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Effect of Plant Growth-Promoting Local Rhizobacteria on Rhizome Development and Plant Growth of *Trachystemon orientalis*

Bitki Büyümesini Teşvik Eden Yerel Rizobakterilerin *Trachystemon orientalis*'in Rizom Gelişimi ve Bitki Büyümesine Etkisi

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ABSTRACT

One of the most important strategies to increase agricultural productivity and sustainability is to use a variety of local plant growth-promoting rhizobacteria (PGPB) and their improved consortium formulations. This study aimed to determine the effect of local PGPB on plant growth and rhizome development of *Trachystemon orientalis*, which has great potential as an alternative food source. Six different combined microbial consortia prepared from 11 different rhizobacteria isolated from tea rhizosphere, based on their multiple beneficial effects, were applied to the rhizomes of *Trachystemon orientalis* planted in 1:1 (v/v) peat: perlite medium. The experiment was set up according to the randomized complete block design with three replications, five rhizomes in each replication, and rhizomes not subjected to any treatment were used as controls. PGPB consortia were detected to be effective on 14 parameters examined related to rhizome development and plant growth. The correlation heatmap revealed a strong relationship between the applications and all the parameters examined. The best result was obtained from the P4 application containing a mixture of *Pseudomonas putida* and *Bacillus* spp. This microbial cocktail can be a good alternative to chemical fertilizers, as it is both beneficial and profitable, especially in seedling cultivation of *Trachystemon orientalis*.

Keywords: Rhizobacter, Quality, Microbial community, Sustainable agriculture, *Bacillus* spp., *Pseudomonas* spp.

ÖZ

Tarımsal verimliliği ve sürdürülebilirliği artırmanın en önemli stratejilerinden biri, çeşitli yerel bitki büyümesini teşvik eden rizobakterileri (PGPB) ve bunların geliştirilmiş konsorsiyum formülasyonlarını kullanmaktır. Bu çalışmada, bitkilerin topraktan daha fazla besin maddesi almasını sağlayan *Trachystemon orientalis*'in bitki büyümesi ve rizom gelişimi üzerine etkisinin belirlenmesi amaçlanmıştır. Çay rizosferinden izole edilen 11 farklı rizobakteriden çok sayıdaki yararlı etkileri göz önüne alınarak hazırlanan altı farklı mikrobiyal konsorsiyum kombinasyonu 1:1 (v/v) torf:perlit ortamına dikilen *Trachystemon orientalis* rizomlarına uygulanmıştır. Deneme tesadüf blokları deneme desenine göre üç tekrarlamalı olarak kurulmuş olup, her tekrarlamada beş rizom yer almış ve herhangi bir uygulama yapılmayan rizomlar kontrol olarak kullanılmıştır. Çalışma sonucunda PGPB konsorsiyumlarının rizom gelişimi ve bitki büyümesi ile ilgili incelenen 14 parametre üzerinde etkili olduğu tespit edilmiştir. Korelasyon ısı haritası, uygulamalar ile incelenen tüm parametreler arasında güçlü bir ilişki olduğunu ortaya koymuştur. En iyi sonuç *Pseudomonas putida* ve *Bacillus* spp. karışımı içeren P4 uygulamasından elde edilmiştir. Mikrobiyal karışım, *Trachystemon orientalis*'in özellikle fide yetiştiriciliğinde faydalı ve karlı olması nedeniyle uygulanabilir olduğu ortaya koyulmuştur.

Anahtar Kelimeler: Rizobakteri, Kalite, Mikrobiyal topluluk, Sürdürülebilir tarım, *Bacillus* spp., *Pseudomonas* spp.

