J. Micro. Sci. Vol. 4(1), 21-29, **2025,**
https://doi.org/10.38211/jms.2025.04.76

OPEN ACCESS

Research Article

Available on: Innovative Science and Technology Publishers
www.isciencepress.com
Journal of Microbiological Sciences
(JMS)
ISSN 2959-975X (Online); ISSN 3079-0980 (Print)

Growth and Yield Enhancement in Wheat (*Triticum aestivum* L.) Through Rhizobacterial Biopriming and Variable Phosphorus Applications

Saibrina Sethar[1,2*], Saeed Akhter Malik[2,4], Zia-Ul-Hassan Shah[2], Nizamuddin Depar[3], Mahendar Kumar Sootahar[1], Muhammad Aslam Panhwar[1]

[1]Soil Fertility Research Institute Tandojam, Agriculture Research Center, Tandojam
[2]Department of Soil Science, Sindh Agriculture University Tandojam
[3]Soil & Environmental Sciences Division, Nuclear Institute of Agriculture Tandojam
[4]Agriculure Extension Wing Agriculture Supply and Prices Department
*Corresponding author: Saibrina Sethar; saibnoor 90@gmail.com

Article Received 14-11-2024, Article Revised 24-12-2024, Article Accepted 15-01-2025

ABSTRACT

Plant-microbe relations in the rhizosphere play a vital role in enhancing soil fertility and improving crop output. Phosphorus (P) is one of the most vital nutrient for plants, yet its availability for plant in various types of soil is inadequate due to fixation. Plant growth promoting rhizobacterial (PGPR) inoculation, especially containing ACC-deaminase and phosphate-solubilizing activities, has emerged as a promising approach to mitigate phosphorus deficiency and enhance crop growth. This study investigates the consequence of PGPR having single activity (ACC-deaminase only), and PGPR having dual activity (ACC-deaminase and phosphate-solubilizing) , on wheat growth with three phosphorus levels(P_1= 00 kg ha^{-1}, P_2= 45 kg ha^{-1}, and P_3= 90kg ha^{-1} P_2O_5). The experiment was conducted in a randomized complete block split-plot design using three rhizobacterial treatments combined with phosphorus doses. The treatments were $RB_{1=}$no inoculation (control), RB_2 = inoculated with *Pseudomonas fluorescens* having single activity of ACC-deaminase, and $RB_{3=}$ inoculated with *Pseudomonas fluorescens having dual ability (*ACC-deaminase & phosphate-solubilizing). Results demonstrated that the best treatment (90 kg ha^{-1} with Rhizobacterial inoculation having dual ability) significantly improved biological yield by 62.86%, grain yield by 87.72% and straw yield by 46.34% over the control and increased biological yield by 8.53%, grain yield by 12.41% and Straw yield by 5.10% was noted as compared to other (45 kg ha-^{1} with $RB_{3)}$,.Specifically plant height, Spike plant^{-1},Spikelet spike^{-1}, 1000 grain weight, Harvest index, Grain straw ratio, Total P concentration, Grain P concentration and straw P concentration were also enhanced to a significant levels, showing an increase of 3.17% to329.91% over control and 0.86 % to 50.08% compared to 45 kg ha-^{1} with RB_3. We conclude that the PGPR with dual activity inoculation was found to be more efficient over PGPR with single activity under P deficient and adequate conditions.

Keywords: Wheat, Phosphorus, rhizobacteria inoculation, *Pseudomonas fluorescens*, ACC-deaminase, PSB.

INTRODUCTION

Wheat (*Triticum aestivum L.)* world's imperative food crop, is consumed about 30% of the world population as a major food (Rashwan *et al.*, 2019).In Pakistan, wheat serves as a staple food crop and is cultivated annually on more than 9.0 million hectares(Shah *et al.*, 2024).The essential nutrients for wheat cultivation include macronutrients such as phosphorus (P),nitrogen (N), and potassium (K). Nitrogen is particularly crucial for promoting vigor, vegetative progress, and floral growth(Kumar *et al.*, 2017). While P is the second major nutrient required after Nitrogen for better crop growth especially for Wheat crop (Afzal & Bano, 2008). Pakistani soils are approximately 90% N deficient, and <10mg kg^{-1} P available, including Olsen P (0.02%) and total P (0.5%) in the fertile layer (Vishandas *et al.*, 2006; Zia-ul-hassan *et al.*, 2024). It consumes more than 50% of P fertilizers used annually (GoP, 2021; Sial, *et al.*, 2018). Furthermore, more than 90% of the total P is fixed by calcium, which results in low P in the soil solution and causes the soluble P fertilizers to precipitate or get fixed, particularly in alkaline-calcareous soils(Ibrahim *et al.*, 2022). In Pakistan P efficient fertilization becomes indispensable for crop production (Zia-ul-hassan *et al.*, 2024). Similarly, (Balemi & Negisho, 2012) noticed that by the end of 2050 reservoir of P may be depleted from the world. However, in Pakistan the hype in price of fertilizers that is not approachable to farmers(Zia-ul-hassan *et al.*, 2024). It was reported that the P efficiency is also reducing up <25% of crop production. Hence, its availability in plants become very serious issue, which contribute about 30-40% loss if P become deficient (Kaya *et al.*, 2020). In nutrients deficient soils, microorganisms play a vital role in nutrients availability to plants. One of the useful group of bacteria called Plant Growth Promoting Rhizobacteria (PGPR)shows potential approach to enhance nutrient availability and promote plant growth through various mechanisms (Etesami & Glick, 2023).Among these, bacteria that possess ACC (1-aminocyclopropane-1-carboxylic acid) deaminase phosphate-solubilizing bacteria have gained considerable attention in the research field. ACC deaminase phosphate solubilizing bacteria solubilized P which is unavailable to plants (Pii *et al.,* 2015). Ethylene is a hormone that is produced under stress conditions and inhibit plant growth and rhizobacteria have the ability to lower this ethylene level through deaminazing ACC (removing ammonia group) which is precursor of ethylene production(Glick & Nascimento, 2021). By lowering ethylene levels, ACC-deaminase can alleviate stress and promote root elongation, which enhances nutrient uptake (Ahmed *et al.,* 2019;Zia-ul-hassan *et al.,* 2024). Phosphate solubilizing bacteria (PSB) along with ACC-deaminase activity boosted the yields of many cereals crops (Ro & Mes, 2015), including wheat (Sial*et al.,* 2018; Zia-ul-hassan *et al.,*2024). Inoculation with essential rhizobacteria increasing the development of inoculated plants progress by declining ethylene and allowing plants to established strong and compact root system (Stearns *et al.,* 2012).In addition, PSB can convert insoluble form of phosphorus in the soil into plant-available forms through the production of organic acids and phosphatases (Gupta *et al.,* 2023; Khan *et al.,* 2022). The combined activity of ACC-deaminase and phosphate solubilization offers a synergistic effect, potentially providing a dual benefit for plants growing under phosphorus-limited conditions (Etesami & Glick, 2023). Several studies have shown that inoculation with such multifunctional bacteria can significantly improve plant growth, yield and nutrient uptake, especially under nutrient-stress conditions (Bashir *et al.,* 2021; Glick & Gamalero, 2021; Glick & Nascimento, 2021). However, there is still a need for more research to explore the full potential of these bacteria in different crops and under varying environmental conditions. The current study is to assess the impact of *Pseudomonas fluorescens*, which produces ACC-deaminase, on wheat development and yield under varying phosphorus levels in low phosphorus circumstances, both with and without phosphate-solubilizing activity.

