



Check for
updates



Research Article

Impact of Rhizobacterial Strains on Soil Physicochemical Properties and Wheat (*Triticum Aestivum* L.) Productivity

Rashid Khan¹, Yasar Saleem Ahmed Khan², Tahir Yab Khan³, Sania Ejaz⁴,
Mohammad Ashfaq⁵, Arbaz Hassan^{6*}, Hafiz Munsif Alam⁷, Tariq Ahmad⁸,
Munaza Iram⁹, Hina Javed¹⁰

¹ Department of Agronomy, Gomal University, Dera Ismail Khan, Pakistan.

² Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.

³ Department of Horticulture, Ghazi University Dera Ghazi Khan, Pakistan.

⁴ Department of Phytopathology, Federal University of Lavras, Brazil.

⁵ Department of Soil Science, The Islamia University of Bahawalpur, Pakistan.

⁶ Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan.

⁷ Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan.

⁸ Department of Plant Breeding and Genetics, The University of Agriculture Peshawar Pakistan.

⁹ Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

¹⁰ Soil Chemistry Section, Ayub Agricultural Research Institute, Faisalabad, Pakistan.

ABSTRACT

A field experiment was carried out at the Arid Zone Research Centre, Dera Ismail Khan, in the 2017–18 growing season to evaluate this influence regarding the application of rhizobacterial inoculants. Concerning soil properties, root and shoot structure, and wheat productivity. The study followed a randomized complete block design (RCBD) was implemented with five treatments, including a control and four bacterial inoculants: *Enterobacter asburiae*, *Enterobacter mori*, *Pseudomonas aeruginosa*, and *Pseudomonas putida*. These microbial strains were isolated from the rhizosphere of lentil plants. Crops, identified, as well as used for seed application of microbial inoculants. Various soil, development and productivity metrics were analyzed. These findings revealed that beneficial rhizobacteria (PGPR) had a notable impact on plant height as well as leaf length. Traits such as fresh and dry root weight, root surface area, root extent and root length concentration were notably affected as a result of the applied treatments. Among them, *Enterobacter mori* (T₂) led to the highest fresh and dry root weight and root area, while *Pseudomonas putida* (T₄) enhanced root length and root length density. Yield-related parameters, including thousand-grain weight, grain production, and straw output, were also substantially impacted. The maximum grain and straw productivity were recorded in the treatment with *Enterobacter asburiae* (T₁), whereas *Pseudomonas aeruginosa* (T₃) resulted in the maximum 1000-grain weight. Soil characteristics, including organic matter composition and overall nitrogen levels, and extractable phosphorus showed significant improvement in treatments inoculated with *Pseudomonas putida* (T₄) and *Enterobacter mori* (T₂), respectively. Overall, the results suggest that inoculation with *Pseudomonas putida*, *Enterobacter asburiae*, *Enterobacter mori*, and *Pseudomonas aeruginosa* improves soil nutrient content while enhancing wheat growth and productivity.

Keywords: Rhizobacteria, PGPR, Wheat, Biofertilizer.

INTRODUCTION

Rhizobacteria, naturally inhabiting the plant root zone, play a vital role in nutrient cycling by breaking down soil-bound essential elements, thereby enhancing their bioavailability to plants (Qin et al., 2024). While microbial communities in the rhizosphere have long been recognized, recent research has underscored their critical role in plant development and productivity (Zhuang et al., 2024). Among



Correspondence

Arbaz Hassan

hassanarbaz40@gmail.com

Article History

Received: December 09, 2024

Accepted: February 28, 2025

Published: March 17, 2025



Copyright: © 2024 by the authors.

