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# EVALUATION OF PGPR ISOLATES AS BIOSTIMULANTS FOR ENHANCING GROWTH IN COMMON BEAN (*Phaseolus vulgaris* L.)

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***Abstract***
*Green Bean is an important floriculture plant worldwide. This study was conducted to investigate the effects of seed-inoculated PGPR (Plant Growth-Promoting Rhizobacteria) applications on the growth of common bean (Phaseolus vulgaris L.). The experiment was carried out under unheated greenhouse conditions using a commercial NPK fertilizer (18:18:18) and five different PGPR isolates: Bacillus megaterium U2-1, Pseudomonas putida 9-4-2, Bacillus thuringiensis 2B-2-2, Bacillus spp. 2B-3-1, and Bacillus pumilus EU-20. The bacterial treatments were applied by soaking the seeds for one minute in bacterial suspensions at a concentration of $1\times10^3$ cfu; the control group was treated with sterile distilled water under the same conditions. Observations were conducted until the beginning of the flowering stage to evaluate the effects of PGPR on plant development. The results revealed that bacterial applications significantly enhanced plant height, stem diameter, fresh and dry plant weight, root fresh and dry weight, root length, leaf area, and leaf number compared to the control. Overall, the effects of different rhizobacterial isolates on growth parameters were found to be comparable to those of commercial fertilizer applications. These findings highlight the potential of PGPR formulations as promising biostimulants agents in vegetable production and their role in promoting sustainable and environmentally friendly cultivation practices.*

*Keywords: Bacillus, Biostimulants, Pseudomonas, PGPR*

## 1. INTRODUCTION

The green bean (*Phaseolus vulgaris* L.) is a widely cultivated legume species of global importance due to its dual role in human nutrition and soil fertility enhancement. With its high protein content, it serves as a significant dietary protein source. Furthermore, through symbiotic nitrogen fixation, common bean contributes to improved soil fertility and plays a crucial role in sustainable agricultural systems. *Phaseolus vulgaris*, a warm-season vegetable species belonging to the Fabaceae family, is cultivated both as a fresh vegetable (green bean) and in dry form (dry bean) (Gepts, 1998). In Turkey, all commercially cultivated bean varieties belong to the P. vulgaris species (Balkaya & Yanmaz, 2003). However, its production can be adversely affected by various factors, including environmental stresses, low soil fertility, and plant diseases. According to data from the Turkish Statistical Institute (TÜİK), dry bean production in 2023 amounted to 240,000 tons, reflecting an 11.1% decrease compared to the previous year. The total cultivated area for dry beans in 2023 was recorded at approximately 97,000 hectares. Meanwhile, fresh bean production in 2024 increased by 0.4% compared to the previous year, reaching 507,061 tons. Nevertheless, there has been a gradual decline of approximately 130,000 tons in fresh bean production over the last


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nine years, dropping from 632,000 tons in 2013 to 519,000 tons in 2022 (Balkaya & Yanmaz, 2003).

Ensuring sustainability in agriculture and reducing the reliance on chemical inputs are among the most critical goals of modern crop production systems. In this context, microbial applications that support plant growth have emerged as environmentally friendly alternatives. Plant growth-promoting rhizobacteria (PGPR) are beneficial microorganisms that inhabit the rhizosphere and enhance plant development through a variety of mechanisms. These mechanisms include nitrogen fixation, phosphorus solubilization, phytohormone production, siderophore production, and biocontrol of plant pathogens (Yadegari, 2010; Saharan, 2011; Ahemad & Kibret, 2014). In recent years, the use of biostimulants has gained increasing attention as a means to reduce the application of chemical fertilizers and pesticides, thereby promoting sustainable agriculture and protecting human health (Kauffman et al., 2007). Biostimulants are classified into seven main groups: humic and fulvic acids, protein hydrolysates and other nitrogen-containing compounds, seaweed extracts and plant-derived substances, chitosan and other biopolymers, inorganic compounds, beneficial fungi (e.g., arbuscular mycorrhizal fungi - AMFs), and beneficial bacteria (e.g., PGPRs) (Du Jardin, 2015).