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Introduction

The center of genetic diversity of *Trachystemon orientalis* in the Boraginaceae family of the order Boraginales is in the Mediterranean basin and the Middle East, and its distribution extends to Europe and tropical Africa (Selvi & Bigazzi, 2001). The Trachystemon genus, belonging to the Boraginaceae family, represented by one species, is broadly distributed in the Black Sea region of Türkiye (Akçin et al., 2004). *Trachystemon orientalis* (L.) G. Don, with the diploid chromosome number 56, is a perennial and herbaceous plant with rhizomes, hairy, blue-red flowers, 30-40 cm tall (Akçin et al., 2004; Coppi et al., 2006; Özer & Aksoy, 2019). The venation in the broad and hairy leaves of *Trachystemon orientalis* is reticulate and the veinlets branch out, networking towards the edge of the leaf (Selvi & Bigazzi 2001; Koca et al., 2015). It has been used for medicinal purposes in the mountainous regions along the Türkiye-Georgia border in the Western Lesser Caucasus Region, which is also consumed as wild edible plants (WEPs) by local people in the Black Sea region (Kazancı et al., 2020; Üstün et al., 2018). With its rich phenolic compounds and high antioxidant activity, it has anticarcinogenic, antidiabetic, antioxidant, antifungal, and herbicide properties (Demirel Ozbek et al., 2024). This species, also known by local names such as Hodan, Galdirek, Kaldırık, Kalduruk, Tamara, Zılbıt, and Burğı in Türkiye (Akçin et al., 2004), is of great economic importance for the local people as well as in terms of human nutrition (Üstün et al., 2018). The flowering branches, rhizomes, leaves and stems of the plant are consumed as vegetables in different regions of the Black Sea (Akçin et al., 2004). The rhizome structures are covered with several layers of scaly leaves that protect the apical meristem and help push the soil. The thick, horizontally oriented rhizomes located underground are the main axis of the plant, producing roots downwards and shoots towards the top of the soil. The buds on the rhizome produce adventitious roots to expand the root system of the plants (Guo et al., 2021). *Trachystemon orientalis* is ordinarily found in relatively cool environments such as wetlands and shady areas in deep valleys and mountain slopes (Novák et al., 2019). Since this species has not yet been cultured, it is collected from its natural habitats by ranchers and offered to consumers in local markets (Özer & Aksoy, 2019; Demirel Ozbek et al., 2024). The natural plantations of this perennial species are in regional areas with a unique microbiota adapted to the environment. Its cultivation requires special applications aimed at environmentally friendly production and preservation of the ecological balance.

Plant growth-promoting rhizobacteria promotes plant growth by positively contributing to rhizosphere microbiota change and rhizosphere microbial consortia formation (Zhang et al., 2019). PGPB, free-living microbes on or around roots, promotes plant growth due to their capacity to form stable endospores and can also suppress plant diseases. In addition, they play a role in the carbon, nitrogen, and sulfur cycle and the transformation of nutrients (Lin et al., 2019). However, for naturally occurring PGPB in soil to be effective on plants, it must successfully colonize the rhizome and compete with other resident bacteria (Zhu et al., 2024). Although there is still limited information on PGPB-plant interactions, some of these bacteria are used commercially to promote plant growth in agricultural practices (Glick, 2012).

This study aimed to (i) evaluate the impact of the different plant growth-promoting local rhizobacteria plant growth-promoting rhizobacteria on *Trachystemon orientalis* rhizome development and plant growth, (ii) identify the correlations among plant growth-promoting rhizobacteria composition, rhizome development and plant growth, and (iii) assess the benefits of combining native plant growth-promoting rhizobacteria.

Methods

The research was conducted in a greenhouse covered with polycarbonate and a laboratory during the growing season of 2024 at the Recep Tayyip Erdogan University, Faculty of Agriculture.

Experimental Material

The rhizomes of *Trachystemon orientalis*, a naturally disturbing plant in the Black Sea region of Türkiye, were provided from the Pazar district of Rize province.

Plant growth-promoting rhizobacteria (PGPB) was provided by SoilBiom Biotechnology Research and Development Co. Ltd. PGPB that support plant growth were strains isolated from roots of tea (*Camellia sinensis* L.) grown in Rize province (Yıldız et al., 2024). Plant growth-promoting rhizobacteria (PGPB) used as material in this study were obtained from tea genotypes in the Recep Tayyip Erdoğan University National Tea Gene Pool. Six different combined microbial consortiums prepared according to their multiple beneficial effects from a total of 11 different rhizobacteria isolated belonging to the tea rhizosphere were used in the study. Active components and proportions of PGPB cocktails are presented in Table 1.

Table 1.
The characteristics, active components and proportions of plant growth-promoting rhizobacteria (PGPB) used in the study

Code	Characteristics	Active components	Proportions (CFU/ml)
P1	Siderophores production	*Bacillus toyonensis, Lysinibacillus fusiformis*	1 x 10^7
P2	Phosphate solubilization	*Bacillus proteolyticus, Pseudomonas batumici*	1 x 10^7
P3	Cocktail	*Bacillus cereus, Pseudomonas putida, Bacillus toyonensis, Bacillus safensis*	1 x 10^7
P4	IAA production	*Bacillus toyonensis, Pseudomonas putida*	1 x 10^7
P5	Potassium solubilization	*Pseudomonas lini, Bacillus safensis*	1 x 10^7
P6	N_2 fixer	*Pseudomonas konensis, Bacillus thuringiensis*	1 x 10^7

Experimental Design

The rhizomes, collected from the garden in January, were grown in a 1:1 (v/v) ratio of peat: perlite medium until the rhizomes reached an average length of 10 cm. The plants were uprooted in May 2024; the shoots were removed from the rhizomes. They were transplanted in styrofoam boxes (50x29.7x15 cm) containing peat:perlite (1:1, v/v) mixture. PGPB applications were carried out with six different mixtures, as outlined in Table 1. The application was made once at following the planting. 20 ml of bacterial suspension (containing approximately 1 x 10^7 CFU/ml in sterile water) was inoculated into the rhizosphere for each rhizome. As a control, the rhizomes were treated with an equal volume of sterile distilled water. The trial was laid out as a randomized block design with three replicates and five rhizomes in each replicate.

Plant and Rhizome Growth Index Measurement

Morphological observations were taken at the end of 90 days from the establishment of the experiment. Plant height (PH) was measured with a ruler from the root collar to the tip. Leaf width (LWDT) was determined by measuring the widest part of the leaf that completed its development, and leaf length (LLGN) was determined in cm by measuring the longitude from the tip of the leaf's blade to the stem with a ruler. The number of leaves (LNUM) was recorded by counting all the leaves formed on the plant (number plant^{-1}). Leaf chlorophyll content (SPAD) was estimated using a portable hand-held chlorophyll meter (Minolta SPAD-502 Plus). Leaf color was measured by identifying CIE L, a, and b values with a colorimeter (CR-400, Minolta Corporation, Ltd., Osaka, Japan). Plant fresh weight (PFW) was determined by weighing with a precision scale after the plant material above the soil was harvested. Plant dry weight (PDW) was determined after drying the samples in the oven at 72°C until they reached a constant weight. The root length (PRL) formed on the rhizome was measured with a ruler. The density of hairy roots (RFD) at the branch roots' base was observed morphologically using a rating scale (1=very little, 3=little, 5=medium, 7=dense, 9=very dense). Rhizome development was recorded by calculating the differences between the rhizome's (RLG) lengths measured with a ruler and their diameters (RD) measured with a digital vernier caliper before and after the experiment.

Statistical Analyses

The study's data collected in the study were subjected to variance analysis using the JUMP 13.0 statistics program. Statistically significant differences between the applications were determined using the LSD (Least Significant Difference) multiple comparison test. A correlation heatmap graph was generated using OriginPro Version 2021.

Results

In the study conducted to determine the effects of PGPB on both plant growth and rhizome development of *Trachystemon orientalis*, it was found that the difference in the parameters examined between PGPB inoculated and uninoculated treatments was significant. The effect of PGPB was detected in all growth and development parameters examined. However, it was observed that plant growth responses were variable, and PGPB showed different effects depending on their plant growth-promoting properties (Figure 1).