MATERIAL AND METHODS

Experimental Soils: The field experimentation was conducted Nuclear Institute of Agriculture (NIA) experimental site (25.4216° N, 68.5415° E), located at Tandojam, Sindh, Pakistan. The climatic zone is subtropical. The physio-chemical characteristics (Table 1) of the soil samples taken from the experimental region were examined using the conventional procedures of Ryan et al. (2001). In addition to being non-saline and slightly alkaline, the experimental soil had low levels of organic matter (0.69%), nitrogen (0.23%), and accessible P (<4 mg kg-1).

The wheat SAU-1genotype was selected for the study. Before the experiment land was prepared and wheat seed priming with PGPR was done before drilling. There was 25×25 cm row to row distance and drilling was performed manually. The first irrigation was given after 25 days of sowing and other irrigations were applied as per requirement of the crop and Cultural practices done throughout growing season

Table 1. Soil Physio-chemical properties

Parameters	Value	Method used
Electrical Conductivity (dS/m)	0.90	Conductivity meter (Richards, 1954)
pH	7.6	pH meter (Jackson, 1973)
Soil Organic Matter (%)	0.69	Walkley & Black, 1934
Nitrogen (%)	0.23	Kjeldahl method (Bremner, 1965)
Available Phosphorus (mgkg^{-1})	1.25	Olsen method (Olsen et al., 1954)
Soil Texture	Clay Loam	Hydrometer method (Bouyoucos, 1962)

Experimental treatments and Design: The research was carried out with three replications utilizing a two-factor randomized complete block split-plot design. Phosphorus (P) was presented in three doses in Factor 1: P_1 (control, no P fertilizer), P_2 (45.0 kg P ha^{-1}, 50% of the recommended dose), and P_3 (90.0 kg P ha^{-1}, 100% of the recommended dose). The three rhizobacterial treatments, Factor 2 were RB_1 (control, no microbial inoculation), RB_2 (*Pseudomonas fluorescens* with ACC-deaminase activity), and RB_3 (*Pseudomonas fluorescens* with both ACC-deaminase and phosphate-solubilizing action). Each plot in the experiment was 3 m × 6 m (18 m²). Pre-isolated strains of *Pseudomonas fluorescence* were used for seed priming. Soil application of Triple Super Phosphate (TSP) fertilizer (51% P_2O_5) was used as a phosphorus supplement. The recommended N and K doses were applied to the wheat crop, with half of the nitrogen (N), along with phosphorus (P) and potassium (K), incorporated as a basal application. The remaining nitrogen was supplied in stages during different irrigation periods.

Preparation of bacterial inoculum and seed priming: Based on a variety of characteristics that promote plant development, including nitrogen fixation, nutrient solubilization, plant Harmon production, antibiotic synthesis, and ACC deaminase production, the phosphate solubilizing rhizobacterial strain and ACC-deaminase rhizobacteria were selected. Selected rhizobacteria inoculum of 10 mL was mixed with 50 g peat and 50 g muck soil. The mixture was incubated at 28 ± 1°C for 24 hours before being used for seed coating, maintaining a 1:1 (w/w) ratio of muck to peat soil, as described by (Shaharoona et al., 2008).

Wheat growth Yield: Randomly 10 plants from each experimental unit were selected to record the parameters i.e.growth parameters, plant height (cm), spike plant^{-1}, spikelet spike^{-1}, and yield parameters, 1000-grain weight (g), grain yield, straw yield (kg ha^{-1}) and Harvest index= (grain yield/ biological yield) x 100 were recorded.

Quality Parameters: Phosphorus in grain and straw of mature wheat plants, obtained from each experimental unit, was determined following the method of single acid digestion method (Westermen1990; Zia *et al.*, 2024).

