursor to other flavonoids (Liu, et al., 2021), and in this study, it was only detected and quantified in the 42P4 and Fertilized treatments. For this reason, the 42P4 strain may promote naringenin production, thereby increasing the synthesis of other flavonoids in growing seedlings. This hypothesis is confirmed by the fact that naringin concentrations were also higher in the 42P4 treatment.

292 The seedlings inoculated with 42P4, 60I1 and Fertilized had the highest
293 concentrations of (+)-catechin. The role of (+)-catechin in plants remains
294 controversial, the response is different between the plant species (Bais et al.,
295 2010). Different authors have demonstrated that application of catechins increased
296 biomass accumulation, leaf area, leaf thickness, net photosynthetic rate, stomatal
297 conductance, and indole acetic acid (IAA) concentration in *Arabidopsis thaliana*
298 plants (Rani et al., 2011), and also alleviated oxidative stress (Yiu et al., 2011) and
299 cold acclimatization (Ding et al., 2019).

300 The data obtained in the present study suggest that the inoculated treatments
301 behave similarly to the Fertilized treatment, stimulating the production of (+)-
302 catechin, which might induce a positive response in growth and in the
303 accumulation of photosynthetic pigments. Similar results were observed in tea
304 plants inoculated with *Bacillus megaterium* that increased the concentration of
305 catechins and other compounds, such as peroxidase, chitinase, β-1,3-glucanase
306 and phenylalanine ammonia-lyase (Chakraborty et al., 2015).

307 Another flavonoid that had interesting behavior was (-)-epigallocatechin gallate. We
308 observed that the Control treatment doubled its concentration compared to the
309 other treatments. This compound has a high antioxidant capacity, and it has been
310 reported that, applied in some concentrations, could reduce seed germination rate
311 and biomass accumulation in seedlings because it modifies the antioxidant activity
312 and gibberellins/abscisic acid ratio (Ahammed et al., 2020). Therefore, we suggest
313 that the high concentrations of (-)-epigallocatechin gallate in the Control treatment
314 could have limited seedling growth. Nevertheless, applications of lower doses of (-

315)-epigallocatechin gallate increased seed germination and growth of stressed
316 tomato seedlings (Ahammed et al., 2020; Li et al., 2019). Therefore, we can
317 suggest that the *Pseudomonas* 42P4 strain may act by modifying or modulating the
318 levels of (-)-epigallocatechin gallate, favoring the accumulation of biomass and
319 increasing the contents of photosynthetic pigments as in the Fertilized treatment.

320 Phloridzin was only detected in the Control treatment. It is known that this
321 compound stimulates the action of indole acetic acid (IAA) oxidases involved in the
322 degradation of auxins (Dare et al., 2013b). Furthermore, phloridzin is easily
323 degraded by soil microorganisms (Stanišić et al., 2019). Recent studies
324 demonstrated that *Ochrobactrum haematophilum* produces IAA and degrades
325 phloridzin in apple rhizosphere soil (Jiang et al., 2022). This could help to explain
326 why phloridzin was not detected in the inoculated treatments, since it could be
327 degraded by bacteria that are known to produce IAA which would promote the
328 growth of seedlings (Pérez-Rodriguez et al., 2020a).

329 In this current study, the most abundant phenolic compound was gallic acid,
330 quantified in the Control treatment. Gallic acid has shown a potent capacity to
331 inhibit seed germination, radicle and hypocotyl growth, and the fresh and dry
332 weight of *Cucumis sativus* seedlings (Muzaffar et al., 2012). We may suggest that
333 the combined effect of gallic acid, hydroxytyrosol, tyrosol, phloridzin, and the
334 exacerbated production of (-)-epigallocatechin gallate contributed synergistically to
335 limit the growth of the Control seedlings.

336 In the stilbenoids group, the Control treatment showed the highest total
337 concentration of these family compounds. We suggest that the deflection of the

photoassimilates product of the primary metabolism towards the synthesis of stilbenoids seems to be a strategy that limits the growth of the non-inoculated seedlings. It is possible that peat, as a low nutrient inert substrate, induces the seedlings to produce those types of compounds. It is known that the production of stilbenoids in plants is a defense mechanism that overcomes insect and pathogen attacks, although their synthesis can also be induced in response to a wide range of biotic and abiotic stressors, particularly in the leaves (Riviere et al., 2012). In the Control treatment the abundance of polydatin contrasts with the low concentrations of trans-resveratrol. Polydatin is a glycoside of resveratrol and it is possible that the low concentration of trans-resveratrol in the Control is due to that compound being in a glycosylated form as polydatin. While in the treatment inoculated with the 42P4 strain, polydatin was catabolized to trans-resveratrol, which gave other types of stilbenoids through oxidation, such as ε-viniferin, and pterostilbene, which have been reported in other strains. The *Bacillus natto* strain can induce the transformation of polydatin into resveratrol in *Pediomelum cuspidatum* plants (Fan et al., 2021).

5. Conclusion

Overall, the present study suggests that all PGPR used can promote the growth of pepper seedlings. However, *Pseudomonas* 42P4 and *Cellulosimicrobium* 60I1 were more effective in increasing nitrogen uptake and improving the morphological, biochemical, and physiological parameters in pepper seedlings. The higher concentration of flavonoids, such as naringin and (-)-gallocatechin gallate, quantified in the Fertilized and inoculated treatments are correlated with a major

seedlings' growth. The combined effect of gallic acid, hydroxytyrosol, tyrosol, phloridzin, and the exacerbated production of (-)-epigallocatechin gallate may contribute synergistically to limiting the growth of Control seedlings. Finally, it can be suggested that PGPR applied in this study could be used as biofertilizers, reducing the use of nitrogen fertilizers, and the time and cost of production of quality seedlings for horticulturists and nursery.