PGPB inoculations prompted a significant increase in plant height except for the P5 (production of potassium) and P2 (phosphate solubilization) treatments. The N_2 fixer (P6) PGPB produced the tallest plants, 23.53 cm, followed by Cocktail (P3), with 22.27 cm. In contrast, the shortest plants were observed in the P5 treatment, 18.73 cm. Leaf width and leaf lenght were varied considerably among the applications. While the P3 application significantly increased in leaf size, in the P5 application had much smaller leaves than in the other applications. P3 application increased leaf width by 36.42% and leaf length by 30.25% compared to the control. The number of leaves were ranged between 2.70 and 4.53. While the highest leaf formation was observed in the P3 application, the lowest leaf production was detected in the P2 application. The P3 application resulted in a 30.55% increase in leaf number compared to the control group, while the P2 application showed a decrease of 22.19% in leaf number.

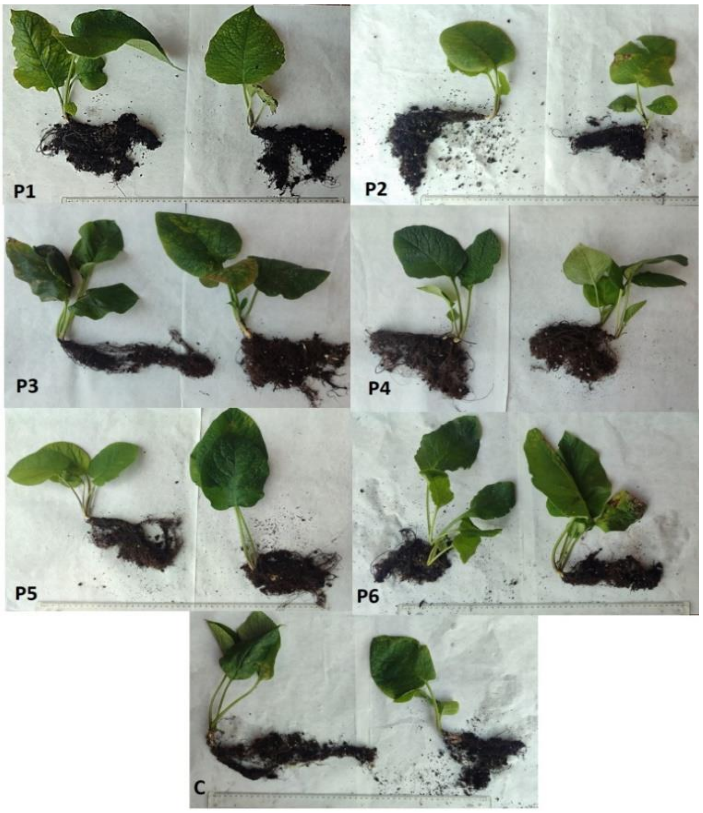

Figure 1.

The effect of PGPB mixtures inoculated and non-inoculated treatments on rhizome development and plant growth of Trachystemon orientalis. P1: Siderophores production, P2: Phosphate solubilization, P3: Cocktail, P4: IAA production, P5: Potassium solubilization, P6: N_2 fixer, C: Control (non-treated)

The chlorophyll content in the leaf blade, referring to SPAD value, was higher in the P3 treatment than in other treatments. CIE L* value was also high in the same application as parallel. It was revealed that the leaves were brighter in the P3 application compared to the others. P1 and P5 applications did not significantly affect on CIE a* value compared to the control, but the other applications caused marked decreases. There was a 25% decrease in the P3 application. A decrease occurred in the CIE b* value in all applications compared to the control and P2. However, the lowest value was measured in the P3 application. In this application, a 55.60% decrease in CIE b* value was detected compared to the control. P3 application contributed significantly to fresh and dry plant weight, and the highest values were obtained from this application. There was an increase of 77.86% for plant fresh weight and 29.73% for plant dry weight. On the other hand, it was determined that there was a significant decrease in the values in other PGPB applications compared to the control, except for P3 and P1 for plant fresh weight and P3 and P4 for plant dry weight. P1, P3, and P5 also contributed positively to the elongation of rhizomes; the longest rhizome was measured in the P3 application. In addition, compared to the pre-trial measurement, the highest elongation was settled in the P4 application. The applications other than P3 caused an increase in rhizome diameter. Compared with the pre-experimental measurement, it was revealed that the P3 application contributed the most to the increase in rhizome diameter (Table 2).

Table 2.

The effects of various mixtures of PGPB on rhizome development and plant growth of Trachystemon orientalis

Code	PH (cm)	LWDT (cm)	LLGN (cm)	LNUM (number $plant^{-1}$)	SPAD	MNTL	MNTa	MNTb	PFW (g $plant^{-1}$)	PDW (g $plant^{-1}$)	PRL (cm)	RFD	RLG (cm)	RD (mm)
P1	21.57± 0.74^{bc}	10.03± 0.31^{b}	12.30± 0.46^{c}	3.20± 0.35^{b}	32.67± 0.40^{d}	55.43± 0.55^{de}	17.93± 0.64^{a}	23.00± 0.44^{b}	36.67± 0.58^{b}	5.46± 0.28^{c}	19.80± 0.53^{b}	4.47± 0.23^{b}	1.17± 0.06^{bc}	0.00± 0.00^{e}
P2	19.43± 0.45^{d}	10.30± 0.26^{b}	13.17± 0.21^{b}	2.70± 0.17^{c}	27.57± 0.55^{f}	55.00± 0.78^{e}	16.60± 0.17^{c}	24.30± 0.44^{ab}	34.40± 1.22^{c}	4.97± 0.06^{c}	16.47± 0.31^{e}	5.66± 0.23^{a}	1.37± 0.08^{b}	0.70± 0.10^{d}
P3	22.27± 0.31^{b}	11.50± $0,36^{a}$	15.33± 0.23^{a}	4.53± 0.23^{a}	43.3± 0.32^{a}	67.77± 0.15^{a}	12.90± 0.20^{f}	11.13± 0.31^{e}	67.00± 1.00^{a}	9.73± 1.26^{a}	23.53± 0.42^{a}	5.26± 0.23^{a}	1.37± 0.10^{b}	5.07± 0.15^{a}
P4	21.57± 0.35^{bc}	8.90± 0.20^{c}	11.63± 0.15^{e}	3.53± 0.12^{b}	33.27± 0.71^{d}	61.93± 0.55^{b}	14.40± 0.44^{e}	13.40± 0.70^{d}	31.67± 1.53^{d}	7.21± 0.42^{b}	17.47± 0.23^{d}	4.33± 0.23^{bc}	3.16± 0.15^{a}	3.20± 0.20^{b}
P5	18.73± 0.12^{d}	7.20± 0.20^{d}	9.87± 0.12^{f}	3.33± 0.12^{b}	29.87± 0.67^{e}	58.67± 1.27^{c}	17.40± 0.44^{ab}	18.90± 0.36^{c}	20.00± 1.00^{f}	5.20± 0.61^{c}	19.87± 0.64^{b}	4.73± 0.23^{b}	1.23± 0.15^{b}	1.33± 0.20^{c}
P6	23.53± 1.21^{a}	8.83± 0.40^{c}	12.17± 0.25^{cd}	3.27± 0.23^{b}	37.63± 0.61^{c}	60.97± 0.90^{b}	15.13± 0.25^{d}	15.17± 0.25^{d}	29.67± 1.53^{e}	5.30± 0.36^{c}	16.00± 0.20^{f}	3.27± 0.23^{d}	0.57± 0.06^{d}	1.23± 0.08^{cd}
C	20.63± 0.21^{c}	8.43± 0.23^{c}	11.77± 0.15^{de}	3.47± 0.12^{b}	38.77± 0.15^{b}	56.63± 0.31^{d}	17.23± 0.21^{bc}	25.07± 2.81^{a}	37.67± 0.58^{b}	7.50± 0.53^{b}	19.06± 0.31^{c}	4.93± 0.23^{c}	0.63± 0.06^{cd}	1.37± 0.09^{c}
LSD	1.105	0.516	0.468	0.387	0.846	1.300	0.687	2.014	1.784	0.968	0.366	0.439	0.150	0.168
CV (%)	2.95	3.04	2.14	6.35	1.37	1.23	2.42	6.05	2.73	8.40	1.09	5.46	6.17	5.12