Statistical Analysis: Analysis of variance (ANOVA) and mean separation were performed on the data using SPSS (version 24.0). Tukey's Honestly Significant Difference (HSD) test was used to compare treatment means at a significance threshold of α = 0.05 in a two-factor randomized complete block split-plot design for ANOVA.

RESULTS

Rhizobacteria biopriming and phosphorus doses effect on Wheat growth parameters: The wheat field experiment was conducted at NIA field experimental, and data was recorded during the cropping period. The growth parameters, plant height (cm), spike plant^{-1} and spikelet spike^{-1}were varied among the various treatments presented in Table 2. The results demonstrated that the different P application doses of 45 and 90 kg ha^{-1}(50% and 100% recommended P) along with seed inoculated with rhizobacterial strains significantly enhanced the wheat growth parameters. Moreover, the maximum plant height (105.9cm), spike plant^{-1}(14.3) and spikelet spike^{-1}(19.5) were recorded at 90kg ha^{-1}P_2O_5 with RB_3 treatment among all treatments. Furthermore the 45 and 90 kg ha^{-1} P application increased plant height (7.35% and 14.33% cm), spike plant^{-1}(9.68 and 32.26%) and spikelet spike^{-1}(25.81% and 44.35%) respectively over control, whereas 90 kg ha^{-1} increased plant height (3.43%cm), spike plant^{-1}(10.92%) and spikelet spike^{-1}(6.82%) over 45 kg ha^{-1}. Additionally, RB_2 and RB_3 treatments improved plant height (8.17% and 12.54% cm), spike plant^{-1}(14.02% and 23.36%) and spikelet spike^{-1}(16.99% and 25.49%) respectively over RB_1 (control), whereas RB_3treatment improved plant height (4.03% cm), spike plant^{-1} (8.20%) and spikelet spike^{-1} (7.26%) over RB_2treatment.

Table 2. Rhizobacteria biopriming and phosphorus doses effect on plant height, spike plant^{-1} and spikelet spike^{-1}

Rhizobacteria	Phosphorus dose			Rhizobacteria mean
	00	45	90	
	Plant height (cm)			
RB_1	85.7	92.0	98.0	91.8 B
RB_2†	95.7	99.0	103.3	99.3 A
RB_3‡	99.0	105.0	105.9	103.3 A
Phosphorus dose mean	93.4 B	99.0 AB	102.4 A	
	Spike plant^{-1}			
RB_1	9.3	10.2	12.3	10.7 C
RB_2†	11.3	12.3	13.0	12.2 B
RB_3‡	12.3	13.1	14.3	13.2 A
Phosphorus doses mean	11.0 B	11.9 AB	13.2 A	
	Spikelet spike^{-1}			
RB_1	12.4	15.6	17.9	15.3 B
RB_2†	16.8	17.9	18.9	17.9 A
RB_3‡	18.9	19.2	19.5	19.2 A
Phosphorus dose mean	16.0 B	17.6 AB	18.8 A	

†: *Pseudomonas fluorescence* with single activity, i.e. ACC-deaminase activity
‡: *Pseudomonas fluorescence* with dual activities, i.e. ACC-deaminase activity and phosphate solubilizing activity

Rhizobacteria biopriming and phosphorus doses effect on 1000 grain weight (g), harvest index (%) and grain straw ratio of Wheat crop: The growth parameters, 1000 grain weight (g), harvest index (%) and grain to straw ratio were varied and significantly affected by the various treatments presented in Table 3. The data depicted that the maximum 1000 grain weight (44.2 g), harvest index (46.1%) and grain straw ratio (0.86:1) recorded at 90 kg ha^{-1} P_2O_5 with RB_3(ACC-deaminase & PS inoculation) among all treatments. Moreover, the application of both recommended doses of P 45 and 90 P_2O_5 kg ha^{-1}(50% and 100% recommended P) increased 1000 grain weight (13.5% and 24 %), harvest index % (0.73% and 6.57%) and grain straw ratio (1.43% and 11.43%) respectively over control. The latter 90 kg ha^{-1}P treatment increased 1000 grain weight (9.3%), harvest Index (5.8%) and grain straw ratio (9.86%) over 45 kg ha^{-1}P application. Furthermore, both rhizobacterial inoculation treatments (RB_2 and RB_3) increased 1000 grain weight (5.52% and 13.26%), harvest index % (4.95% and 7.67%) and grain straw ratio (7.35% and 14.71%) respectively over control (RB_1). Additionally, RB_3treatmentincreased 1000 grain weight (7.33%), harvest Index (2.59%) and grain straw ratio (6.85%) over RB

Table 3. Rhizobacteria biopriming and phosphorus doses effect on 1000 grain weight (g), harvest index (%) and grain straw ratio of wheat crops

Rhizobacteria	**Phosphorus dose**			**Rhizobacteria mean**
	00	**45**	**90**	
	1000-grain weight (g)			
RB_1	30.0	37.6	41.1	36.2B
RB_2†	34.5	38.0	42.0	38.2B
RB_3‡	38.1	40.7	44.2	41.0A
Phosphorus dose mean	34.2C	38.8B	42.4A	
	Harvest index (%)			
RB_1	40	39.3	41.8	40.4C
RB_2†	41.8	41.9	43.4	42.4B
RB_3‡	41.4	43.1	46.1	43.5A
Phosphorus doses mean	41.1B	41.4B	43.8A	
	Grain to straw ratio			
RB_1	0.67	0.65	0.72	0.68A
RB_2†	0.72	0.71	0.77	0.73AB
RB_3‡	0.71	0.76	0.86	0.78A
Phosphorus doses mean	0.7B	0.71B	0.78A	

†: *Pseudomonas fluorescence* with single activity, i.e. ACC-deaminase activity
‡: *Pseudomonas fluorescence* with dual activities, i.e. ACC-deaminase activity and phosphate solubilizing activity

Rhizobacteria biopriming and phosphorus doses effect on biological yield, grain yield and straw yield of wheat crops: The results showed that the different phosphorus and rhizobacterial treatments significantly affected the parameters including biological, grain and straw yield. The data showed the maximum biological yield (11066 kg ha^{-1}), grain yield (5105 kg ha^{-1}) and straw yield (4961 kg ha^{-1}) found at 90 kg ha^{-1} phosphorus dose with RB_3 application and minimum biological yield (6795 kg ha^{-1}), grain yield (2720 kg ha^{-1}) and straw yield (4075 kg ha^{-1}) recorded at control (no Phosphorus and no rhizobacterial application).Moreover, the application of both recommended doses of P 45 and 90 P_2O_5 kg ha^{-1}(50% and 100% recommended P) increased biological yield (27.51% and 46.58%), grain yield (25.22% and 52.72%) and straw yield (28.97% and 42.29% respectively over control. The latter 90 kg ha^{-1}P treatment, increased biological yield (14.93%) grain yield (22.07%) and straw yield (10.34%) over 45 kg ha^{-1} P application. Furthermore, both rhizobacterial inoculation treatments (RB_2 and RB_3) increased biological yield (11.66% and 21.51%), grain yield (16.85% and 31.16%) and straw yield (8.12% and 14.96%) respectively over control (RB_1). Additionally, RB_3 treatment increased biological yield (8.83%), grain yield (12.23%) and straw yield (6.33%) over RB_2

Table4. Rhizobacteria biopriming and phosphorus doses affect biological yield, grain yield and straw yield of wheat crop

Rhizobacteria	**Phosphorus dose**			**Rhizobacteria mean**
	00	**45**	**90**	
	Biological yield (kg ha^{-1})			
RB_1	6795	8660	9952	8469 C
RB_2†	7994	9751	10584	9455 B
RB_3‡	8976	10827	11066	10290 A
Phosphorus doses mean	7922 C	9758 B	10534 A	
	Grain yield (kg ha^{-1})			
RB_1	2720	3406	4156	3427C
RB_2†	3339	4084	4590	4004B
RB_3‡	3715	4661	5105	4494A
Phosphorus doses mean	3258C	4050B	4617A	
	Straw yield (kg ha^{-1})			
RB_1	4075	5254	5796	5042B
RB_2†	4655	5703	5994	5451AB
RB_3‡	5261	6166	5961	5796A
Phosphorus doses mean	4664B	5708A	5917A	

†: *Pseudomonas fluorescence* with single activity, i.e. ACC-deaminase activity
‡: *Pseudomonas fluorescence* with dual activities, i.e. ACC-deaminase activity and phosphate solubilizing activity

Rhizobacteria biopriming and phosphorus doses effect on total P content, grain P content and straw P content in wheat crop: The nutritional parameters, total P content, grain P content and straw P content were varied among the various treatments presented in Table 5.The results showed that different phosphorus and rhizobacterial treatments significantly affected total P concentration, grain P concentration, and straw P concentration in wheat crop. The data showed that the maximum total P concentration (9.5 g kg^{-1}), grain P concentration (4.90 g kg^{-1}), and straw P concentration (4.60 g kg^{-1}) at 90 kg ha^{-1} phosphorus dose with RB3 application, while the minimum total P concentration (2.6 g kg^{-1}), grain P concentration (2.13 g kg^{-1}), and straw P concentration (1.07 g kg^{-1}) were recorded at control (no phosphorus and no rhizobacterial application). Moreover, the application of both phosphorus doses, 45 and 90 kg ha^{-1} P_2O_5 (50% and 100% recommended P), increased total P concentration (58.16% and 122.49%), grain P concentration (50.25% and 116.92%), and straw P concentration (69.34% and 132.12%), respectively, over control. The 90 kg ha^{-1} P treatment further increased total P concentration (40.82%), grain P concentration (44.37%), and straw P concentration (37.07%) over 45 kg ha^{-1} P application. Furthermore, both rhizobacterial inoculation treatments (RB2 and RB3) increased total P concentration (29.49% and 52.76%), grain P concentration (9.20% and 22.70%), and straw P concentration (34.09% and 74.43%), respectively, over control (RB1). Additionally, RB3 treatment increased total P concentration (17.98%), grain P concentration (12.36%), and straw P concentration (30.08%) over RB2.

Table 5. Rhizobacteria biopriming and phosphorus doses effect on total P content, grain P content and straw P content in wheat crop.

Rhizobacteria	Phosphorus dose			Rhizobacterial Mean
	00	**45**	**90**	
	Total P concentration (g kg^{-1})			
RB_1	2.6	4.17	6.23	4.34C
RB_2†	3.5	5.53	7.8	5.62B
RB_3‡	4.04	6.33	9.5	6.63A
Phosphorus doses mean	3.38C	5.34B	7.52A	
	Grain P concentration (g kg^{-1})			
Control	1.53	2.57	3.63	2.58C
RB_1	2.13	3.10	4.53	3.26B
RB_2†	2.37	3.40	4.90	3.56A
Phosphorus doses mean	2.01C	3.02B	4.36A	
	Straw P concentration (g kg^{-1})			
RB_1	1.07	1.60	2.60	1.76C
RB_2†	1.37	2.43	3.27	2.36B
RB_3‡	1.67	2.93	4.60	3.07A
Phosphorus doses mean	1.37C	2.32B	3.18A	

†: *Pseudomonas fluorescence* with single activity, i.e. ACC-deaminase activity
‡: *Pseudomonas fluorescence* with dual activities, i.e. ACC-deaminase activity and phosphate solubilizing activity

Analysis of variance P-values of various parameters of wheat as affected by different phosphorus levels and rhizobacterial strains: Table6 shows the p-values from the analysis of variance for wheat traits affected by phosphorus (P) doses and rhizobacterial strains. Both factors had a highly significant impact ($p < 0.01$ to $p < 0.001$) on all traits, including 1000-grain weight, grain yield (kg ha^{-1}), straw yield (kg ha^{-1}), grain P concentration and straw P concentration. while their interaction remained non-significant ($p > 0.05$) for all traits.

