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دور البكتريا المحفزة لنمو النبات في تعديل ميكروبيوم منطقة الجذور في الطماطم (*Solanum lycopersicum*)

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المستخلص:
يلعب التنوع الميكروبي في منطقة جذور الطماطم (الرايزوسفير) دورًا حاسمًا في نمو النبات وإنتاجيته من خلال تعزيز امتصاص العناصر الغذائية (وخاصة الفوسفور)، وتحفيز المقاومة الجهازية ضد الأمراض الفيروسية والبكتيرية، وتحسين صحة التربة، مما يؤدي إلى نباتات أقوى وإنتاجية أعلى. ويشمل ذلك استخدام الكائنات الحية الدقيقة النافعة (PGPR) كبديل للمبيدات الكيميائية، خاصة في مواجهة أمراض مثل التبقع البكتيري والذبول، وفقًا للدراسات البحثية.
في العراق، تشير الدراسات إلى أن التنوع الميكروبي في منطقة الجذور يؤثر بشكل كبير على نمو الطماطم وإنتاجيتها، حيث تعمل الكائنات الدقيقة النافعة (مثل فطر Trichoderma وبكتيريا مثل *Bacillus subtilis*) على تعزيز نمو النبات وزيادة مقاومة الأمراض الفطرية مثل ذبول الفيوزاريوم، مما يزيد من وزن النبات وطوله وإنتاجيته في الظروف الحقلية، لا سيما استخدامها مع إلمحسنات مثل السماد العضوي (الكمبوست). وتعد هذه الدراسات حيوية لتحسين الزراعة المستدامة في البلاد لمواجهة التحديات المناخية.

١:- تعزيز امتصاص العناصر الغذائية:
من خلال استخدام إذابة الفوسفور: يمكن لبعض البكتيريا إذابة الفوسفور المرتبط في التربة ، مما يجعله متاحًا لامتصاص النبات، وهو أمر حيوي للنمو وتكوين الثمار، كما أثبتت الدراسات في العراق.

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وكذلك من خلال تثبيت النيتروجين؛ باستخدام الكائنات الحية الدقيقة التي يمكنها تثبيت النيتروجين الجوي وتحويله إلى اشكال يمكن للنبات الاستفادة منها.

٢- المكافحة الحيوية للأمراض:

من خلال تحفيز المقاومة الجهازية المستحثة(ISR) ، إذ تقوم بعض البكتيريا (مثل أنواع Pseudomonasالمحلية) بتحفيز النباتات لتطوير مقاومة داخلية ضد مسببات الأمراض مثل فيروس تجعد واصفرار أوراق الطماطم(TYLCV) .

كذلك التنافس مع المسببات الممرضة ، حيث تشغل الكائنات الحية الدقيقة النافعة المساحات الموجودة على الجذور وتستهلك العناصر الغذائية، مما يمنع مسببات الأمراض من التكاثر.

3:- تحسين الصحة العامة للتربة:

تساهم الكائنات الحية الدقيقة في تكوين المادة العضوية في التربة وزيادة قدرتها على الاحتفاظ بالماء والعناصر الغذائية. كما انها تساعد في تقليل الإجهاد على الجذور وتحسين قدرتها على امتصاص الماء والمغذيات.

الكلمات المفتاحية: البكتيريا المحفزة لنمو النبات(PGPR)، نبات الطماطم (*Solanum lycopersicum*)، ميكروبيوم المنطقة المحيطة بالجذور ، الاجناس البكتيرية Bacillus & Pseudomonas ، تحفيز نمو النبات .

Role of Plant Growth-Promoting Bacteria in Modulating the Rhizosphere Microbiome of Tomato (*Solanum lycopersicum*)

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Abstract

Microbial diversity in the tomato root zone (rhizosphere) plays a crucial role in plant growth and production by enhancing nutrient uptake (especially phosphorus), stimulating systemic resistance to viral and bacterial diseases, and improving soil health, resulting in stronger plants and higher

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productivity. This approach includes using beneficial microorganisms (PGPR) as an alternative to chemical pesticides, especially in the face of diseases such as bacterial spot and wilt, according to research.

In Iraq, studies indicate that microbial diversity in the root zone significantly affects tomato growth and productivity, as beneficial microbes (such as *Trichoderma fungi* and bacteria such as *Bacillus subtilis*) promote plant growth and resistance to fungal diseases such as Fusarium wilt, increasing plant weight, height, and productivity under field conditions, especially when used with amendments such as compost. These studies are vital to improving sustainable agriculture in the country and meeting climate challenges.

1. Enhancing nutrient uptake: Through using phosphorus solubility, some bacteria can solubilize bound phosphorus in the soil, making it available for plant uptake, which is vital for growth and fruit formation, as demonstrated in studies in Iraq. Also, using nitrogen fixation, microorganisms can fix atmospheric nitrogen and convert it into forms that plants can utilize.