Group means were compared with LSD test (*p* <.05). P: Plant growth-promoting rhizobacteria P1: Siderophores production, P2: Phosphate solubilization, P3: Cocktail, P4 IAA production, P5: Potassium production, P6: N2 fixer, C: Control (non-treated), PH: Plant height, LWDT: Leaf width, LLGN: Leaf length, LNUM: number of leaves, SPAD: Leaf chlorophyll content, MNTL: Leaf color CIE L value, MNTa: Leaf color CIE a value, MNTb: Leaf color CIE b value, PFW: Plant fresh weight, PDW: Plant dry weight, PRL: Length root formed on the rhizome, RFD: Density of hairy roots, RLG: Differences between the lengths of the rhizomes before and after the experiment, RD: Differences between the diameters of the rhizomes before and after the experiment.

Differences in the development of *Trachystemon orientalis* rhizomes before and after inoculation with various PGPB mixtures are shown with horizontal lines in Figure 2. PGPB, characterized by siderophore production (P1), only contributed to the elongation of rhizome length. Nevertheless, other applications contributed to the increase in length and diameter of rhizome development. P4 and P5 made a balanced contribution to both length and diameter development, while P3 played a greater role in increasing the diameter. The changes in rhizome diameters were 270.07% for the P3 treatment and 133.58% for the P4 treatment, compared to the control group.

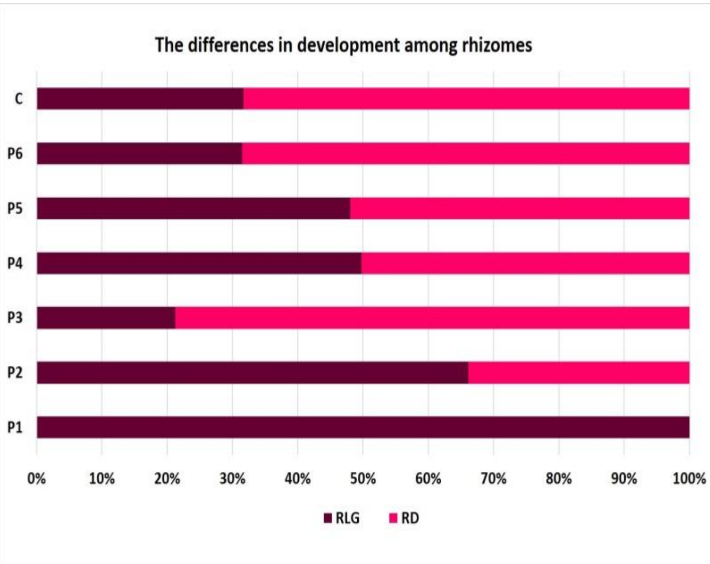

Figure 2.

Differences in the development of Trachystemon orientalis rhizomes before and after inoculation with various PGPB mixtures. P: Plant growth-promoting rhizobacteria P1: Siderophores production, P2: Phosphate solubilization, P3: Cocktail, P4 IAA production, P5: Potassium solubilization, P6: N_2 fixer, C: Control (non-treated). RLG: Differences between the lengths of the rhizomes before and after the experiment, RD: Differences between the diameters of the rhizomes before and after the experiment

The possible relationships between the investigated parameters are displayed with the correlation heatmap graph. Pearson's correlation coefficient was computed to quantify linear associations between variables ($p<.0001$). Orange and blue colors indicate direct and inverse correlation, respectively. The treatments were strongly associated with all parameters. The plant's fresh weight was positively correlated, with Pearson's correlation coefficients of 0.92 for leaf length and 0.84 for leaf width. In contrast, a negative relationship was found in between plant fresh weight and Leaf color CIE a (r=-0.64) and Leaf color CIE b (r=-0.38) (Figure 3).