Table 6. Analysis of variance P-values of various parameters of wheat as affected by different phosphorus levels and rhizobacterial strains

Parameter	**Phosphorus dose (P)**	**Rhizobacterial strains (R)**	**P × R**
1000-grain weight (g)	0.0009	0.0001	0.0812
Grain yield (kg ha^{-1})	0.0009	0.0000	0.7990
Straw yield (kg ha^{-1})	0.0004	0.0022	0.1971
Grain P concentration (g kg^{-1})	0.0000	0.0000	0.4611
Straw P concentration (g kg^{-1})	0.0003	0.0000	0.1287

DISCUSSION

Microorganisms particularly bacteria and microbial population in plant nutrition has a positive effect and play a significant role in enhancing plant growth, yield and quality of several crops and considered as the best option under nutrient stress and normal conditions (Ro & Mes, 2015), including wheat (Rashwan *et al.*, 2019; Sial*et al.*, 2018) and Mungbean (Rahman *et al.*, 2018; Zia-ul-hassan *et al.*, 2024). We have summarized that through the process of mineralization P-solubilizing bacteria significantly increased the phosphorus nutrition by solubilizing of

P (Bashan *et al.,* 2013). The field experiment of this study reveals that using rhizobacteria with interaction of phosphatic fertilizers significantly increased the growth yield and phosphorus accumulation on wheat crop. We have also noticed from this experiment that both phosphorus nutrition and rhizobacterial inoculants significantly ($p < 0.05$ to < 0.0001) increased the different growth parameters and phosphorus content in the wheat crop.

Additionally, both main sources of variation were found to interact significantly with respect to plant height, spike plant^{-1}, spikelet spike^{-1}, 1000 grain weight, straw weight, grain yield, and phosphorus concentration in grain and straw of wheat plants, indicating that these wheat plant traits were governed by the two sources of variation in an interactive manner. Similarly, it was observed P-solubilizing rhizobacteria significantly increasing P uptake (Bargaz *et al.,* 2021; Hassan *et al.,* 2017), however over study reveals that the P-solubilization was observed higher where application dual inoculation of ACC-deaminase rhizobacterial strains of *Pseudomonas fluorescens* were applied. Our experiments also showed that phosphorus nutrition and rhizobacterial inoculation enhance wheat growth and yield. Above results declared that P 45 and 90 kg ha^{-1}effectively enhanced parameters like plant height, tiller count, biomass, and yield. The related results of P fertilization enhancement on root development, nutrient uptake, and overall plant vigor found in previous research (Rezakhani *et al.,* 2019). Similarly, Plant growth-promoting (PGP) rhizobacteria found from the hilly regions of Madhya Pardesh India have high potential in improving nutrients accessibility and increase plant development through soil mechanism (Singh *et al.,* 2020).

Pseudomonas fluorescens strains producing ACC-deaminase, with or without phosphate-solubilizing traits, significantly enhanced wheat growth, yield, and P assimilation. In addition, P fertilization with ACC-deaminase-producing *Pseudomonas fluorescens* inoculation showed prominent impact on wheat growth. The ACC-deaminase rhizobacteria promote root enlargement for nutrient uptake and enhance nutrient acquisition by lowering the levels of stress-induced ethylene in plants (Ahmed *et al.,* 2019; Orozco *et al.,* 2020; Zafar *et al.,* 2020). These findings support our study that these bacteria potentially raised various plant traits by 1.4 to 38%under both phosphorus-sufficient and phosphorus-deficient conditions.

Increasing PSB efficiency co-inoculation with other beneficial microbes such as mycorrhizal fungi are beneficial for PSB reported by (Sharma *et al.,* 2016). Similarly, Malik et al. (2012) concluded that rhizobacterial inoculation significantly raised the germination percentage, number of tiller and spike, grain weight, and yield of Wheat crop. It is observed by different scientist that, use of PSB such as N-Fixing and P-solubilizing bacteria with NPK fertilization may reduce the P fertilization in Wheat crop and can also be more effective in other cereal crops (Saber *et al.,* 2012; Turan *et al.,* 2010). Additionally, Bargaz et al. (2021) highlighted that these bacteria improve plant nutrient uptake by solubilizing phosphorus and producing growth-promoting substance such as siderophores and indole-3-acetic acid (IAA) (Wahid *et al.,* 2020).

It was also observed that bio-inoculation significantly enhanced P content in plants across different fertilization levels, emphasizing the role of these microbes in nutrient uptake and reducing the need for fertilizers (Bechtaoui *et al.,* 2020; Rezakhani *et al.,* 2019). Similarly, PSB applications have been shown to lower phosphate fertilizer requirements by 50–75% in various systems, such as sugarcane in Brazilian Latosol soils and tea cultivation in Chinese Troporthents and Rhodustuts soils (Rosa *et al.,* 2020, Tennakoon *et al.,* 2019).