Improving the physical, chemical, and biological properties of soils requires long-term strategic planning aimed at increasing soil organic matter levels to ensure sustainability. Excessive use of inorganic fertilizers, a common input in traditional agricultural systems, is now recognized to contribute to soil degradation and environmental pollution. Therefore, in response to growing global demand for organic agriculture and to promote sustainable soil use and environmental protection, organic fertilizers should be prioritized over synthetic nitrogen and phosphorus fertilizers. While widely accepted and used, farmyard manure is a relatively costly organic material in terms of procurement and application. However, a wide range of alternative organic materials can serve as substitutes or supplements. These materials are essential inputs in agricultural production that support human health and well-being. Therefore, PGPR applications have emerged as a promising research focus in bean cultivation, aiming to enhance yield and improve plant tolerance to stress conditions. Recent studies have demonstrated that PGPR can accelerate root development, increase biomass, and promote nodule formation in beans. Moreover, some PGPR strains have been reported to improve resistance against both biotic and abiotic stresses.

This study aimed to evaluate the plant growth-promoting effects of PGPR bacteria on common bean and to compare their effectiveness with that of widely used commercial NPK fertilizers. Plant growth parameters were assessed from germination to the flowering stage. The findings of this study aim to highlight the potential of microbial applications such as PGPR in sustainable agricultural practices..

## 2. MATERIALS AND METHODS

### Plant Material

The experiment was conducted in 2024 in an unheated polyethylene greenhouse belonging to the Department of Horticulture, Faculty of Agriculture, Erciyes University. As plant material, the bush-type green bean cultivar 'ALBENİ', one of the most commonly preferred commercial varieties in green bean cultivation, was used.

### Preparation of Rhizobacteria

In this study, rhizobacterial strains previously identified at the Faculty of Agriculture, Erciyes University, through 16S rRNA sequencing and genome analysis, and characterized for gene


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presence via PCR analysis, were used. The experiment included six treatments: a control (no treatment), NPK fertilizer application (18-18-18 commercial fertilizer), and five different Rhizobium sp. isolates: Bacillus megaterium U2-1, Pseudomonas putida 9-4-2, Bacillus thuringiensis 2B-2-2, Bacillus spp. 2B-3-1, and Bacillus pumilus EU-20 (Ünlü et al., 2024) (Table 1). For activation, bacterial strains preserved at -80°C in stock culture were used. Initial cultures were obtained by streaking onto nutrient agar (NA) solid medium. After observing colony growth, the bacteria were further purified. The activated rhizobacteria were then cultured in Luria–Bertani (LB) liquid medium and incubated overnight at 35–37°C with shaking at 180 rpm. Bacterial suspensions were prepared to a final concentration of $1\times10^3$ CFU/mL (Yılmaz, 2010). These suspensions were diluted 1:2 with sterile distilled water and applied to the seeds.

***Table 1. Bacterial codes, species, and concentrations used in the study***

| Treatment | Rhizobacteria Code | Rhizobacteria Species | Concentration (CFU/mL) |
|---|---|---|---|
| **1** | U2-1** | *Bacillus megaterium* | $1x10^3$ |
| **2** | 9-4-2* | *Pseudomonas putida* | $1x10^3$ |
| **3** | 2B-2-2* | *Bacillus thurigiensis* | $1x10^3$ |
| **4** | 2B-3-1** | *Bacillus* spp. | $1x10^3$ |
| **5** | EU-20** | *Bacillus pumilus* | $1x10^3$ |
| **Control** | commercial fertilizer | 18:18:18 | 5g (each pod) |

*NCBI record numbers identified by MALDI TOF, **identified by SANGER sequencing

**Experimental Design**

The experiment was conducted in an unheated polyethylene greenhouse using a randomized complete block design (RCBD) with three replications. The plants were grown in 3-liter plastic pots with a diameter of 17.5 cm. The growth medium was prepared by mixing peat, garden soil, and perlite in a 1:1:1 ratio. Prior to mixing, peat and garden soil were sterilized by autoclaving at 121 °C for 25 minutes.

**Determination of Plant Growth Parameters**

To evaluate the effects of rhizobacterial treatments on plant growth, the following morphological parameters were measured: plant height, stem diameter, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, root length, leaf area, and number of leaves. For the measurement of leaf area and leaf number, five fully developed, medium-sized leaves were randomly selected from each pot. Leaf area, which serves as a reliable indicator of treatment-induced physiological responses in plant growth, was assessed using a leaf area meter. For this purpose, ten leaves randomly sampled from each replicate were measured, and the mean value was expressed in $cm^2$.