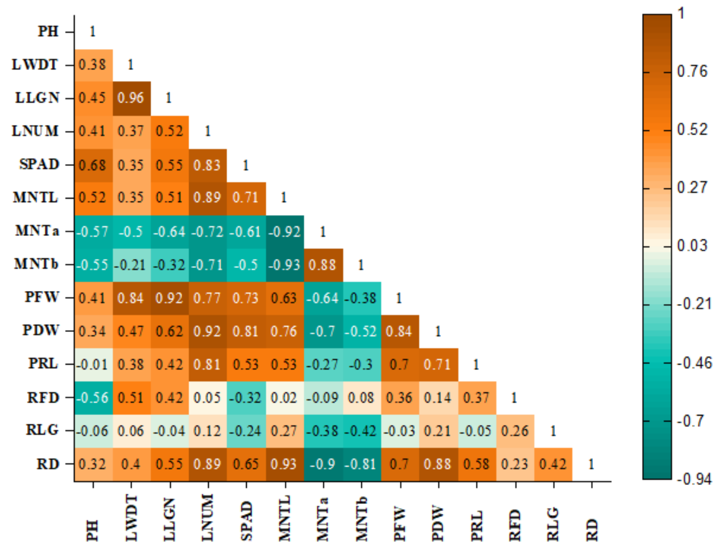

Figure 3.

Correlation heatmap of Trachystemon orientalis rhizome development and plant growth parameters data collected from untreated and treated mixtures of PGPB. PH: Plant height, LWDT: Leaf width, LLGN: Leaf length, LNUM: Number of leaves, SPAD: Leaf chlorophyll content, MNTL: Leaf color CIE L, MNTa: Leaf color CIE a, MNTb: Leaf color CIE b, PFW: Plant fresh weight, PDW: Plant dry weight, PRL: Length root formed on the rhizome, RFD: Density of hairy roots, RLG: Differences between the lengths of the rhizomes before and after the experiment, RD: Differences between the diameters of the rhizomes before and after the experiment.

Discussion

The collection, protection, and sustainability of wild edible vegetables are critical issues on the agenda, and their cultivation as an alternative food source is also key to providing food security (Borelli et al., 2020; Kidane & Kejela, 2021). Sustainable collection programs are needed to ensure supplies of edible wild plants. However, their natural distribution area is threatened by adverse environmental conditions caused by climate change, industrialization, and urbanization (Taghouti et al., 2022). *Trachystemon orientalis* is an important edible wild species used for human nutrition and has potential industrial products for the pharmaceutical and cosmetic industries (Biyik et al., 2023; Chrzanowska et al., 2024). This study provides important findings regarding the PGPB application that may increase the success rate of the *Trachystemon orientalis* cultivation process.

Plant growth-promoting bacteria (PGPB) are generally effective in supporting growth by communicating with the host plant. They play a role in nitrogen fixation, the solubilization of inorganic phosphate and potassium, IAA, and the production of siderophore molecules (Santoyo et al., 2021; Vocciante et al., 2022). In this study, the relationships of local PGPB with the specified characteristics of the plant were revealed in the environment where no nutrients were added to the growth medium, and there was no competition with other endophytic bacteria. The differences in these relationships on the growth and development of plants were evident.

Research indicates that when a large amount of chemical fertilizer is applied to soil, beneficial bacteria that aid plant growth by supplying nitrogen or phosphorus may not benefit the plants (Glick, 2012). In this study, the effectiveness of plant growth promoting rhizobacteria in a nutrient-free medium was investigated. However, no positive impact on plant development was observed in most traits measured compared to the control group. These results may relate to the structure of the growing medium.