Relating these researches the crop management with lowering environmental pollution and farming costs, especially in arid and semiarid regions PSB integration could be useful techniques (Mallin & Cahoon, 2020). Producing phytohormones particularly IA Arhizobacteria shows great influence on lateral roots and root hairs formation witsh nutrient absorption improved (Qin & Huang, 2018; Gusain & Bhandari, 2019). Furthermore relevant studies by Maldonado et al. (2020) emphasized the consequence of PGPR for improving phosphorus fertilization efficiency, crop productivity, and quality while simultaneously reducing production costs. Similar, finding by Zafar et al. (2020)confirmed the additive benefits of PGPR strains with dual traits such as ACC-deaminase and P-solubilization on wheat growth and yield. Bacteria with these combined traits showed superior performance compared to single-trait strains, highlighting their potential for increased phosphorus uptake and crop yield.

For instance, Pseudomonas fluorescens with multiple activities improved wheat grain yield by 16.8–31.1%, a result ascribed to its capacity to solubilize phosphorus into plant-available forms (Baig *et al.,* 2012; Khan *et al.,* 2020). These conclusion support with previous studies, which constantly show that PSB enhances phosphorus accessibility and crop performance under inadequate phosphorus environment (Damo *et al.,* 2024). Combined application of both mineral and organic phosphorus with PSB in amended soils and inoculated crops has been exposed to be agronomicalcost-effective. This PSB impact on agro ecosystem productivity and P fertilization demonstrated the microbial diversity and crop efficient traits related to phosphorus acquisition benefits (Bargaz *et al.,* 2021). Bacillus dual traits performed conspicuously better than mono featured. Furthermore, multi-strain inoculation with PGPR containing ACC-deaminase activity was more efficient than single-strain inoculation in promoting wheat growth and yield. Earlier, (Ahmed et al., 2019) reported that ACC-deaminase bacteria sustain plant growth and development by reducing ethylene production, particularly under environmental stress. Plant gene expressions modification techniques by contributing these bacteria results in high growth and flexibility. Introducing ACC deaminase bacteria inoculation results in a crucial role in long term agro-sustainability through abiotic stress management. Additionally, studying the key PGPR strains mechanistic actions will augment the probable applications of this knowledge (Shahid *et al.*, 2023).Wheat farming in P deficient and under stress soils, inclusion of PSB and ACC-deaminase strains demonstrated the significant benefits in soil health, nutrient cycling, wheat growth and physiological traits improvement. Additionally, exploring the broader applicability of these microbial solutions in other crops and environmental contexts could further enhance sustainable agricultural practices.

CONCLUSION

This research concluded that *Pseudomonas fluorescens,* with dual activity (ACC- deaminase and P solubilizing) rhizobacteria inoculation under both P deficient and P adequate is more beneficial and effective over rhizobacteria having single activity of ACC deaminase and control. Rhizobacterial inoculation with optimal phosphorus doses showed great enhancement in the physiological characteristics and yield of wheat as well as phosphorus accumulation under both phosphorus-deficient and P adequate conditions. Therefore, we recommend that further study should be focused on beneficial rhizobacterial strains that can be utilized for essential nutrients accumulation and agro-sustainable practices enhancement.

AUTHORS' CONTRIBUTION

Saibrina Sethar, conducted all aspects of the research, including data collection, manuscript preparation. Work was carried under the supervision of Dr. Zia-ul-Hassan Shah and Dr. Nizamuddin Depar, who provided critical guidance and over site throughout the research process. Saeed Akhter Malik carried out data analysis. Mahendar Kumar Sootahar and Muhammad Aslam Panhwar edited the manuscript and improve the English language also collected and inserted the literature review for the manuscript.

AUTHORS' CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES

Afzal, A., & Bano, A. (2008). Rhizobium and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (Triticum aestivum). *International Journal of Agriculture and Biology*, *10*(1), 85–88.

Ahmed, T., Shahid, M., Noman, M., Hussain, S., Khan, M. A., Zubair, M., Ismail, M., Manzoor, N., Shahzad, T., & Mahmood, F. (2019). Plant Growth-Promoting Rhizobacteria as Biological Tools for Nutrient Management and Soil Sustainability. *Plant Growth Promoting Rhizobacteria for Agricultural Sustainability*, 95–110.

Baig, K. S., Arshad, M., Shaharoona, B., Khalid, A., & Ahmed, I. (2012). Comparative effectiveness of Bacillus spp. possessing either dual or single growth-promoting traits for improving phosphorus uptake, growth and yield of wheat (Triticum aestivum L.). *Annals of Microbiology*, *62*(3),

Balemi, T., & Negisho, K. (2012). Management of soil phosphorus and plant adaptation mechanisms to phosphorus stress for sustainable crop production: A review. *Journal of Soil Science and Plant Nutrition*, *12*(3), 547–561.

Bargaz, A., Elhaissoufi, W., Khourchi, S., Benmrid, B., Borden, K. A., & Rchiad, Z. (2021). Benefits of phosphate solubilizing bacteria on belowground crop performance for improved crop acquisition of phosphorus. *Microbiological Research*, *252*(8),

Bashan, Y., Kamnev, A. A., & de-Bashan, L. E. (2013). Tricalcium phosphate is inappropriate as a universal selection factor for isolating and testing phosphate-solubilizing bacteria that enhance plant growth: A proposal for an alternative procedure. *Biology and Fertility of Soils*, *49*(4),

Bashir, S., Basit, A., Abbas, R. N., Naeem, S., Id, S. B., Ahmed, N., Saeed, M., Id, A., Ilyas, M. Z., Id, Z. A., Alotaibi, S. S., El-shehawi, A. M., & Id, Y. L. (2021). *Combined application of zinc-lysine chelate and zinc-solubilizing bacteria improves yield and grain biofortification of maize (Zea mays L .)*. 1–14.

