

Volume: 4 Issue: 2 Year: 2024 Research Article e-ISSN: 2822-4167



# **Assessment of the Effect of Plant Growth Promoting Rhizobacteria (PGPR) Strains on Rooting in Rose Cuttings**

## **Akife DALDA-SEKERCİ1\* Kanykei KANATBEKOVA1 Emel UNLU[2](https://orcid.org/0000-0002-1047-7828)**

<sup>1</sup> Erciyes University, Faculty of Agriculture, Department of Horticulture, Kayseri, Türkiye

<sup>2</sup> Erciyes University, Graduate School of Natural and Applied Sciences, Horticulture Department, Kayseri, Türkiye



#### **Bitki Gelişimini Teşvik Eden Rizobakteri (PGPR) Suşlarının Gül Çeliklerinde Köklenme Üzerine Etkisinin Değerlendirilmesi**



#### **To cite this article:**

Dalda-Sekerci, A., Kanatbekova, K., & Unlu, E. (2024). Assessment of the effect of plant growth promoting rhizobacteria (PGPR) strains on rooting in rose cuttings. *Ereğli Tarım Bilimleri Dergisi, 4*(2), 65-75. <https://doi.org/10.54498/ETBD.2024.34>

**\*Sorumlu Yazar:** Akife DALDA-SEKERCİ, *akidal\_@hotmail.com*



#### **INTRODUCTION**

The rose (*Rosa* L.), belonging to the genus *Rosa* in the family Rosaceae, is a valuable ornamental plant used both in landscape design and as a cut flower. It ranks first among globally cultivated ornamental plants due to its flowers, essential oil, and other value-added products. Modern roses are predominantly hybrids derived from diploid and tetraploid species native to Asia and Europe (Zhang et al. 2013).

Research and development of new rose varieties continue worldwide. The vegetative propagation of varieties bred in accordance with breeding objectives is generally carried out through the method of cutting propagation. The cutting propagation method is extensively preferred in the vegetative propagation of ornamental plants due to the identical genetic structure between the parent and the newly produced plant. However, several factors influence the process of cutting production. Among these factors are the age and characteristics of the parent plant from which the cutting is taken, the season of cutting collection, the type of cutting, the storage conditions until planting, chemical substances used to accelerate rooting, the physical and chemical properties of the rooting medium, the temperature of the rooting medium, sterilization substances used to prevent fungal infections (Dalda-Sekerci and Ünlü, 2023).To hasten root formation and promote healthy root development in plants propagated via cuttings, various chemical substances and hormonal treatments are frequently utilized. However, in recent years, the importance of microorganisms has risen within the context of sustainable agricultural practices. Research is increasingly focusing on the use of these microorganisms for biocontrol of plant diseases, enhancement of plant growth, bio-fertilization, and improvement of rooting efficiency in cuttings (Antoun and Prevost, 2006; Mehmood et al., 2018; Ünlü et al., 2023). Studies conducted thus far have underscored the primary role of bacteria among the key beneficial microorganisms utilized in plant production (Higa and Paar, 1994; Bloemberg and Lugtenberg, 2001; Esitken et al., 2003; Lugtenberg and Kamilova, 2009).

These studies have revealed that the application of rhizobacteria species, including *Bacillus, Pseudomonas, Agrobacterium, Streptomyces*, and *Alcaligenes*, as well as the use of Indole Acetic Acid (IAA)-producing bacteria with genes responsible for IAA production, significantly promotes rooting in cuttings (Esitken et al., 2003; Kisvarga et al., 2022). Studies have demonstrated that Plant Growth-Promoting Rhizobacteria (PGPRs) are utilized as plant growth regulators in ornamental plant cultivation worldwide due to their capacity to enhance plant growth, yield, and soil quality (Srivastava and Govil, 2007; Sharma and Kaur, 2010; García-Fraile et al., 2012; Flores-Félix et al., 2013; Zulueta-Rodriguez et al., 2014; Karagöz et al., 2016). Typically, these bacteria colonize the root system, supporting plant development and suppressing harmful microorganisms. PGPRs facilitate plant growth by producing growth hormones, regulating microbial balance in the rhizosphere, and enhancing mineral uptake, thereby positively influencing plant development (Siddiqui, 2006; Şevik, 2010). Additionally, growthpromoting rhizobacteria stimulate rooting and enable the production of high-quality seedlings in a shorter time frame (Ruzzi and Aroca, 2015). Rhizobacteria not only promote rooting but also contribute to healthier seedlings through faster and superior root formation (Şekerci and Ünlü, 2023).

In this study, three distinct rhizobacterial formulations (*Bacillus subtilis*, *Bacillus cereus*, and mix of *Bacillus subtilis* and *Bacillus cereus*) were applied to assess their impact on the rooting of green rose cuttings.

#### **MATERIAL AND METHODS**

#### **Plant Material**

Semi-hardwood cuttings, each containing 3-4 buds, obtained from pink garden roses, were

employed as the plant material.

### **Rhizobacteria Isolation from Soil**

Sixty soil samples were collected from various locations across Central Anatolia, Turkey, for the isolation of rhizobacteria. These samples were transferred into bottles containing sterile water (0.9% NaCl) and Luria-Bertani (LB) Broth medium. After incubation, aliquots from each sample were spread onto solid LB and NB (Nutrient Broth) media using the spread plate technique. The plated samples were then incubated at 35°C for 12-24 hours to facilitate the growth of primary bacterial cultures. Then, isolates from distinct colonies on the petri dishes were selected and purified. Purified strains were further cultured and transferred to NB medium (Upadhyay et al., 2009).

#### **DNA Isolation from Rhizobacteria**

The bacterial DNA was extracted according to the procedure of Wilson (2001). Bacterial isolates were cultured in 10 mL of nutrient broth (NB) and incubated for 24 hours. After incubation, 1.5 mL of the bacterial culture was suspended in TE (Tris-EDTA) buffer (pH 8.0). To each suspended pellet, 30 µL of 10% SDS (sodium dodecyl sulfate) and 3 µL of proteinase K were added, mixed thoroughly, and then incubated at 37 $\degree$ C for 1 hour. Following the incubation, 100 µL of 5M NaCl and 80 µL of CTAB/NaCl solution were added to each sample. The samples were then washed sequentially with chloroform/isoamyl alcohol (24/1, v/v), phenol/chloroform/isoamyl alcohol (25/24/1, v/v/v), and isopropanol, and finally dissolved in TE (Tris-EDTA) buffer.

### **PCR Amplification and Sequencing of the 16S rDNA Gene**

The DNA obtained from the bacteria was initially tested with IAA primers F 5'- CCAACATCATCAAGCTGCCGAACA-3' and R 5'-AGACCTTCATCATCGTGGCCTTCA-3', and bacterial strains possessing the IAA gene region were identified. The identification of the isolates based on 16S rDNA sequence analysis involved the following steps: isolation of genomic DNA, amplification using universal 16S forward (5'-3') and 16S reverse (3'-5') primers (targeting 16S rDNA regions; 16S forward 5'-AGA GTT TGA TCC TGG CTC AG-3' and 16S reverse 5'-CCG TCA ATT CCT TTG AGT TT-3') according to Edwards et al. (1989), sequencing of the amplified regions, and comparison of the obtained sequences with the base sequences of microorganisms available in the database.

### **Preparation and Activation of Rhizobacteria**

Different rhizobacteria solutions were prepared from bacteria identified as carrying the IAA (indole acetic acid) gene through PCR analysis. These rhizobacteria were reactivated from stock cultures stored at -80°C, resulting in three distinct formulations (Table 1). Nutrient Agar (NA) and Nutrient Broth (NB) media were employed for the reactivation and cultivation of the rhizobacteria (Yılmaz, 2010; Ünlü et al., 2023). Bacterial suspensions were prepared at a concentration of  $3x10^7$  cfu/ml.



### **Table 1**

*Formulations created by activating stock bacterial strains used in the study*

\* Defined by MAlDI TOF

#### **Application of Bacteria to Cuttings**

Semi-hardwood cuttings of pink garden roses, prepared with 3-4 buds, were soaked in rhizobacteria solution for 5 minutes before planting. In the control group, the cuttings were soaked in water. The rose cuttings were planted in growth containers filled with a 1:1 mixture of peat and perlite, with each container holding 15 cuttings and replicated three times. After planting, the cuttings were watered twice with 5 ml  $(3x10^7 \text{ cft/ml})$  of bacterial solution mixed into 1 liter of irrigation water at 15day intervals.

#### **Statistical Analyses**

Rooting rate measurements were recorded at the end of the 10th week to assess the effects of different bacterial formulation treatments. The obtained data were analyzed using analysis of variance (ANOVA) in SAS software (version 9.00). Means were compared using the Duncan test at significance levels of 0.05.

#### **RESULTS AND DISCUSSION**

Propagation via cuttings is a widely used method in rose cultivation. However, this method often results in seedling losses due to the proliferation of fungal and bacterial diseases. In this study, three different rhizobacterial formulations were tested to determine their effects on the rooting of semihardwood cuttings of pink garden roses. PGPR bacteria, isolated from various soils, had their DNA extracted and subjected to PCR analysis using IAA primers. Subsequently, DNA fragments containing the target 16S rDNA gene were extracted. The analysis revealed that the rhizobacterial species used in the study possessed the IAA gene region (Figure 1).

#### **Figure 1**

*PCR gel images of Bacillus subtilis and Bacillus cereus species indole acetic acid (IAA) gene region*



Rhizobacterial treatments provided a statistically significant increase in rooting compared to the control group (Table 2, Figure 2). The rooting success rates were as follows; control group 40.75%, *B. cereus* treatment 62.50%, *B. subtilis* treatment 50.25%, and mixed application of *B. cereus* and *B. subtilis* strains 74.75%. These results indicate that *Bacillus* spp. positively affect rooting in rose cuttings (Figure 3).

## **Table 2**





## **Figure 2**

*Visual of the effect of 1st formulation (B. subtilis), 2nd formulation (B. cereus), and 3rd formulation (B. subtilis and B. cereus) and control on rooting in rosa.*



## **Figure 3**

*Graphical Representation of the Impact of Various PGPR Bacterial Strains on Rose Rooting Parameters*



The present study clearly demonstrates the positive effects of different *Bacillus* species on the rooting of rose cuttings. In recent years, there has been a noticeable increase in the use of rhizobacterial applications in ornamental plant cultivation, a trend that extends throughout the horticultural sector. Despite advancements in vegetative propagation techniques, the industry still faces economic challenges due to suboptimal rooting efficiency. Previous studies have highlighted the need for further research to identify biostimulants that can enhance root development (Ahkami et al., 2009). The findings of this study align with the literature, providing consistent data with past research. Numerous studies have confirmed the effectiveness of rhizobacterial applications in promoting root growth and natural plant development, suggesting a promising alternative to commercial hormones.

Positive outcomes from the application of plant growth-promoting rhizobacteria (PGPR) have been documented in various commercially important ornamental plants, showing beneficial effects on both rooting processes and agronomic characteristics. Research has explored the use of PGPR bacteria in ornamental plants belonging to various botanical families, including *Asteraceae* (e.g., chrysanthemum, aster, and zinnia) and *Geraniaceae* (e.g., geranium) (Göre and Altın, 2006), *Iridaceae* (iris) and *Oleaceae* (jasmine) (Damodaran et al., 2014), *Solanaceae* (petunia) (Hoda and Mona, 2014), *Crassulaceae* (kalanchoe) (Dalda-Sekerci and Ünlü, 2023) and *Poaceae* (turfgrass) (Okumus et al., 2024). The results of these studies are consistent with previous research, emphasizing the positive impact of PGPR applications on rooting across a variety of ornamental plants. For instance, earlier studies showed that certain strains of *Pseudomonas fluorescens* enhanced rooting in zinnia flowers (Yuen and Schroth, 1986). In firethorn cultivation, experiments combined indole-3-butyric acid with *Azospirillum brasilense* strains to promote early shoot rooting, encouraging rooting at an early developmental stage (Larraburu et al., 2007). Similarly, another study revealed that the presence of *Agrobacterium rubi* and *Serratia liquefaciens* significantly increased both fresh and dry root weight during the rooting process of hardwood cuttings derived from Forsythia intermedia plants (Kır, 2010). Sezen et al. (2014) examined the effects of *Agrobacterium rubi*, *Pseudomonas putida,* and *Bacillus subtilis* on the rooting process of *Ficus benjamina* L. cuttings and found that Bacillus subtilis exhibited the highest efficacy compared to the other bacterial species.

Similarly, Alkaç et al. (2022) investigated bacterial applications on aster flowers and observed varied effects; notably, the application of *Pseudomonas putida* (ZE-12) resulted in a 12% increase in germination compared to the control group, while *Acinetobacter calcoaceticus* (ZE-13) led to a significant 32.9% increase in seedling height. It is well-documented that *Bacillus* spp. promote plant growth by synthesizing plant growth regulators such as indole-3-acetic acid (IAA), gibberellins, and cytokinins. The biosynthesis of IAA in bacteria is crucial in regulating various aspects of plant growth and development, including differentiation of root vascular tissue, lateral root formation, and root gravitropism, thus playing a fundamental role in shaping plant root structure (Aloni et al., 2006). Furthermore, a study on *Rosa canina* reported the highest rooting rate with the application of *Bacillus megaterium* and *Pseudomonas fluorescens* bacteria (Kınık, 2014). In summary, the use of PGPR bacteria in agriculture is of great significance and continues to be a focus of research due to its potential benefits.

### **CONCLUSIONS**

In recent times, there has been a concerted effort among researchers to devise an integrated strategy aimed at mitigating the adverse impacts of synthetic chemicals utilized in agricultural practices. Within this framework, biostimulants have emerged as pivotal contributors, particularly within the realms of horticulture and ornamental plant cultivation. Additionally, biostimulants are progressively gaining traction for their role in bolstering tolerance to both biotic and abiotic stresses, as well as in enhancing sexual and asexual reproduction, seedling development, and overall yield. Among the noteworthy applications of biostimulants, treatments involving Plant Growth-Promoting Rhizobacteria (PGPR) offer manifold benefits, including conservation of soil and water resources, mitigation of environmental pollution arising from pesticides and chemical fertilizers, management of diseases and pests, augmentation of nutrient uptake by plants, and alleviation of biotic and abiotic stresses in plants. Recent studies have yielded significant findings demonstrating the capacity of PGPRs to stimulate root formation, increase plant height, expand leaf area, enhance shoot and root dry weights, modulate flowering time, augment flower and branch numbers, and extend flowering durations in ornamental plants.

This investigation, specifically, revealed that formulations containing bacterial strains from the *Bacillus* genus notably enhanced rooting in rose cuttings. Hence, it is anticipated that future research endeavors will further refine the efficacy of biostimulants and widen their adoption in commercial settings, thereby unveiling their substantial potential within the agricultural sector.

#### **Ethics Statement**

This study was produced from the TUBITAK 2209-A student project submitted by Kanykei KANATBEKOVA under the supervision of Dr. Akife DALDA ŞEKERCİ.

#### **Author Contributions**

Research Design Author 1 (%100)

Data Collection Author 1 (%40) Author 2 (%30) Author 3(%30)

Research- Data Analysis Author 1 (%20) Author 2(%40) Author 3(%40)

Writing the article Author 1 (%80) Author 3(%20)

Revisions and Improvement of the textAuthor 1 (%80) Author 3(%20)

#### **Finance**

We would like to thank TÜBİTAK for funding this study with project 2209-A and Erciyes University Scientific Research Projects Coordination Office (BAP) for providing support in the identification of Rhizobacteria with project no. FDK-2021-11088.

#### **Conflict of Interest**

No conflict of interest.

#### **Sustainable Development Goals (SDG)**

12 Responsible Production and Consumption

13 Climate Action

#### **REFERENCES**

- Ahkami, A. H., Lischewski, S., Haensch, K. T., Porfirova, S., Hofmann, J., Rolletschek, H., Hajirezaei, M. R. (2009). Molecular physiology of adventitious root formation in *Petunia hybrida* cuttings: involvement of wound response and primary metabolism. New Phytologist, 181(3), 613-625.
- Alkaç, O.S., Sabriye Belgüzar, S., Öndeş, E., Okatar, F., Kayaaslan, Z. (2022). Farklı kök bakterisi ve mikoriza uygulamalarının yıldız çiçeği (*Dahlia variabilis*) fidelerinin büyüme ve gelişimine etkileri. Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi 27 (2):331-339, 2022. DOI:10.37908/mkutbd.1092636
- Aloni, R., Aloni, E., Langhans, M., Ullrich, C. I. (2006). Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. Annals of botany, 97(5), 883-893.
- Antoun, H., and Prevost, D. (2006). Ecology of plant growth promoting printed in the Netherlands: PGPR: Biocontrol and biofertilization rhizobacteria, Ed.: Siddiqui, Z.A., Printed in the Netherlands pp: 1-38.
- Bloemberg, G. V., Lugtenberg, B. J. J.(2001). Molecular Basis of Plant Growth Promotion And Biocontrol By Rhizobacteria, Current Opinion in Plant Biology 4, 343–350.
- Dalda-Sekerci, A., Ünlü, E. (2023). The effect of plant growth promoting rhizobacteria (PGPR) applications on rooting and seedling quality of cuttings Kalanchoe (*Kalanchoe blossfeldiana*). Erciyes Tarım ve Hayvan Bilimleri Dergisi, 6(1), 73-78.
- Damodaran, T., Rai, R. B., Jha, S. K., Kannan, R., Pandey, B. K., Sah, V., Sharma, D. K. (2014). Rhizosphere and endophytic bacteria for induction of salt tolerance in gladiolus grown in sodic soils. Journal of plant interactions, 9(1), 577-584.
- Edwards, U., Rogall, T., Blöcker, H., Emde, M., & Böttger, E. C. (1989). Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic acids research, 17(19), 7843-7853.
- Esitken, A., Karlidag H., Ercisli S., Turan M. and Sahin F. (2003). The effect of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot (*Prunus armeniaca* L. cv. Hacihaliloglu). Aust. J. Agric. Res., 54: 377-380.
- Flores‐Félix, J.D., Menéndez, E., Rivera, L.P., Marcos‐García, M., Martínez‐Hidalgo, P., Mateos, P.F., Rivas, R., (2013). Use of Rhizobium leguminosarum as a potential biofertilizer for *Lactuca sativa* and *Daucus carota* crops, Journal of Plant Nutrition and Soil Science, 176(6), 876-882.
- García-Fraile P., Carro L., Robledo M., Ramírez-Bahena M.H., Flores-Félix J.D., Fernández M.T., Velázquez E. (2012). Rhizobium promotes non-legumes growth and quality in several production steps: towards a biofertilization of edible raw vegetables healthy for humans. PLoS One 7(5): e38122.
- Göre, M. E., Altin, N. (2006). Growth promoting of some ornamental plants by root treatment with specific fluorescent pseudomonads. J. Biol. Sci, 6(3), 610-615.
- Higa, T., Parr, J. F. (1994). Beneficial and effective microorganisms for a sustainable agriculture and environment (Vol. 1). Atami: International Nature Farming Research Center.
- Hoda, E. E., Mona, S. (2014). Effect of bio and chemical fertilizers on growth and flowering of *Petunia hybrida* plants. American journal of plant physiology, 9(2), 68-77.
- Karagöz, F.P., Dursun, A., Kotan, R., Ekinci, M., Yıldırım, E. and Mohammadi, P. (2016). Assessment

of the effects of some bacterial isolates and hormones on corm formation and some plant properties in saffron (*Crocus sativus* L.). Journal of Agricultural Science, 22: 500-511.

- Kınık, E. (2014). Bazı Odunsu Süs Bitkilerinin Çelikle Çoğaltılmaları Üzerine Oksin, Mikoriza ve Bakteri Uygulamalarının Etkileri. Ondokuz Mayıs Üniversitesi. Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, 87.
- Kır, Ö. (2010). Ekonomik öneme sahip bazı süs çalılarının köklendirilmesi üzerine hormonların ve bakterilerin etkileri. Fen Bilimleri Enstitüsü, Van.
- Kisvarga, S., Farkas, D., Boronkay, G., Neményi, A., & Orlóci, L. (2022). Effects of biostimulants in horticulture, with emphasis on ornamental plant production. Agronomy, 12(5), 1043.
- Larraburu, E. E., Carletti, S. M., Rodríguez Cáceres, E. A., Llorente, B. E. (2007). Micropropagation of photinia employing rhizobacteria to promote root development. Plant Cell Reports, 26, 711-717.
- Lugtenberg, B., Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. Annual review of microbiology, 63, 541-556.
- Mehmood, U., Inam-ul-Haq, M., Saeed, M., Altaf, A., Azam, F., Hayat, S. (2018). A brief review on plant growth promoting rhizobacteria (PGPR): a key role in plant growth promotion. Plant protection, 2(2), 77-82.
- Okumuş O., Gün B., Yılmaz S., Uzun S. (2024). The Effects of Bio-Priming on Seed Germination and Seedling Growth of Italian Ryegrass (*Lolium multiflorum* Lam.), Journal of Erciyes Agriculture and Animal Science, 7(2):111-114
- Ruzzi, M., Aroca, R. (2015). Plant growth-promoting rhizobacteria act as biostimulants in horticulture. Scientia Horticulturae, 196, 124-134.
- Sezen, I., Kaymak, H.Ç., Aytatlı, B., Dönmez, M.F. and Ercişli, S. (2014). Inoculations with Plant Growth Promoting Rhizobacterıa (PGPR) Stimulate Adventitious Root Formation On Semi-Hardwood Stem Cuttıngs of *Ficus benjamina* L. Propagation of Ornamental Plants, 14 (4),152- 157.
- Sharma S., Kaur M. (2010). Antimicrobial activities of rhizobacterial strains of *Pseudomonas* and *Bacillus* strains isolated from rhizosphere soil of carnation (*Dianthus caryophyllus* cv. Sunrise). Indian J. Microbiol. 50(2): 229-232.
- Sıddıqui, Z.A. (2006). Prospective Biocontrol Agents of Plant Pathogens. PGPR: Biocontrol and Biofertlization. Edited by Zaki A. Sıddıqui. S 111- 142., Springer, The Netherlands.
- Srivastava R., Govil M. (2007) Influence of biofertilizers on growth and flowering in *Gladiolus* cv. American beauty. Acta Hortic. 742(742): 183-188.
- Şevik M.A. (2010). Bitki virüs hastalıklarına karşı kullanılan bitki gelişimini teşvik eden rhizobakteriler (PGPR). Elektronik Mikrobiyoloji Dergisi, 08: 31-43.
- Upadhyay, S. K., Singh, D. P., & Saikia, R. (2009). Genetic diversity of plant growth promoting rhizobacteria isolated from rhizospheric soil of wheat under saline condition. Current Microbiology, 59, 489-496.
- Ünlü, E., Şekerci, A. D., Yılmaz, S., Yetişir, H. (2023). Fıeld trıal of PGPR, *Bacillus megaterium* E-U2- 1, on some vegetable species. Journal of Applied Biological Sciences, 17(1), 125-137.
- Wilson, K. (2001). Preparation of genomic DNA from bacteria. Current protocols in molecular biology, 56(1), 2-4.
- Yılmaz S. (2010). Çeşitli Habitatlarda izole edilen *Bacillus thuringiensis* suşlarının moleküler karakterizasyonu ve bazı zararlı böceklere karşı mücadelede kullanımı. Doktora Tezi, Erciyes Üniversitesi Fen Bilimleri Enstitüsü, Kayseri ,144
- Yuen, G. Y., Schroth, M. N. (1986). Interactions of *Pseudomonas fluorescens*strain E 6 with ornamental plants and its effect on the composition of root-colonizing microflora. Phytopathology, 76(2), 176-180.
- Zhang, J., Esselink, G. D., Che, D., Fougère-Danezan, M., Arens, P., & Smulders, M. J. M. (2013). The diploid origins of allopolyploid rose species studied using single nucleotide polymorphism haplotypes flanking a microsatellite repeat. The Journal of Horticultural Science and Biotechnology, 88(1), 85-92.
- Zulueta-Rodriguez R., Cordoba-Matson M.V., Hernandez-Montiel L.G., Murillo-Amador B., Rueda-Puente E., Lara L. (2014) Effect of *Pseudomonas putida* on growth and anthocyanin pigment in two poinsettias (*Euphorbia pulcherrima*) cultivars. Sci. World J. 2014: 810192.