Licensee: Roots Press, Rawalpindi, Pakistan.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license: <https://creativecommons.org/licenses/by/4.0>

these, Plant Growth-Promoting Rhizobacteria (PGPR) have attracted significant attention due to their ability to enhance crop growth through diverse mechanisms. However, their field performance remains inconsistent across varying soil types and climatic conditions, limiting their large-scale adoption. This study aims to address this gap by evaluating the effectiveness of PGPR under different environmental settings. These bacteria not only increase nutrient bioavailability but also interact synergistically with other beneficial soil microbes. For instance, phosphate-solubilizing bacteria have been reported to enhance the effects of arbuscular mycorrhizal fungi in wheat, leading to a 92.8% increase in grain yield (Saxena et al., 2013). Additionally, PGPR improve crop health by suppressing pathogens, producing bioactive compounds, and stimulating plant growth through phytohormone synthesis (Ahemad and Kibret, 2014). Their role extends beyond crop yield enhancement, as they also contribute to soil fertility restoration and function as biological control agents against plant pathogens (Glick, 2012).

Ongoing research focuses on optimizing PGPR strains for higher efficiency and adaptability across different soil conditions (Wani and Khan, 2010). Biological nitrogen fixation (BNF) remains a major contributor to natural nitrogen cycling, accounting for two-thirds of nitrogen fixation globally, whereas industrial processes like Haber-Bosch support synthetic nitrogen conversion (Rubio and Ludden, 2008). Various bacterial genera play a key role in nutrient solubilization, soil structure enhancement, and stress tolerance, further improving overall plant resilience (Khan et al., 2009). Additionally, certain PGPR species, such as *Pseudomonas*, exhibit resistance to environmental stressors, including extreme temperatures, detergents, and antibiotics, allowing them to thrive in diverse conditions (Raja et al., 2006). While synthetic fertilizers have been widely used to boost soil fertility, their adverse effects on soil health and surrounding ecosystems have raised environmental concerns. PGPR inoculation presents a sustainable and eco-friendly alternative, as it enhances soil productivity and plant growth by facilitating nutrient mobilization and improving plant stress tolerance (Dobbelaere et al., 2003). Various microbial species residing in the rhizosphere contribute to plant growth through multiple biochemical pathways, collectively termed as PGPR (Vessey, 2003).

Wheat (*Triticum aestivum*), a globally cultivated cereal, serves as a staple food source in many regions, including Pakistan. It is a primary supplier of carbohydrates and protein (Ali and Khan, 2014). However, Pakistan's wheat yield remains significantly lower than its potential, with countries like Germany achieving much higher production levels (5960–8088 kg/ha), whereas Pakistan struggles to match these figures (Byerlee and Siddiq, 1994). Studies have shown that certain PGPR strains enhance wheat grain yield and improve its protein content (Saubidet et al., 2002). The effectiveness of PGPR in wheat production stems from their ability to solubilize phosphate, regulate quorum sensing (QS), produce phytohormones, and exhibit antifungal properties. Since chemical fertilizers negatively impact soil and biodiversity, PGPR have emerged as a sustainable alternative to conventional fertilizers (Bhattacharyya, 2012). Despite their numerous benefits, variability in PGPR effectiveness under different environmental conditions has restricted their large-scale adoption (Vessey, 2003). Therefore, this study investigates the impact of various PGPR strains on wheat growth, yield improvement, and soil enhancement, aiming to provide insights into their practical application in sustainable agriculture.

Objectives

This study was conducted with the following objectives:

To isolate and characterize different strains of rhizobacteria obtained from lentil crops for seed inoculation.

To evaluate the effects of various Plant Growth-Promoting Rhizobacteria (PGPR) inoculants on wheat growth and yield performance.

To analyze the impact of PGPR application on soil physicochemical properties.

MATERIALS AND METHODS

Research Site

A controlled field experiment was conducted during the winter season of 2017 at the Arid Zone Research Centre, Dera Ismail Khan, to evaluate the response of wheat to different rhizobacterial strains. The study was structured using a Randomized Complete Block Design (RCBD) with five distinct treatments. Punjab-11 wheat cultivar was selected, and nitrogen (120 kg/ha) and phosphorus (90 kg/ha) were uniformly applied across all plots.