Bechtaoui, N., Raklami, A., Benidire, L., Tahiri, A. I., Göttfert, M., & Oufdou, K. (2020). Effects of PGPR co-inoculation on growth, phosphorus nutrition and phosphatase/phytase activities of faba bean under different phosphorus availability conditions. *Polish Journal of Environmental Studies*, *29*(2),

Damo, J. L. C., Pedro, M., & Sison, M. L. (2024). Phosphate Solubilization and Plant Growth Promotion by Enterobacter sp. Isolate. *Applied Microbiology*, *4*(3), 1177–1192.

Etesami, H., & Glick, B. R. (2023). Current Research in Biotechnology Exploring the potential : Can mycorrhizal fungi and hyphosphere silicate-solubilizing bacteria synergistically alleviate cadmium stress in plants. *Current Research in Biotechnology*, *6*(November), 100158.

Glick, B. R., & Gamalero, E. (2021). Recent Developments in the Study of Plant Microbiomes. *Microorganisms*, *9*, 1533.

Glick, B. R., & Nascimento, F. X. (2021).

Pseudomonas 1-aminocyclopropane-1-carboxylate (Acc) deaminase and its role in beneficial plant-microbe interactions. *Microorganisms*, *9*(12).

Government of Pakistan. (2021). *Overview of the Economy Despite*.

Gupta, H., Mishra, U. S., & Sirothia, P. (2023). *Unveiling the Synergistic Impact of N & P Levels Alone and in Conjunction with Bio-Inoculants (Azotobacter and PSB) on Growth and Yield of Wheat*. *35*(21), 569–575.

Gusain, P., & Bhandari, B. S. (2019). Rhizosphere associated PGPR functioning. *Journal of Pharmacognosy and Phytochemistry*, *8*(5), 1181–1191.

Hassan, W., Bashir, S., Hanif, S., Sher, A., Sattar, A., Wasaya, A., Atif, H., & Hussain, M. (2017). Phosphorus solubilizing bacteria and growth and productivity of mung bean (Vigna radiata). *Pakistan Journal of Botany*, *49*(Special Issue), 331–336.

Ibrahim, M., Iqbal, M., Tang, Y. T., Khan, S., Guan, D. X., & Li, G. (2022). Phosphorus Mobilization in Plant–Soil Environments and Inspired Strategies for Managing Phosphorus: A Review. *Agronomy*, *12*(10), 1–17.

Kaya, C., Şenbayram, M., Akram, N. A., Ashraf, M., Alyemeni, M. N., & Ahmad, P. (2020). Sulfur-enriched leonardite and humic acid soil amendments enhance tolerance to drought and phosphorus deficiency stress in maize (Zea mays L.). *Scientific Reports*, *10*(1), 1–13.

Khan, H., Ali, W., Shah, Z., Ur, H., Taj, A., & Alatalo, J. M. (2022). Heliyon Coupling phosphate-solubilizing bacteria (PSB) with inorganic phosphorus fertilizer improves mungbean (Vigna radiata) phosphorus acquisition , nitrogen fi xation , and yield in alkaline-calcareous soil. *Heliyon*, *8*(January), e09081.

Khan, S., Shahid, M., Khan, M. S., Syed, A., Bahkali, A. H., Elgorban, A. M., & Pichtel, J. (2020). Fungicide-tolerant plant growth-promoting rhizobacteria mitigate physiological disruption of white radish caused by fungicides used in the field cultivation. *International Journal of Environmental Research and Public Health*, *17*(19), 1–26.

Kumar, A., Dhyani, B., Rai, A., & Kumar, V. (2017). Effect of timing of vermicompost application and different level of NPK on growth, yield attributing characters and yield of rice in rice-wheat cropping system. *International Journal of Chemical Studies IJCS*, *5*(55), 2034–2038.

Maldonado, S., Rodríguez, A., Ávila, B., Morales, P., González, M. P., Araya Angel, J. P. A., Olalde, V., Bravo, J., Jana, C., Sierra, C., & Stoll, A. (2020). Enhanced Crop Productivity and Sustainability by Using Native Phosphate Solubilizing Rhizobacteria in the Agriculture of Arid Zones. *Frontiers in Sustainable Food Systems*, *4*(December), 1–14.

Malik, A. U., Malghani, A. L., & Hussain, F. (2012). Growth and yield response of wheat (Triticum aestivum L.) to phosphobacterial inoculation. *Russian Agricultural Sciences*, *38*(1), 11–13.

Mallin, M. A., & Cahoon, L. B. (2020). The Hidden Impacts of Phosphorus Pollution to Streams and Rivers. *BioScience*, *70*(4), 315–329.

Muhammad, S., Saleem, M., Riaz, M., Iqbal, Z., & Ashraf, M. (n.d.). *Author ' s personal copy PGPR with varied ACC-deaminase activity induced different growth and yield response in maize (Zea mays L .) under fertilized conditions*.

Orozco-Mosqueda, M. del C., Glick, B. R., & Santoyo, G. (2020). ACC deaminase in plant growth-promoting bacteria (PGPB): An efficient mechanism to counter salt stress in crops. *Microbiological Research*, *235*(January), 126439.

Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S., & Crecchio, C. (2015). Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. *Biology and Fertility of Soils*, *51*(4), 403–415.

Qin, H., & Huang, R. (2018). Auxin controlled by ethylene steers root development. *International Journal of Molecular Sciences*, *19*(11).

Rashwan, E., El-Gohary, Y., & Hafez, E. (2019). Impact of different levels of Phosphorus and seed inoculation with Arbuscular mycorrhiza (AM) on growth, yield traits and productivity of wheat. *Egyptian Journal of Agronomy*, *41*(1), 11–20.