PGPB affects plants' root properties, especially in root length, biomass, and volume, as reported in various studies (Grover et al., 2021). PGPB influenced the root length, the density of hairy roots, and the lenght and diameter of rhizomes formed in the rhizome of *Trachystemon orientalis*. However, the impacts were varied; some consortia had great impacts on root architecture, while others had no impact or the impacts were negative way. The PGPB, Cocktail, mixture of *Bacillus toyonensis* and *Lysinibacillus fusiformis*, and mixture of *Pseudomonas lini* and *Bacillus safensis* contributed positively to the increase of hairy roots. In contrast, the others, except mixture of *Bacillus toyonensis* and *Lysinibacillus fusiformis*, contributed to the increase of rhizome diameter. N2 fixer caused a decrease in root length. Although the N2 fixer bacteria (P6) contributed to the extension of plant height, no positive contribution was detected for other parameters. It may be due to a lack of nitrogen or insufficient nutrients in the growth medium. Similarly, Ghaffari et al. (2018) reported a strong relationship between the activity of Pseudomonas bacteria and the presence and amount of nitrogen in the medium. On the other side, bacteria belonging to the *Bacillus* and *Pseudomonas* genera can perform beneficial interactions in the rhizosphere thanks to their enzymes called adhesins, which facilitate their attachment to plant roots (Santoyo et al., 2021). Therefore, the cocktail, including Bacillus and Pseudomonas, may have contributed more positively to growth and development.

Auxin-producing PGPB may play an important role in improving plant growth by triggering various metabolic processes that directly or indirectly control different aspects of plant growth and development. These processes may provide higher root biomass and reduce stomata size and density (Borah et al., 2023). Compatible with this information, it was observed that the IAA-producing PGPB used in this study actually increased root mass compared to the control and caused a decrease in leaf chlorophyll content.

The discovery of different Pseudomonas strains that support plant growth has brought their use as a safe alternative to chemical fertilizers to the agenda. Pseudomonas strains, in particular, increase the plant's resistance by ensuring the production of defense-related chemicals and stress-protective proteins in the host plant and contribute to the increase in plant height and biomass (Singh et al., 2024). In this study, the mixtures prepared using four different isolates of Pseudomonas (*Pseudomonas batumici*, *Pseudomonas putida*, *Pseudomonas lini*, *Pseudomonas konensis*), the *Pseudomonas putida* isolate had a significant positive effect on plant growth and biomass increase was observed. The positive effect of *P. putida*, a Gram-negative rhizobacteria, on the development of different plant species were also reported in previous studies (Naserzadeh et al., 2018; Arslan & Akkaya, 2020). The presence of different strains of Bacillus together with *Pseudomonas putida* in the most effective mixtures for the growth of *Trachystemon orientalis* indicates that these two families of promoting bacteria may have made an important contribution together. Dimkić et al. (2022) relayed that bacteria belonging to the genera Bacillus and Pseudomonas are known producers of secondary metabolites, enzymes, and other bioactive compounds that can benefit plants. Bacillus promotes plant growth and development and provides biocontrol of plant pathogens. This strain's usage for more sustainable agricultural practices is becoming increasingly widespread (Borriss, 2015). It was evident by Esitken et al. (2010) that the application of Bacillus and Pseudomonas to the roots and leaves of strawberry plants enhances plant growth, yield, and microelement content. They reported that these bacteria could serve as a promising alternative biofertilizer for fruit and vegetable production in sustainable and organic farming systems.

Conclusion and Recommendations

In conclusion, plant growth-promoting rhizobacteria (PGPB) affected the growth and rhizome development parameters of *Trachystemon orientalis*. In the growth medium, where there was no plant nutrient and no competition with other bacteria, the cocktail, including a mixture of Bacillus and Pseudomonas isolates, promoted plant growth. This cocktail has great potential to be used, especially in cultivating seedlings of *Trachystemon orientalis*. However, the effects of PGPB will undoubtedly differ in the soil and the environments where nutrients are added. Therefore, it cannot be simply claimed that mixtures other than the cocktail are ineffective. The efficiency of these bacterial mixtures should also be tested in the soil with different physical, chemical, and biological characteristics. The effects of PGPB applications should be determined according to each environment and condition.

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