2. Biological disease control: through the simulation of systemic and resistance (ISR): Some bacteria (such as endemic Pseudomonas) stimulate plants to develop internal resistance against pathogens like TYLCV (Tylochrome Turbidity Virus), also adding through pathogen competition: Beneficial microorganisms occupy spaces on roots and consume nutrients, preventing pathogens from multiplying.

3. Improved overall soil health: Microorganisms contribute to the formation of organic matter in the soil and increase its water and nutrient retention capacity. They help reduce root stress and improve their ability to absorb water and nutrients.

Keywords: PGPR, Tomato (*Solanum lycopersicum*), Rhizosphere microbiome, Bacillus & Pseudomonas, Plant growth promotion.

Introduction

Plant Growth-Promoting Bacteria (PGPR) play a crucial role in modulating the rhizosphere microbiome of tomato (*Solanum lycopersicum*),

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shifting it toward a more beneficial, diverse, and stable community. By colonizing the root zone, PGPR (such as Bacillus, Pseudomonas, Streptomyces, and Enterobacter) act as biofertilizers, biostimulants, and biocontrol agents, enhancing nutrient uptake and providing resistance against biotic and abiotic stresses. A wide range of PGPR are known to be associated with the rhizosphere of tomatoes and belong to the following genera:

Bacillus, Pseudomonas, Streptomyces, Micrococcus, Azotobacter, Acinetobacter, and Flavobacterium (Ottesen et al., 2013; Romero et al., 2014).

PGPR enhances the growth of tomatoes and other plants directly or indirectly via the production of phytohormones, biological nitrogen fixation, solubilizing nutrients such as phosphorus, potassium, and iron, and making more of them available to plants via the release of enzymes, siderophores, and organic acids (Ahmad & Zaib, 2020; Ahmed et al., 2017; El-Rahman et al., 2020).

The PGPR have been the focus of extensive research on their potential to enhance tomato growth across a range of environmental conditions.

There are Several PGPR strains, including (*Pseudomonas fluorescens*, *Pseudomonas palleroniana*, *Pseudomonas koreensis*, *Pseudomonas sp*. NK2, *Bacillus subtilis*, *Bacillus velezensis*, *Bacillus atrophaeus*, *Bacillus megaterium*, and *Bacillus siamensis*), adding to others, that have shown significant promise in improving tomato plant growth (Agarwal et al., 2020; Bai et al., 2024; Cochard et al., 2022; Cordero et al., 2018; Guo et al., 2021; Islam et al., 2019).

These bacteria achieve this by enhancing nutrient availability, boosting soil microbial diversity, and inducing plant resistance to both abiotic stresses, such as drought and salinity, and biotic stresses, including pathogens (Hamid et al., 2021; Morcillo & Manzanera, 2021).

For example, *B. megaterium* has been reported to enhance phosphorus solubilization (Zhao et al., 2021), while P. Koreensis contributes to increased

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tomato growth by producing plant hormones like auxins and cytokinins, which are crucial for tomato growth (Guo et al., 2021).

These findings underscore the broad applicability of PGPR across different climates and soils, especially in regions like Ethiopia, where such bacteria can thrive.

Many of studies have demonstrated the effects of microorganisms on tomatoes regarding in their size and development, adding to proper seed multiplication, nutrition, disease resistance, and seedling development (De Coninck et al., 2021; Patil & Fauquet, 2021).

Because of these interrelationships, introduced microbes are nonetheless rarely sustained in the new environment they occupy (Odelade & Babalola, 2019).

The ET is considered a crucial phytohormone that promotes the ripening and rotting of tomato plant fruits (Tao et al., 2021), in addition to being the only phytohormone that happens to be a gas. Also, it can be produced mostly in every tissue of the plant and can diffuse out of the plant. This procedure induces the stimulation of 1-amino cyclopropane-1-carboxylic acid, which is an ethylene precursor and modifies ACC oxidase activity, in Thiobacillus and other reported PGPRs (Ankati & Podie, 2018; Kalam, Basu & Podile, 2020). It is beneficial for microbes in the soil community; bacterial species are the most abundant and helpful. Saravanan et al. (2020) gave the following full details of the action of bacteria in the soil: all this helps stimulate plant growth after the production of a certain phytohormone responsible for the development of plants; they return nutrients to the plants by fixing nitrogen back to the soil; they promote soil structure; they act against spoilage organisms, which can destroy the crop plants. Naturally, PGPR is more beneficial to the soil that they colonize.

2 MATERIALS AND METHODS

The complete scientific classification of tomato licorice(*Solanum lycopersicum*)

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Solanaceae, which also includes potatoes, eggplants, and peppers. It is botanically classified as a fruit-bearing herbaceous plant, and it is distinguished as the main vegetable crop grown in warm and temperate places, and grows either upright or climbing.

Taxonomy of tomatoes:

Kingdom: Plants (Plantae), **Section**: Tracheophyta, **Division**: Magnoliophyta

Rank: Solanales, **Family**: Solanaceae, **Genus**: Solanum,

Type: tomato (*S. lycopersicum*),

Scientific name: *Solanum lycopersicum* or *Lycopersicon esculentum*

The most important agricultural divisions for tomatoes:

According to growth, it is divided into determinate varieties that stop growing after a period, and indeterminate varieties that continue to grow and bloom.

According to al-Thumrah, it is considered botanically as "fruit" (because it contains seeds), but it is sold and consumed as "vegetable".

Classification: Tomato classification according to APG (2009)

Div: Spermatophytae, Sub Div: angiospermae, Class: eudicotyledoneae, Sub Class: Dillenidae, Order: Solanales, Family: Solanaceae, Genre: Lycoperscom, Esp: *Lycoperscom esculentum Mill*, Var: Heintz

Morphological description of tomato pulp

The tomato plant is considered to be one of the seasonal woody plants of the two-leafed plants, which are naturally pollinated, having different forms, the stems branching from the stem and the fixed stem in the soil, modern agriculture, and it belongs to the short-day plants and the crops of the cold seasons, and the three-carbon plants of the nature of germination.

2.1 Bacterial strain and culture conditions

Three PGPR isolates (*BIA1*, *PIA2*, and *PIA3*) that had been previously isolated from the tomato rhizosphere in our laboratory and displayed multi-trait plant growth promotion (*PGP*) (phosphate solubilization, nitrogen fixation, indole-3-acetic acid production) were subjected to greenhouse tests. *PIA2* and *PIA3* belonged to the genus Pseudomonas, while BIA1 was

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identified as a Bacillus isolate. They were kept in 20% glycerol at −80°C before being reactivated on *Nutrient Agar* (NA) plates.

2.2 Preparation of bacterial inoculum

The PGPR isolates (*BIA1*, *PIA2*, and *PIA3*) were streaked on NA plates and cultured for 48 h at 28 ± 2°C. Sterilized distilled water (*SDW*) was used to harvest the colonies, and a spectrophotometer was used to adjust the cell suspension to an OD of 0.2 at 620 nm, approximately 2.6 × 108 CFU/mL (Kurabachew & Wydra, 2013).

2.3 Plant growth conditions and inoculation

Tomato seeds of the Melkesalsa variety (TC2) and Maya variety (TC1) (commonly used varieties in the agro-climatic conditions in the study area) were obtained from the Iraq Agricultural Research Centre (*IARC*). The surface of tomato seeds was disinfected using successive 3-min immersions in 70% (v/v) ethanol and 1% sodium hypochlorite solution. The seeds were then cleaned three times in aseptic conditions with sterile distilled water (Anith et al., 2015). Sterilized 3 kg mix of potting soil, including loam soil and sand in a 2:1 weight ratio, was made and placed into plastic pots that had been cleaned and disinfected with 1% sodium hypochlorite. Seeds were planted in plastic pots in a greenhouse.

Four-week-old seedlings of each tomato plant were uprooted and immersed for 60 min in the selected *PGPR* suspension, and then replanted in plastic pots with around 3 kg of sterilized potting soil. Seedlings used as controls were immersed in SDW (Mekonnen et al., 2022).

Tomato varieties treated with only *SDW* serve as controls. In this case study, we used a completely randomized design with three replications was used us for the greenhouse experiments.

In a greenhouse with a temperature range of (25°–29°), a relative humidity range of (75%–85%), and 12 h of darkness and 12 h of light, plants were raised. Distilled water was used to water the plants when required. The treatments were as follows:

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T1: BIA1 + TC1, T2: PIA2 + TC1, T3: PIA3 + TC1, T4: BIA1 + PIA2 + TC1, T5: BIA1 + PIA3 + TC1, T6: PIA2 + PIA3 + TC1, T7: Control (C1), T8: BIA1 + TC2, T9: PIA2 + TC2, T10: PIA3 + TC2, T11: BIA1 + PIA2 + TC2, T12: BIA1 + PIA3 + TC2, T13: PIA2 + PIA3 + TC2, and T14: Control (C2). Here, TC1 is the Maya variety, TC2 is the Melkesalsa variety, C1 is the Maya variety control, and C2 is the Melkesalsa variety control.

2.4 Plant growth promotion assessment

One month after transplanting, plant growth metrics were assessed. The height of tomato plants was measured before their removal from the greenhouse. The tomato plants were uprooted, washed with tap water to remove any remaining soil, blot dried, and weighed to determine their fresh weight of shoots and roots. Moreover, the dry weights of the shoots and roots were determined after 48 h of oven drying at 80°C. The efficiency of PGPR isolates in promoting growth, as measured by the height and dry weight of the tested tomato varieties, was calculated using the formula (Singh et al., 2012):

$$GPE = \frac{(Treatedgroup - Control)}{Control} \times 100$$

where *GPE* is the growth promotion efficacy, the treated group is the plants treated with *PGPR* isolates, and the control is the plants treated with only *SDW*.

2.5 Data analysis

The results of the greenhouse experiment were examined using SPSS statistical software, Version 25. The parameters were compared across at least three independent groups using a one-way analysis of variance. Tukey's test, with a significance level of $\alpha = 0.05$, was employed to compare the treatment means for the same tomato variety.

-The effect of bacteria on productivity and the relationship between plant length and productivity: The results showed that the recruitment of Bacillus (*BIA1*) isolates led to a significant increase in the indicators of

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Pseudomonas (*PIA*) and vegetative growth (length, dry and wet weight of roots and stems). This increase in vegetative growth, especially plant length and biomass, is a strong indicator of improving the plant's representative efficiency and its ability to absorb nutrients such as phosphorus and nitrogen. The increase in the length of the plant and its development in the first stages enhances its future ability to produce fruits and increase the overall productivity, as the growth-promoting bacteria (*PGPR*) contribute to the improvement of general plant health and stress resistance. This is reflected positively in the existence of the final result.

-The stage of growth in which treatment is completed: The treatment of plants in the four-week-old seedling stage by tinning the seedlings and immersing the roots for 60 minutes in the selected bacterial suspension (*PGPR*) with a concentration of approx. (2.6 x 10/CFU mL), then recaptured in plastic pots to monitor the effect of treatment on growth indicators.

3 RESULTS

3.1 The effect of PGPR isolates on the height of tomato plants

The effect of *PGPR* isolates on the height of tomatoes under greenhouse conditions is displayed in Table 1.

There was a statistically significant difference between all single inoculation treatments and one combination treatment (*BIA1 + PIA2*), where BIA1-treated plants had the maximum height in both tomato cultivars compared to the control ($\alpha = 0.05$).

Treatment of the *TC1* with *BIA1* displayed the highest height (57.27 cm), followed by PIA2 (50.13 cm) in the same variety. Furthermore, the *BIA1* treatment resulted in the highest height (55.82 cm) in the *TC2*, followed by the *PIA2* treatment (49.06 cm).

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TABLE 1.
Effect of (PGPR) Plant Growth-Promoting Rhizobacteria isolates on tomato plant height under greenhouse conditions.

	Plant height (cm)	
Treatment	**Maya variety (mean ± SE)**	**Melkesalsa variety (mean ± SE)**
BIA1	57.27 ± 0.63d	55.82 ± 0.65e
PIA2	50.13 ± 0.91c	49.06 ± 0.33d
PIA3	47.77 ± 0.81bc	46.11 ± 0.30c
BIA1 + PIA2	46.66 ± 0.66bc	44.31 ± 0.31bc
BIA1 + PIA3	41.30 ± 0.52a	40.46 ± 0.49a
PIA2 + PIA3	45.43 ± 0.25b	41.39 ± 0.85ab
Control	40.89 ± 1.04a	38.44 ± 0.20a

Note: The mean in the column that is followed by a small letter is not significantly different according to Tukey's test ($\alpha = 0.05$). Comparisons between treatments using the same cultivar are denoted by small letters.
Abbreviation: SE, standard error.

3.2 The effect of *PGPR* isolates on the fresh and dry weight of tomato plants

The effect of PGPR isolates on tomato fresh weight in greenhouse conditions is shown in Table 2. The treatment with BIA1 and PIA2 isolates significantly increased the fresh weight of shoots and roots of tomato varieties when compared to the control.

The BIA1 treatment showed maximum efficiency in increasing shoot and root fresh weight by 29.05 and 3.72 g in the *TC1*, followed by PIA2 by 25.03 and 3.26 g in the same variety, respectively. For the *TC2*, the BIA1 treatment caused rises in shoot and root fresh weight of 28.69 and 2.76 g, respectively, followed by PIA2 at 24.69 and 2.37 g. Among the combination treatments, *BIA1 + PIA2*

resulted in a significant effect on the fresh weight of the shoots in the Maya and Melkesalsa varieties by 22.59 and 20.44 g, respectively, compared

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to the control. The combined BIA1 and *PIA3* treatment had the least impact on the fresh weight shoots and roots of tomato varieties when compared to the control.

TABLE 2.
Effect of (PGPR) Plant Growth-Promoting Rhizobacteria on the fresh weight of tomato plants under greenhouse conditions.

Treatments	Maya variety fresh weight (g)		Melkesalsa variety fresh weight (g)	
	Shoot (mean ± SE)	Root (mean ± SE)	Shoot (mean ± SE)	Root (mean ± SE)
BIA1	29.05 ± 0.41d	3.72 ± 0.15c	28.69 ± 0.38e	2.76 ± 0.02b
PIA2	25.03 ± 1.33c	3.26 ± 0.04bc	24.69 ± 0.64d	2.37 ± 0.09b
PIA3	23.16 ± 0.51bc	3.01 ± 0.29ab	21.04 ± 0.35c	2.23 ± 0.27ab
BIA1 + PIA2	22.59 ± 0.53bc	2.99 ± 0.16ab	20.44 ± 0.62bc	2.20 ± 0.05ab
BIA1 + PIA3	20.50 ± 0.44ab	2.74 ± 0.30ab	18.69 ± 0.51ab	1.99 ± 0.07a
PIA2 + PIA3	21.30 ± 0.48ab	2.87 ± 0.06ab	19.02 ± 0.41abc	2.17 ± 0.13ab
Control	19.17 ± 0.72a	2.47 ± 0.05a	17.21 ± 0.34a	1.89 ± 0.06a

- Note: According to Tukey's test (α = 0.05), the mean in the column that is followed by a small letter is not substantially different. Comparisons between treatments using the same cultivar are denoted by lowercase letters. Abbreviation: SE, standard error.

The effect of *PGPR* on the dry weight of shoots and roots is shown in Table 3. The results of the study showed that *BIA1* and *PIA2* significantly

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varied from the control in terms of the dry weight of the shoots and roots in both tomato varieties across all treatments.

The treatment with *BIA1* and *PIA2* isolates significantly increased the dry weight of shoots and roots of tomato varieties compared to the control.

Treatment with *BIA1* increased the dry weight of the shoots and roots of the TC1 by 5.64 and 0.87 g, respectively, while *PIA2* increased the dry weight of the same variety by 5.01 and 0.84 g. Furthermore, *BIA1* had the greatest impact on raising the dry weight of shoots and roots in the *TC2* by 5.61 and 0.76 g, respectively, while *PIA2* had the second-highest effect with increases of 4.87 and 0.67 g, respectively, in the same variety.

In comparison with all other treatments, the combined *BIA1* and *PIA3* treatment had the least impact on the dry weight shoots and roots in both tomato varieties compared to the control.

TABLE 3

Effect of (PGPR) Plant Growth-Promoting Rhizobacteria isolates The dry weight of the tomato under greenhouse conditions.

Treatment	Maya variety dry weight (g)		Melkesalsa variety dry weight (g)	
	Shoot (mean ± SE)	Root (mean ± SE)	Shoot (mean ± SE)	Root (mean ± SE)
BIA1	5.64 ± 0.21c	0.87 ± 0.11b	5.61 ± 0.35c	0.76 ± 0.01b
PIA2	5.01 ± 0.22ab	0.84 ± 0.09b	4.87 ± 0.32bc	0.67 ± 0.07b
PIA3	4.68 ± 0.43abc	0.75 ± 0.04ab	4.23 ± 0.06ab	0.65 ± 0.03b
BIA1 + PIA2	4.64 ± 0.29abc	0.72 ± 0.07ab	4.19 ± 0.22ab	0.63 ± 0.02ab
BIA1 + PIA3	4.26 ± 0.14ab	0.68 ± 0.02ab	3.79 ± 0.28ab	0.59 ± 0.06ab
PIA2 + PIA3	4.47 ± 0.06ab	0.70 ± 0.01ab	3.88 ± 0.21ab	0.60 ± 0.02ab
Control	3.85 ± 0.15a	0.49 ± 0.01a	3.62 ± 0.07a	0.45 ± 0.01a

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- Note: The mean followed by a small letter in the column is not significantly different according to Tukey's test (α = 0.05). Lowercase letters refer to comparisons between treatments with the same cultivar.
- Abbreviation: SE, standard error.

3.3 Efficacy of *PGPR* isolates on tomato plant height and dry weight

The efficacy of *PGPR* isolates as growth promoters on the height and dry weight of the *TC1* is shown in Table 4. The efficacy of *PGPR* isolates in promoting plant growth was determined by calculating plant biomass and height, which demonstrated differences between tomatoes treated with *PGPR* isolates and the control.

This study demonstrated that *BIA1* increased the height by 40.1%, followed by *PIA2* by 22.6% in the *TC1*. Besides, treatment with *BIA1* and *PIA2* improved shoot dry weight by 46.6% and 30.2%, and root dry weight by 73.3% and 68.7% in the *TC1*, respectively. The combination treatment of BIA1 and *PIA2* showed better efficacy on height (14.1%), dry weight of shoots (20.4%), and dry weight of roots (43.3%) in the *TC1* than other consortium treatments. The combination treatment of *BIA1* and *PIA3* had the least impact on the efficacy of height and dry weight of shoots and roots in the *TC1*.

TABLE 4.

Efficacy of (PGPR) Plant Growth-Promoting Rhizobacteria isolates growth of the Maya variety under greenhouse conditions.

Treatment	Plant height (%) ± SE	Dry weight of shoot (%) ± SE	Dry weight of root (%) ± SE
BIA1	40.06 ± 1.55	46.58 ± 1.45	73.33 ± 2.31
PIA2	22.60 ± 2.22	30.21 ± 1.67	68.66 ± 1.87
PIA3	16.83 ± 1.96	21.47 ± 2.10	50.66 ± 0.82
BIA1 + PIA2	14.11 ± 1.62	20.43 ± 1.70	43.33 ± 1.50
BIA1 + PIA3	1.00 ± 1.28	10.65 ± 3.64	36.66 ± 2.90
PIA2 + PIA3	11.09 ± 0.61	16.19 ± 1.60	40.66 ± 2.40
Control	–	–	–

- Abbreviation: SE, standard error.

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The efficacy of *PGPR* isolates as a growth promotion on the height and dry weight of the TC2 is displayed in Table 5. *BIA1* increased the height by 45.2%, followed by *PIA2* by 27.6% in the TC2. Moreover, treatment with *BIA1* and *PIA2* increased shoot dry weight by 54.9% and 34.4%, and root dry weight by 68.1% and 48.89% for the same variety, respectively. The combination treatment of *BIA1* and *PIA2* showed better efficacy on height (15.3%), dry weight of shoots (15.9%), and roots (40.7%) in the *TC2* than other consortium treatments.

The combination treatment of *BIA1* and *PIA3* had the least impact on efficacy in terms of height, dry weight of shoots, and roots in the TC2.

TABLE 5.
Efficacy of (*PGPR*) Plant Growth-Promoting Rhizobacteria isolates of Melkesalsa variety under greenhouse conditions.

Treatment	Plant height (%) ± SE	Dry weight of shoot (%) ± SE	Dry weight of root (%) ± SE
BIA1	45.21 ± 1.68	54.88 ± 2.80	68.15 ± 1.48
PIA2	27.64 ± 0.87	34.44 ± 3.90	48.89 ± 1.56
PIA3	19.96 ± 0.79	16.94 ± 1.83	45.18 ± 0.76
BIA1 + PIA2	15.27 ± 0.52	15.93 ± 3.26	40.74 ± 2.12
BIA1 + PIA3	5.25 ± 1.27	4.60 ± 2.92	32.59 ± 1.25
PIA2 + PIA3	7.69 ± 2.20	7.18 ± 1.87	34.07 ± 0.78
Control	–	–	–

Abbreviation: SE, standard error.

4 DISCUSSION

In this study, Bacillus isolates (*BIA1*) and Pseudomonas isolates (*PIA2* and *PIA3*) were evaluated for the effectiveness of *PGP* on two tomato varieties.

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The results revealed that the highest height was displayed with BIA1 and PIA2 treatments in the Maya and Melkesalsa varieties. and these study is agreed in the line with the study by Almaghrabi et al. (2013), who reported that treatment of tomatoes with P. fluorescens, B. amyloliquefaciens, and P. putida showed the highest heights of 50.66, 48, and 48 cm under greenhouse conditions, respectively. Similarly, phosphate-solubilizing bacterial isolates PB-1 and VC-01 recorded the highest height of 40 and 39.2 cm, respectively, under greenhouse conditions (Pathak et al., 2017).

The biomass of tomato plants can be increased by inoculating them with Pseudomonas and Bacillus spp. (Widnyana & Javandira, 2016). The current findings demonstrated that treatment with *BIA1* and *PIA2* isolates showed the highest fresh weight of shoots and roots in the Maya and Melkesalsa varieties.

This study showed that the shoots and roots increased in higher fresh and dry weight than findings by Tan et al. (2013), in which treatment of tomato with Bacillus amyloliquefaciens strains *CM-2* and *T-5* showed an increase in the fresh weight of shoots by 8.95 and 8.41 g, respectively, under greenhouse conditions. Moreover, treatment with phosphate-solubilizing rhizobacteria *PB-1* and *VC-01* explains a significant effect on the fresh weight of shoots by 55.91 and 57.11 g and the fresh weight of the roots by 11.65 and 12.01 g under greenhouse conditions (Pathak et al., 2017).

The variation in the fresh weight of shoots and roots from greenhouse experiments could be attributed to the bacterial strains used, the tomato varieties, and the time of harvesting tomato plants.

The combination treatments, particularly *BIA1* + *PIA2* and *BIA1* + *PIA3*, did not significantly outperform the single isolate treatments in terms of fresh weight. In fact, the *BIA1* + *PIA3* combination had the least impact on both shoot and root fresh weight, suggesting a potential antagonistic interaction.

Similar findings have been reported in other studies, where combining PGPR strains did not always enhance plant growth and, in some cases, even reduced effectiveness (Myresiotis et al., 2012). This may be due to antagonistic interactions between strains, such as competition for nutrients or

the production of inhibitory metabolites (e.g., antibiotics), which limit their growth-promoting potential (He et al., 2024). Although the mechanisms underlying these interactions are not always well understood, they represent an important area of research, particularly when developing multi-strain PGPR formulations. Interestingly, the *PIA2* + *PIA3* combination was also relatively ineffective compared to the BIA1 and *PIA2* treatments alone, further suggesting that synergy between PGPR strains may not always be straightforward. *PGPR* strains may employ different mechanisms for promoting growth (e.g., phosphate solubilization, nitrogen fixation, indole-3-acetic acid production), and when combined, these mechanisms may either fail to synergize or interfere with each other's activity (Islam et al., 2013; Lobo et al., 2022). While *BIA1* and *PIA2* likely promote plant growth through mechanisms such as indole-3-acetic acid production, nitrogen fixation, and phosphate solubilization, *PIA3* may exert an antagonistic effect due to a less compatible mode of action, diminishing the overall impact when combined with other strains.

In comparison to the control, the Maya and Melkesalsa varieties treated with *BIA1* and *PIA2* isolates had the highest dry weight of shoots and roots. Similar to this, Kurabachew and Wydra (2013) found that under pot trials, *BC1AW* (*Bacillus cereus*) and PP3WT (*Pseudomonas putida*) demonstrated the greatest drying shoot weights of 5.6 and 5.2 g in KK 2 and 4.4 g and 4.2 g in the L390 tomato variety, respectively. Besides, the treatment of phosphate-solubilizing rhizobacteria *PB-1* and VC-01 increased the dry weight of roots by 0.96 and 0.91 g, and the dry weight of shoots by 6.2 and 6.22 g, respectively, under greenhouse conditions (Pathak et al., 2017).

Treatment of tomatoes with *Serratia marcescens* and *Pseudomonas putida* showed the highest shoot dry weight of 43 and 34.33 g, respectively, under greenhouse conditions (Almaghrabi et al., 2013), which is larger than the findings of this study. This variation could be due to the tomato variety and the treated bacterial strains.

Bacterial consortia for *PGP* have been proposed to boost the activity and viability of rhizobacteria. Each of the consortium constituent strains

outcompetes the others for rhizosphere establishment while also complementing them functionally for PGP (Yanti et al., 2018). In this study, consortium treatment of *BIA1* and *PIA2* resulted in a significant effect on the fresh weight of shoots in the Maya and Melkesalsa varieties.

A similar study found that a combination treatment of the *UM96* (Bacillus isolate) and *UM256* (Pseudomonas isolate) on tomato plants showed a significant growth-promoting effect on tomato seedlings under greenhouse conditions (Rojas-Solís et al., 2016).

Moreover, the current study showed that other consortium treatments showed positive effects on the fresh and weight drying of shoots and roots, but not a significant difference compared to the control treatments. Studies have demonstrated that mixed inoculation of beneficial rhizobacteria causes a competitive process, resulting in less effective *PGP* (Gamalero et al., 2002; Gamalero et al., 2004).

A similar study also found that three combination Bacillus-Pseudomonas treatments (*UM96 + UM16*, *UM96 + UM240*, and *UM96 + UM270*) did not promote seedling development under greenhouse conditions (Rojas-Solís et al., 2016).

In contrast to the strong effects of individual treatments with BIA1 and PIA2, the combination treatments (*BIA1 + PIA2*, *BIA1 + PIA3*, and *PIA2 + PIA3*) did not result in significant improvements in dry weight. Specifically, the *BIA1 + PIA3* combination had the least impact on both shoot and root dry weight, with increases of 4.26 and 0.68 g for Maya shoots and roots, respectively, which were not significantly different from the control group. Similarly, other combination treatments also failed to outperform individual isolates in terms of dry weight. This lack of synergy is consistent with research indicating that not all *PGPR* combinations are beneficial (Myresiotis et al., 2012).

In fact, combinations can sometimes lead to antagonistic effects, where one strain inhibits the growth-promoting activity of another, often due to competition for resources, production of inhibitory metabolites, or incompatible mechanisms of action (Islam et al., 2013; Puvanasundram et al., 2021).

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The absence of significant improvements in dry weight from combination treatments, especially those involving *PIA3*, suggests these interactions may not be as beneficial for tomato growth as initially hypothesized.

Future proteomic analyses should explore the specific mechanisms underlying these interactions.

The *PGPR* isolates increased tomato growth in both tomato varieties, with varying efficacy in all growth indices (height and dry weight). The current study revealed that BIA1 and *PIA2*-treated tomato plants significantly increased the tomato varieties in height in both of them compared to the other group to the control. Agarwal et al. (2020) reported that Bacillus velezensis *GL3*-treated tomato plants increased their height by 43.2% more than the control group in a pot experiment. Furthermore, inoculating three tomato cultivars, Kochero, Maya, and Melkesalsa, with Bacillus isolates *B1*, *B2*, and *B5*, increased their height by 65%, 85%, and 62%, respectively, under greenhouse conditions (Mengistie & Awlachew, 2022), which is consistent with current findings.

The results of the current study revealed that treatment with the *BIA1* and *PIA2* isolates increased the dry weight of shoots and roots in the Maya and Melkesalsa varieties compared to the control. According to Singh et al. (2012), Bacillus subtilis BS-9 had a better ability to promote tomato plant growth, resulting in a significantly higher dry weight of shoots and roots compared to the control and a 25.3% and 82.7% increase in the dry weight of shoots and roots of tomato plants, respectively, compared to the control. Similarly, in pot trials, rhizobacterial isolates of *Bacillus cereus BC1AW* and *Pseudomonas putida PP3WT* from tomato plants increased shoot dry weight by 75%, 62.5% in genotype KK2, and 57.1%, 50% in genotype L390, respectively (Kurabachew & Wydra, 2013). Phosphate solubilization and *IAA* production features of bacterial strains linked to the tomato rhizosphere could be implicated in the mechanism of action during biomass increment.

Rhizobacteria are influenced by numerous variables that interact intricately to affect rhizobacteria in terms of both quantity and quality, including soil

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type, plant species and diversity, cultivar type, climate, agriculture, and fertilization practices (Igiehon & Babalola, 2018; Yagmur & Gunes, 2021).

It is crucial to investigate and identify PGPR strains that could be exploited as possible PGP under particular ecological and environmental circumstances to boost tomato production. The findings of the present investigation showed that treatment with Bacillus isolate (BIA1) and Pseudomonas isolate (*PIA2*) significantly raised the height and fresh and dry weight of shoots and roots in both tomato cultivars under greenhouse conditions.

The effect of bacteria on productivity and the relationship between plant length and productivity.

The findings demonstrated that the recruitment of Bacillus (*BIA1*) isolates significantly increased vegetative growth (length, dry and wet weight of roots and stems) and Pseudomonas (*PIA*) indicators.

An improvement in the plant's representative efficiency and its capacity to absorb nutrients like phosphorus and nitrogen is strongly shown by this rise in vegetative growth, particularly plant length and biomass. Growth-promoting bacteria (PGPR) promote general plant health and stress tolerance, which increases the plant's capacity to produce fruits in the future and boosts overall production. The result's degree of existence is positively impacted by this.

The growth stage at which therapy is finished. Plants in the four-week-old seedling stage were treated by tinning the seedlings and submerging the roots in the chosen bacterial suspension (*PGPR*) for 60 minutes at a concentration of roughly 2.6 x 10/CFU mL. The plants were then recaptured in plastic pots to track the impact of the treatment on growth indicators.

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5 CONCLUSION

The results of this study show that the application of *PGPR* isolates significantly enhanced the growth of tomato plants in terms of height, fresh weight, and dry weight under greenhouse conditions. Among the tested isolates, the Bacillus isolate (*BIA1*) consistently produced the most significant positive effects, leading to the highest increases in plant height, fresh weight, and dry weight in both tomato cultivars (Maya and Melkesalsa). *BIA1* treatment resulted in the greatest improvements in both plant height and root dry weight for both varieties, followed by Pseudomonas isolate (*PIA2*), which also had a notable impact on plant growth. Combination treatments, especially *BIA1* + *PIA2*, also provided substantial benefits, particularly in terms of plant biomass. Conversely, the *BIA1* + *PIA3* combination had the least impact across all growth parameters. Overall, the findings highlight that Bacillus isolate (*BIA1*) and Pseudomonas isolate (*PIA2*) are the most effective *PGPR* isolates for promoting tomato growth, making them strong candidates for enhancing tomato production in controlled environments, given that they were validated in the field.

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