Rezakhani, L., Motesharezadeh, B., Tehrani, M. M., Etesami, H., & Mirseyed Hosseini, H. (2019). Phosphate–solubilizing bacteria and silicon synergistically augment phosphorus (P) uptake by wheat (Triticum aestivum L.) plant fertilized with soluble or insoluble P source. *Ecotoxicology and Environmental Safety*, *173*(October 2018), 504–513.

Ro, M. I. C., & Mes, B. I. O. (2015). Curating communities from plants. In *microbiomes* (pp. 1–2).

Rosa, P. A. L., Mortinho, E. S., Jalal, A., Galindo, F. S., Buzetti, S., Fernandes, G. C., Barco Neto, M., Pavinato, P. S., & Teixeira Filho, M. C. M. (2020). Inoculation With Growth-Promoting Bacteria Associated With the Reduction of Phosphate Fertilization in Sugarcane. *Frontiers in Environmental Science*, *8*(March).

Saber, Z., Pirdashti, H., Esmaeili, M., Abbasian, A., & Heidarzadeh, A. (2012). Response of wheat growth parameters to co-inoculation of plant growth promoting Rhizobacteria (PGPR) and different levels of inorganic nitrogen and phosphorus. *World Applied Sciences Journal*, *16*(2), 213–219.

Shah, S. A. A., Wu, H., Farid, M. F., Tareen, W. U. H., & Badar, I. H. (2024). Climate Trends and Wheat Yield in Punjab, Pakistan: Assessing the Change and Impact. *Sustainability (Switzerland)* , *16*(11).

Shaharoona, B., Naveed, M., Arshad, M., & Zahir, Z. A. (2008). Fertilizer-dependent efficiency of Pseudomonads for improving growth, yield, and nutrient use efficiency of wheat (Triticum aestivum L.). *Applied Microbiology and Biotechnology*, *79*(1), 147–155.

Shahid, M., Singh, U. B., Khan, M. S., Singh, P., Kumar, R., Singh, R. N., Kumar, A., & Singh, H. V. (2023). Bacterial ACC deaminase: Insights into enzymology, biochemistry, genetics, and potential role in amelioration of environmental stress in crop plants. *Frontiers in Microbiology*, *14*(April).

Sharma, S., Kumar, V., & Tripathi, R. B. (2016). Isolation of Phosphate Solubilizing Microorganism (PSMs) From Soil Isolation of Phosphate Solubilizing Microorganism (PSMs) From Soil. *Journal of Microbiology and Biotechnology Research*, *2*(1), 3–9.

Sial, NA., SA Abro, M Abbas, M Irfan, N. D. (2018). Growth and yield of wheat as affected by phosphate solubilizing bacteria and phosphate fertilizer. *Pak J Biotech*, *15*(July), 475–479.

Singh, T. B., Sahai, V., Ali, A., Prasad, M., Yadav, A., Shrivastav, P., Goyal, D., & Dantu, P. K. (2020). Screening and evaluation of PGPR strains having multiple PGP traits from hilly terrain. *Journal of Applied Biology and Biotechnology*, *8*(4), 38–44.

Stearns, J. C., Woody, O. Z., Mcconkey, B. J., & Glick, B. R. (2012). *Effects of Bacterial ACC Deaminase on Brassica napus Gene Expression*. *25*(5), 668–676.

Syed-Ab-Rahman, S. F., Carvalhais, L. C., Chua, E., Xiao, Y., Wass, T. J., & Schenk, P. M. (2018). Identification of soil bacterial isolates suppressing different phytophthora spp. And promoting plant growth. *Frontiers in Plant Science*, *871*(October), 1–18.

Tennakoon, P. L. K., Rajapaksha, R. M. C. P., & Hettiarachchi, L. S. K. (2019). Tea yield maintained in PGPR inoculated field plants despite significant reduction in fertilizer application. *Rhizosphere*, *10*(October 2018), 100146.

Turan, M., Gulluce B, M., Cakmakci, R., Oztas, T., & Sahin, F. (2010). The effect of PGPR strain on wheat yield and quality parameters. *Proceeding of World Congress of Soil Science, Soil Solutions for a Changing World*, *August*, 140–1443. https://www.iuss.org/19th

Vishandas, Zia-Ul-Hassan, Arshad, M., & Shah, A. N. (2006). Phosphorus fertigation at first irrigation due to its unavailability at sowing time prevents yield losses in Triticum aestivum L. *Pakistan Journal of Botany*, *38*(5 SPEC. ISS.), 1439–1447.

Wahid, F., Fahad, S., Danish, S., Adnan, M., Yue, Z., Saud, S., Siddiqui, M. H., Brtnicky, M., Hammerschmiedt, T., & Datta, R. (2020). Sustainable management with mycorrhizae and phosphate solubilizing bacteria for enhanced phosphorus uptake in calcareous soils. *Agriculture (Switzerland)*, *10*(8), 1–14.

Zafar-Ul-Hye, M., Zahra, M. B., Danish, S., Abbas, M., Rehim, A., Akbar, M. N., Iftikhar, A., Gul, M., Nazir, I., Abid, M., Tahzeeb-Ul-Hassan, M., & Murtaza, M. (2020). Multi-strain inoculation with pgpr producing acc deaminase is more effective than single-strain inoculation to improve wheat (Triticum aestivum) growth and yield. *Phyton*, *89*(2), 405–413.

Zia-ul-hassan, Depar, N., Rajput, S. S., Arain, J. A., Jamali, I., Talpur, N. A., Hajra Khan, & Rajpar, I. (2024). Phophorus nutrition of Mungbean (Vigna Radiata L.) in relation to mycorhizobacterial inoculation. *Pakistan Journal of Biotechnology*, *21*(2), 428–434.