Fresh and dry weight measurements were performed on three randomly selected plants per replicate. After gently removing soil particles from the roots, the aboveground (shoot) and belowground (root) parts were separated and weighed to determine the fresh biomass. Subsequently, the samples were oven-dried at 72 °C for 48 hours, and the dry weights were recorded and expressed as grams per plant (g $plant^{-1}$).

**Statistical Analyses**

The experiment was conducted using a randomized complete block design (RCBD) with three replications, with one plant per pot in each replication. Measurements and observations were recorded throughout the growing period. Data obtained before and after harvest were subjected to


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analysis of variance (ANOVA) using the SAS statistical software (version 9.00). Mean comparisons were performed using Duncan's multiple range test at significance levels of $p < 0.05$ and $p < 0.001$.

## 3. RESULTS AND DISCUSSIONS

### Plant Growth Parameters Results

In this study, the effects of PGPR applications used as biostimulants on the plant growth parameters of green bean (*Phaseolus vulgaris* L. cv. Albeni) were investigated in comparison with commercial NPK fertilizer application. The results are presented in Figure 1 and Table 2.

Regarding the effect of PGPR and NPK treatments on plant height, the highest value (58.75 cm) was observed in the U2-1 bacterial strain treatment, followed by 2B-2-2 (57.45 cm), 9-4-2 (56.80 cm), 2B-3-1 (55.25 cm), and EU-20 (52.40 cm) isolates, respectively (Table 2; Figure 1). In the control group, the plant height was measured as 40.15 cm. These findings demonstrate that PGPR applications had a positive influence on plant height. Similar positive effects of rhizobacterial treatments on plant height have been reported in previous studies (Odabaş and Gülümser, 2001; Bildirici, 2003; Uyanöz et al., 2010; Kumar et al., 2014; Angın, 2022; Krawczyk et al., 2022; Dalda-Şekerci et al., 2023). The results obtained in our study are consistent with these earlier findings.

When the findings regarding plant fresh and dry weight were examined, the highest values were obtained from the U2-1 bacterial isolate with 58.63 g fresh weight and 9.65 g dry weight. This was followed by isolates 2B-2-2 (59.75 / 9.41 g), 9-4-2 (57.40 / 9.30 g), 2B-3-1 (56.61 / 5.91 g), and EU-20 (55.30 / 8.97 g), respectively. Considering the mean values among treatments, the U2-1 isolate yielded the most favorable results. In the control treatment, fresh and dry weights were measured as 45.20 g and 8.44 g, respectively (Table 2; Figure 1).

When the data on stem diameter were analyzed, the best result (3.89 mm) was obtained from the U2-1 rhizobacterial isolate. This was followed by the isolates 9-4-2 (3.85 mm), 2B-2-2 (3.75 mm), 2B-3-1 (3.72 mm), and EU-20 (3.60 mm). Based on the average values of the treatments, the highest stem diameter was recorded in the U2-1 isolate with 3.89 mm. In the control treatment, stem diameter was measured as 3.50 mm (Table 2). Similar findings have been reported in other studies as well. For instance, Kokalis-Burelle et al. (2002) and Ibiene et al. (2012) demonstrated that PGPR applications enhanced plant development in tomato seedlings by improving parameters such as root length, stem diameter, and plant height. Likewise, Garcia et al. (2003) reported that PGPRs promoted seedling growth in pepper and tomato.

According to the findings related to leaf area, the largest leaf area (351.23 $cm^2$) was obtained from the U2-1 rhizobacterial isolate. This was followed by the isolates 9-4-2 (348.15 $cm^2$), 2B-2-2 (345.61 $cm^2$), 2B-3-1 (345.37 $cm^2$), and EU-20 (345.15 $cm^2$). In the control group, the leaf area was measured as 175.16 $cm^2$ (Table 2; Figure 1). Statistical analysis revealed that PGPR applications had a highly significant effect on leaf area at the $p<0.001$ level. In a study by Deng et al. (2013), it was reported that Bacillus megaterium and Paenibacillus polymyxa bacteria led to increases in chlorophyll content and leaf area index, particularly enhancing plant height in tomato plants. Similarly, other studies have also demonstrated the positive effects of PGPRs on leaf area (Karakurt et al., 2011; Tuzlacı, 2014).

In terms of the number of leaves, the most favorable results were observed with the U2-1 and 2B-2-2 rhizobacterial isolates, both producing an average of 11 leaves per plant. These were followed by isolate 9-4-2 with 10 leaves per plant, 2B-3-1 with 9 leaves per plant, and EU-20 with 8 leaves per plant. In contrast, the control-NPK application resulted in an average of 7 leaves per plant (Table 2;


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Figure 1). These findings indicate that rhizobacterial isolates had a significant effect on increasing the number of leaves. Similar results were reported in previous studies, where PGPR applications were shown to enhance both leaf number and area (Banchio et al., 2008; Karagöz et al., 2010; Krawczyk et al., 2022).

In terms of root fresh and dry weight, the rhizobacterial isolates U2-1 (50.62 g / 5.91 g) and 2B-2-2 (50.21 g / 5.93 g) were grouped together statistically, followed by isolates 9-4-2 (49.93 g / 5.87 g), 2B-3-1 (49.72 g / 5.60 g), and EU-20 (48.79 g / 5.45 g), which formed a second group. The control group plant (37.46 g / 4.17 g), on the other hand, was placed in a separate statistical group. When evaluating the effect of bacterial isolates on root development, it was observed that all PGPR applications had a positive impact on both root fresh and dry weight compared to the control (Table 2).

When evaluating the effect of PGPR and NPK applications on root length, the highest value was recorded in the 2B-2-2 bacterial isolate (49.92 cm), followed by U2-1 (49.85 cm), 9-4-2 (47.60 cm), 2B-3-1 (47.20 cm), and EU-20 (43.40 cm) (Table 2). In the control treatment, root length was measured at 35.17 cm. These findings indicate that rhizobacterial applications positively influenced root development. Similar results have been reported in previous studies, where bacterial inoculations significantly enhanced root growth. It has been stated that approximately 80% of PGPR strains are capable of producing indole-3-acetic acid (IAA), a key phytohormone involved in root development (Patten and Glick, 2002; Spaepen et al., 2007). Calvo et al. (2010) found that 81% of 63 isolates obtained from the potato rhizosphere produced IAA. Similarly, Ashrafuzzaman et al. (2009) reported that 60% of bacteria isolated from the rice rhizosphere were IAA producers. However, the level of IAA production varies among isolates, and this variation is considered a critical factor affecting PGPR efficacy. Poonguzhali et al. (2006) reported that PGPR isolates from the Brassica campestris rhizosphere produced IAA in the range of 602–2,775 ppm. Likewise, Majeed et al. (2015) found that 53.3% of the PGPR isolates they obtained produced IAA in the range of 1–2,503 ppm.

***Table 2. Effects of Different Rhizobacterial Applications on Growth Parameters of Green Bean***

| Bacterial Code | Plant Height (cm) | Stem Diameter (mm) | Fresh Shoot Weight (g) | Dry Shoot Weight (g) | Fresh Root Weight (g) | Dry Root Weight (g) | Root Length (cm) | Leaf Area (cm²) | Number of Leaves (plant) |
|---|---|---|---|---|---|---|---|---|---|
| **U2-1** | 58.75 a | 3.89 a | 58.63 ab | 9.65 a | 50.62 a | 5.91 a | 49.85 a | 351.23 a | 11 a |
| **2B-2-2** | 57.45 ab | 3.85 ab | 59.75 a | 9.41 a | 50.21 a | 5.93 a | 49.92 a | 348.15 ab | 11 a |
| **9-4-2** | 55.12 b | 3.76 b | 57.40 b | 9.30 ab | 49.93 ab | 5.87 b | 47.60 b | 345.37 ab | 10 ab |
| **2B-3-1** | 55.25 b | 3.72 bc | 56.61 b | 9.17 ab | 49.72 ab | 5.60 b | 47.20 b | 345.61 ab | 9 b |
| **EU-20** | 52.40 c | 3.61 c | 55.30 c | 8.97 b | 48.79 b | 5.45 b | 43.40 c | 302.91 c | 8 bc |
| **Control** | 40.15 d | 3.50 d | 45.20 d | 8.44 c | 37.46 c | 4.17 c | 35.17 d | 175.16 d | 7 c |
| **Significance** | ** | * | ** | ** | ** | ** | ** | ** | ** |

In this study investigating the effects of plant growth-promoting rhizobacteria (PGPR) on common bean (Phaseolus vulgaris L.), the findings revealed that PGPR applications positively influenced plant development. The impact of rhizobacterial treatments was particularly evident in morphological traits, where higher plant growth parameters were observed compared to the control


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group treated with NPK fertilizer. Notably, the U2-1 and 2B-2-2 bacterial strains demonstrated strong potential as effective biostimulants. PGPR applications offer an environmentally friendly alternative for reducing chemical fertilizer use and promoting a transition toward sustainable agriculture.

Significant increases were observed in key growth parameters such as root and shoot length, fresh plant biomass, leaf area, and number of leaves in PGPR-treated plants compared to the controls. These results are consistent with previous studies involving various PGPR strains. For instance, Yadegari et al. (2010) reported that PGPR applications enhanced both plant growth and symbiotic nitrogen fixation in common bean. Similarly, Vessey (2003) highlighted that PGPR promote plant growth through mechanisms such as phytohormone (especially IAA) production, phosphate solubilization, and siderophore synthesis. The pronounced improvement in root development observed in the current study can be attributed to PGPR-mediated regulation of rhizospheric microbial interactions, which enhance root surface area and subsequently improve water and nutrient uptake. Previous studies have consistently demonstrated the beneficial effects of PGPR (plant growth-promoting rhizobacteria) applications on parameters such as shoot fresh and dry weight, root fresh and dry weight, root length, leaf area, stem diameter, and leaf number (Yaman and Sepetoğlu, 1997; Rodriguez and Fraga, 1999; Deka Boruah and Dileep Kumar, 2003; Şevik, 2010; Akkurt, 2010; Al-Askar and Rashad, 2010; Küçük, 2011; Karaca and Uyanöz, 2011; Türkmen et al., 2016; Özturan-Akman, 2017; İmamoğlu, 2019; Angın, 2022). Compared to untreated controls, PGPR treatments have been shown to significantly enhance root and shoot growth, as well as leaf development. The beneficial effects of seed inoculation with rhizobacteria on shoot dry weight and overall plant yield have also been previously reported (Schippers et al., 1987; Guo et al., 2004; Sharafzadeh, 2012; Singh et al., 2014; Fan et al., 2017; Yılmaz et al., 2022; Şekerci and Ünlü, 2023; Chowhan et al., 2023). These outcomes are largely attributed to the bacteria's nitrogen-fixation and phosphate-solubilizing abilities, as well as their capacity to produce plant growth-promoting substances such as indole-3-acetic acid (IAA) (Salantur et al., 2006; Flores-Félix et al., 2013). Additionally, Turan et al. (2021) noted that IAA is commonly produced by PGPR through various metabolic pathways, and that PGPR-mediated indirect activation of the plant auxin pathway further contributes to growth promotion.

Despite these findings, some discrepancies exist between the results of this study and those reported in the literature. These variations are likely due to differences in the bacterial isolates used. The effectiveness of PGPR can vary depending on the bacterial strain, application method, environmental conditions, and plant species (Ahemad and Kibret, 2014). Therefore, it is crucial to evaluate each PGPR strain individually on the target crop to identify the most effective combinations. Overall, the positive outcomes observed in this study support the potential of locally sourced PGPR isolates to be utilized in agriculture as biofertilizers, biostimulants, or biological growth regulators.

## 4. CONCLUSIONS

The findings of this study demonstrate that the bacterial isolates applied had positive effects on plant growth parameters in common bean (*Phaseolus vulgaris* L.). Although the degree of impact varied depending on the specific growth parameter, isolates U2-1 (*Bacillus megaterium*), 9-4-2 (*Pseudomonas putida*), 2B-2-2 (*Bacillus thuringiensis*), 2B-3-1 (*Bacillus* spp.), and EU-20 (*Bacillus pumilus*) exhibited significant potential as biofertilizers. These results highlight the


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potential of PGPR applications as an effective biotechnological approach to promote plant growth in a sustainable and environmentally friendly manner.
However, to confirm these findings and evaluate the full agronomic potential of the tested isolates, further research under open-field conditions over at least two growing seasons is necessary. In particular, yield and yield-related traits should also be assessed. Moreover, optimizing key factors such as bacterial strain selection, application dose, and timing is essential to enhance the efficacy of PGPR applications in agricultural systems.

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