Preparation of Rhizobacterial Cultures

Rhizobacterial strains were isolated from the rhizosphere of harvested lentil fields. These isolates were first cultivated on general-purpose media and subsequently transferred to Yeast Mannitol Agar (YMA) medium for further development. Strain identification was performed by EzBiCloud, a Korean microbial identification platform, before seed inoculation in a broth medium.

Treatment Details and Layout Plan

The experiment consisted of the following treatments:

T₀: Control (Without inoculation)

T1: *Enterobacter asburiae* application

T2: *Enterobacter mori* treatment

T3: *Pseudomonas aeruginosa* application

T4: *Pseudomonas putida* treatment application

Key Growth Parameters of Wheat

Shoot Parameters

Leaf Length (cm)

Measured randomly from selected plants using a ruler.

Leaf Width (cm)

Recorded at the midpoint of the leaf using a measuring scale.

Plant Height (cm)

The tallest plant from each treatment was selected and measured using a scale.

Foliage Surface Area (cm²)

Computed using the following equation: Leaf area of the wheat plant was calculated using its length and width, multiplied by a factor of 0.95 (Thomas, 1975).

Number of Tillers

Counted per square meter across all treatment plots.

Chlorophyll Content (mg/m²)

Measured using a SPAD meter from 4–5 randomly selected leaves per plot.

Root Parameters

Root morphology was analyzed using Root Snap software (version 1.3.2.25), focusing on:

Root length (cm)

Root diameter (mm)

Root area (cm²)

Root length density (cm³)

Root volume (cm³)

Yield Metrics

Grain production (tons/ha)

Straw output (tons/ha)

1000-grain weight (g)

Soil Analysis

Soil Sample Collection and Processing

Post-harvest Soil specimens were obtained from each plot at a depth of 0–15 cm, then air-dried, pulverized, and filtered through a 2 mm sieve. before labeling.

Soil acidity (pH) and Electrical Conductivity (EC) Measurement

pH: Soil pH was determined using a calibrated pH meter, standardized with buffer solutions (pH 4.00, 7.00, and 10.00) to ensure accuracy.

EC: Electrical conductivity (EC) was measured using a conductivity meter calibrated with a 1416 $\mu\text{S}/\text{cm}$ standard solution.

Soil Nutrient Analysis

Nitrogen Content: Quantified through Kjeldahl digestion, distillation, and titration.

Extractable Phosphorus: Measured using Olsen's method (Olsen et al., 1954).

Soluble Potassium and Sodium: Assessed via flame photometry for precise quantification.

Extractable Calcium and Magnesium: Determined through ammonium acetate extraction and EDTA titration.

Statistical Analysis

Data analysis was conducted using Statistix 9.1 software. Treatment differences were evaluated through Analysis of Variance (ANOVA), while mean comparisons were performed using the Least Significant Difference (LSD) test (Steel et al., 1997).

RESULTS AND DISCUSSION

Shoot Structure of Wheat Influenced by Plant Growth-Promoting Rhizobacteria (PGPR) Application

Plant Height (cm)

Rhizobacterial inoculation had a significant impact on plant height. The tallest plants (58 cm) were observed in the control group (T₀), followed closely by T₁ (*Enterobacter asburiae*) and T₃ (*Pseudomonas aeruginosa*) (Table 1). The shortest plants were recorded in T₂ (*Enterobacter mori*). Similar findings have been reported in maize, where PGPR inoculation improved plant height (Noumavo et al., 2013).

Number of Tillers Per Plant

PGPR application had no statistically significant effect on tiller count (Table 1). However, previous studies suggest that *Pseudomonas fluorescens* biotypes enhance tiller production in wheat (Shaharoon et al., 2008).

Leaf Length (cm)

The longest leaves were recorded in the *Pseudomonas aeruginosa* (T₃) treatment, which was statistically similar to other treatments except T₂ (*Enterobacter mori*) (Table 1). PGPR-mediated elongation of leaf structure has been observed in wheat and other crops (Burd et al., 2000).

Chlorophyll Content ($\mu\text{g}/\text{cm}^3$)

variations among treatments were not statistically significant, though the control group exhibited the highest concentration (Table 1). Vafadar et al. (2014) reported similar synergistic effects of *Bacillus polymyxa*, *Pseudomonas putida*, and *Azotobacter chroococcum* on chlorophyll levels in various crops.

Table 1. Effect of Rhizobacteria on growth parameters of wheat (*Triticum Aestivum*).

Treatment	Plant Height (cm)	Leaf Length (cm)	Leaf Area (cm ²)	No. Tillers	Chlorophyll Content (mg/m ²)
T ₀	58	16.3	25.4	4.8	39.2
T ₁	57.2	17.1	26.1	5	38.7
T ₂	55.6	15.7	24.7	4.9	37.5
T ₃	57.5	18.4	27.3	5.2	40.1
T ₄	56.9	17.9	26.8	5.1	39.5

Dry and Fresh Root Biomass (g)

The highest dry root biomass (8.88 g) and fresh root weight (24 g) were observed in *Enterobacter mori* (T₂), whereas *Enterobacter asburiae* (T₁) exhibited the lowest values (5.03 g). These findings are consistent with Abbasi et al. (2011), who reported similar results in wheat

Root Length Density (cm³)

The greatest root length density (7.22 cm³) was recorded in *Pseudomonas putida* (T₄), which was statistically similar to *Pseudomonas aeruginosa* (T₃) and *Enterobacter mori* (T₂). These results align with those of Belimov et al. (2005), who demonstrated that PGPR inoculation enhances root length density.

Root Mass and Thickness

Root volume did not show significant variation across treatments. However, *Pseudomonas putida* (T₄) produced the largest root diameter (6.00 mm). This finding is consistent with Ekinici et al. (2014), who observed a PGPR-induced increase in root diameter in cauliflower (Table 2).

Yield and Soil Properties

Grain Production and 1000-Kernel Weight: Wheat yield parameters showed a positive response to PGPR treatments (Table 3). The highest grain yield (4200 kg/ha) was recorded in *Enterobacter asburiae* (T₁), whereas *Pseudomonas aeruginosa* (T₃) resulted in the heaviest grains (50 g). These results align with those of Yazdani et al. (2013), who reported similar effects of PGPR on maize productivity. Additionally, Belimov et al. (2005) documented comparable findings regarding PGPR-mediated yield improvements.

Table 2. Effect of Rhizobacteria on roots growth parameters of wheat (*Triticum Aestivum*).

Treatment	Root Length (cm)	Root Diameter (mm)	Root Area (cm ²)	Root Volume (cm ³)	Root Length Density (cm/cm ³)	Root Mass Density (g/cm ³)
Control (T ₀)	26.67	4.73	125.5	5.23	4.78	0.98
T1 (<i>Enterobacter asburiae</i>)	28.45	5.12	132.1	5.64	5.75	1.12
T2 (<i>Enterobacter mori</i>)	27.89	4.95	130.3	6.77	6.49	1.23
T3 (<i>Pseudomonas aeruginosa</i>)	30.12	5.34	135.7	6.12	6.78	1.34
T4 (<i>Pseudomonas putida</i>)	32.04	6.00	140.2	6.89	7.22	1.50

Table 3. Effect of Rhizobacterial Inoculants on Yield Parameters.

Treatment	Grain Yield (kg/ha)	Straw Yield kg(kg/ha)	1000-Grain Weight(g)
T ^o Control	2500	4800	35.0
T1 <i>Enterobacter asburiae</i>	4200	5400	49.7
T2 <i>Enterobacter mori</i>	4000	5200	45.0
T3 <i>Pseudomonas aeruginosa</i>	3900	5000	50.0
T4 <i>Pseudomonas putida</i>	4100	5300	48.5

Soil Nutrient Analysis

Post-harvest phosphorus, nitrogen, and organic matter levels were significantly improved in Plant Growth-Promoting Rhizobacteria (PGPR)-treated plots, particularly *Pseudomonas putida* (T4) (Table 4).

Table 4. Effect of Rhizobacterial Inoculants on Soil Physicochemical Properties.

Treatment	Soil pH	EC (ds/m)	Organic Matter (%)	Total Nitrogen (%)	Available P (mg/kg)	Available K (mg/kg)	Soluble Na (mg/kg)
Control (T ₀)	7.85	1.25	0.89	0.12	7.5	95.4	120.3
T1 (<i>Enterobacter asburiae</i>)	7.80	1.18	0.92	0.14	8.2	98.7	122.1
T2 (<i>Enterobacter mori</i>)	7.75	1.22	0.95	0.16	9.1	101.2	125.4
T3 (<i>Pseudomonas aeruginosa</i>)	7.78	1.20	0.94	0.15	8.7	101.1	124.0
T4 (<i>Pseudomonas putida</i>)	7.72	1.15	0.98	0.17	9.5	103.4	127.3

CONCLUSIONS

This study confirms that PGPR inoculation significantly enhances wheat growth, root development, grain yield, and soil fertility. The results highlight that PGPR serve as a sustainable alternative to chemical fertilizers, reducing synthetic nutrient dependency while promoting environmental sustainability. However, the field performance of PGPR remains influenced by soil type, microbial competition, and environmental conditions. Future research should focus on: Optimizing multi-strain PGPR formulations for improved microbial stability.

Conducting long-term studies on PGPR interactions with native soil microbiota.

Evaluating the economic feasibility of PGPR adoption in large-scale wheat production.

ACKNOWLEDGEMENTS

Not applicable.

AUTHOR CONTRIBUTIONS

All the authors contributed equally to this research.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

REFERENCES

- Abbasi, M.K., Sharif, S., Kazmi, M., et al 2011. Isolation of plant growth-promoting rhizobacteria from the wheat rhizosphere and their effect on improving growth, yield, and nutrient uptake of plants. *Plant Biosyst.* 145: 159–168.
- Ahemad, M., Kibret, M. 2014. Mechanisms and applications of plant growth-promoting rhizobacteria: Current perspective. *J. King Saud Univ. Sci.* 26: 1–20.
- Ali, S., Khan, M. 2014. Technical efficiency of wheat production in district Peshawar, Khyber Pakhtunkhwa, Pakistan. *Sarhad J. Agric.* 30: 433–441.
- Andrews, J.H., Harris, R.F. 2000. The ecology and biogeography of microorganisms on plant surfaces. *Annu. Rev. Phytopathol.* 38: 145–180.
- Belimov, A.A., Hontzeas, N., Safronova, V.I., et al 2005. Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). *Soil Biol. Biochem.* 37: 241–250.
- Bhattacharyya, P.N., Jha, D.K. 2012. Plant growth-promoting rhizobacteria (Plant Growth-Promoting Rhizobacteria (PGPR)) emergence in agriculture. *World J. Microbiol. Biotechnol.* 28: 1327–1350.
- Burd, G.I., Dixon, D.G., Glick, B.R. 2000. Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. *Can. J. Microbiol.* 46: 237–245.
- Byerlee, D., Siddiq, A. 1994. Has the green revolution been sustained? The quantitative impact of the seed-nutrient supplement revolution in Pakistan. *Food Policy.* 19: 381–395.
- Dobbelaere, S., Vanderheyden, J., Okon, Y. 2003. Plant growth-promoting effects of diazotrophs in the rhizosphere. *Crit. Rev. Plant Sci.* 22: 107–149.
- Ekinci, M., Turan, M., Yildirim, E., et al 2014. Effect of plant growth-promoting rhizobacteria on growth, nutrient, organic acid, amino acid, and hormone content of cauliflower (*Brassica oleracea* L. var. botrytis) transplants. *Acta Sci. Pol. Hortorum Cultus.* 13: 71–85.
- Glick, B.R. 2012. Plant growth-promoting bacteria: Mechanisms and applications. *Sci. World J.* 2012: 1–15.
- Gray, E.J., Smith, D.L. 2005. Intracellular and extracellular Plant Growth-Promoting Rhizobacteria (PGPR): Commonalities and distinctions in the plant-bacterium signaling processes. *Soil Biol. Biochem.* 37: 395–399.
- Hynes, R.K., Leung, G.C., Hirkala, D.L., et al 2008. Isolation, selection, and characterization of beneficial rhizobacteria from pea, lentil, and chickpea grown in western Canada. *Can. J. Microbiol.* 54: 248–258.
- Khan, M.S., Zaidi, A., Wani, P.A., et al 2009. Role of plant growth-promoting rhizobacteria in the remediation of metal-contaminated soils. *Environ. Chem. Lett.* 7: 1–19.
- Kloepper, J.W., Lifshitz, R., Zablutowicz, R.M. 1989. Free-living bacterial inoculants for enhancing crop productivity. *Trends Biotechnol.* 7: 39–44.
- Noumavo, P.A., Kochoni, E., Didagbé, Y.O., et al 2013. Effect of different plant growth-promoting rhizobacteria on maize seed germination and seedling development. *Am. J. Plant Sci.* 4: 1013–1021.
- Qin, H., Wang, Z., Sha, W., et al 2024. Role of plant growth-promoting rhizobacteria in plant machinery for soil heavy metal detoxification. *Microorganisms.* 12: 700.
- Raja, C.E., Anbazhagan, K., Selvam, G.S. 2006. Isolation and characterization of a metal-resistant *Pseudomonas aeruginosa* strain. *World J. Microbiol. Biotechnol.* 22: 577–585.
- Rubio, L.M., Ludden, P.W. 2008. Biosynthesis of the iron-molybdenum cofactor of nitrogenase. *Annu. Rev. Microbiol.* 62: 93–111.
- Saubidet, M.I., Fatta, N., Barneix, A.J. 2002. The effect of inoculation with *Azospirillum brasilense* on growth and nitrogen utilization by wheat plants. *Plant Soil.* 245: 215–222.
- Saxena, J., Chandra, S., Nain, L. 2013. Synergistic effect of phosphate-solubilizing rhizobacteria and arbuscular mycorrhiza on growth and yield of wheat plants. *J. Soil Sci. Plant Nutr.* 13: 511–525.
- Shaharoona, B., Naveed, M., Arshad, M., et al 2008. Fertilizer-dependent efficiency of *Pseudomonads* for improving growth, yield, and nutrient use efficiency of wheat (*Triticum aestivum* L.). *Appl. Microbiol. Biotechnol.* 79: 147–155.
- Vafadar, F., Amooaghaie, R., Otrushy, M. 2014. Effects of plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungus on plant growth, stevioside, NPK, and chlorophyll content of *Stevia rebaudiana*. *J. Plant Interact.* 9: 128–136.
- Vessey, K.V. 2003. Plant growth-promoting rhizobacteria as bionutrient supplements. *Plant Soil.* 255: 571–586.
- Wani, P.A., Khan, M.S. 2010. *Bacillus* species enhance growth parameters of chickpea. *Environ. Biol.* 31: 647–653.
- Yazdani, M. 2013. Post-anthesis accumulation and remobilization of dry matter in corn (*Zea mays* L.) as affected by bionutrient supplements. *Int. J. Agron. Plant Prod.* 4: 295–303.
- Zhuang, Y., Wang, H., Tan, F., et al 2024. Rhizosphere metabolic cross-talk from plant-soil-microbe tapping into agricultural sustainability: Current advances and perspectives. *Plant Physiol. Biochem.* 205: 108619.