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Table 1. Chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and anthocyanins of pepper seedlings 25 days after treatment with: Fert: Fertilized; 42P4: *Pseudomonas* 42P4; 60I1: *Cellulosimicrobium* 60I1; 64S1: *Enterobacter* 64S1; 53F: *Ochrobactrum* 53F and Control (without bacteria).

Treatments	Chlorophyll a (µg mg^{-1} leaf)	Chlorophyll b (µg mg^{-1} leaf)	Total chlorophyll (µg mg^{-1} leaf)	Carotenoids (µg mg^{-1} leaf)	Anthocyanins (OD_{546} mg^{-1} leaf)
Fertilized	4.03±0.67a	1.90±0.18a	5.93±0.69a	1.20±0.17a	0.12<0.01a
42P4	4.10±0.80a	1.78±0.22a	5.88±0.82a	1.22±0.28a	0.12<0.01a
60I1	3.95±0.55a	1.86±0.25a	5.81±0.60a	1.17±0.25a	0.10<0.01a
64S1	3.98±0.84a	1.92±0.22a	5.90±1.01a	1.12±0.11a	0.11<0.01a
53F	4.01±0.97a	1.27±0.29b	5.28±1.01ab	1.22±0.14a	0.10<0.01a
Control	3.75±0.62b	0.96±0.19b	4.71±0.64b	1.15±0.23a	0.10<0.01a

Values are presented as the mean ± SE of a total of 30 pepper seedlings for each treatment. Different letters indicate significant differences ($P < 0.0001$) according to one-way ANOVA with Duncan's multiple range test.

Table 2. Profile of phenolic compositions observed in pepper seedlings treated with: Fert: Fertilized; 42P4: *Pseudomonas* 42P4; 60I1: *Cellulosimicrobium* 60I1 and Control (without bacteria). Quantitative results expressed in mg g^{-1} of dry material.

Compounds	Control	60I1	42P4	Fertilized
		Phenolics compounds		
Simple phenolic alcohols				
Hydroxytyrosol	1.37±0.18a	1.11±0.12a	0.27±0.02b	0.41±0.08b
Tyrosol	0.55±0.00a	0.30±0.04b	0.41±0.01b	0.38±0.08b
∑Simple phenolic alcohols	**1.92±0.18a**	**1.41±0.04a**	**0.68±0.01b**	**0.79±0.11b**
Flavonoids				
(+)-Catechin	0.10±0.04c	0.26±0.01b	0.47±0.09a	0.31±0.05b
(-)-Epicatechin	0.16±0.01a	n.d.*	0.11±0.01b	0.12±0.01a
Routine	1.37±0.15b	0.19±0.05c	2.22±0.12a	0.18±0.03c
(-)-Gallocatechin gallate	7.89±0.86b	21.41±1.46a	23.71±0.77a	23.30±1.01a
(-)-Epigallocatechin	3.71 ±0.96a	1.08±0.18b	2.55±0.19a	2.51±0.18a
(-)-Epigallocatechin gallate	8.12±1.57a	4.15±0.31b	2.64±0.16c	2.95±0.18c
Quercetin	0.47±0.01b	0.42±0.02b	8.93±1.24a	8.79±0.15a
Astilbine	2.26± 0.35b	n.d.*	3.92±0.27a	n.d.*
Naringin	2.01±0.20c	n.d.*	25.65±0.04a	18.92±0.97b
Naringenin	n.d.*	n.d.*	0.22±0.01a	0.22±0.02a
Myricetin	3.99±0.01c	1.99±0.30d	6.73±0.32b	10.18±0.16a
Phloridzin	1.26±0.10a	n.d.*	n.d.*	n.d.*
Procyanidin B1	n.d.*	n.d.*	n.d.*	8.10±0.33a
∑Flavonoids	**30.05±4.23b**	**29.49±2.30b**	**76.90±0.33a**	**72.61±2.08a**
Phenolic acids				
Gallic acid	48.87±6.40a	n.d.*	n.d.*	4.98±0.33b
Syringic acid	0.12±0.01c	0.14±0.00c	0.40±0.00b	0.55±0.06a
Cinnamic acid	20.73±1.59b	5.46±0.65c	21.63±0.52b	25.66±1.65a
p-coumaric acid	2.10±0.04a	0.34±0.01b	n.d.*	0.41±0.13b
Ferulic acid	n.d.*	0.18±0.03b	n.d.*	4.34±0.38a
∑Phenolic acids	**71.82±6.59a**	**6.12±0.65c**	**22.03±0.52b**	**35.94±1.73b**
Stilbenoids				
Polydatin	21.02±2.81a	1.33±0.11c	1.96±0.23c	4.83±0.19b
Trans-resveratrol	3.10±0.23b	1.65±0.09c	n.d.*	6.13±0.44a
ε-viniferin	n.d.*	n.d.*	0.39±0.03a	0.45±0.06a
Pterostilbene	1.81±0.15a	1.25±0.19c	1.50±0.19b	2.00±0.16a
∑Stilbenoids	**25.93±2.82a**	**4.23±0.23c**	**3.85±0.29c**	**13.41±0.50b**
∑Total phenolics compounds	**130.97±15.77a**	**41.22±3.46c**	**103.66±1.75b**	**125.67±4.23ab**

Data are presented as mean ± SEM of three independent biological replicates. Different letters indicate significant differences ($P < 0.0001$) according to one-way ANOVA with Duncan's multiple range test. ***n.d.: non-detected.**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: