Journal of
Agriculture and Biology
ISSN: 3007-1755 (Print), 3007-1763 (Online)

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Research Article

Dual-Activity ACC-deaminase Phosphate-solubilizing *Pseudomonas* Seed Inoculation Improves Mustard Performance under Phosphorus Deficiency Stress

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ABSTRACT

Phosphorus is a critical macronutrient often limiting crop productivity, particularly in soils with low phosphorus availability. This field study investigated the phosphorus (P) deficiency tolerance of mustard under seed inoculation with 1-aminocyclopropane-1-carboxylic acid (ACC)-deaminase and phosphate-solubilizing strains of *Pseudomonas fluorescens*. The experiment followed a two-factor randomized complete block split plot design with three replications: Factor A included two P doses (0 and 50 kg ha^{-1}), and Factor B comprised three seed treatments (uninoculated control, inoculation with ACC-deaminase-only *P. fluorescens*, and inoculation with dual-activity *P. fluorescens* possessing ACC-deaminase and P-solubilizing traits). The soil was alkaline clay, low in organic matter and Olsen-P. Phosphorus application significantly improved all mustard traits, while rhizobacterial strains significantly influenced all parameters except plant height and P concentration. Their interaction was significant only for number of pods, number of leaves, seed yield, and P concentration. Compared to control, P application enhanced plant height, stem diameter, branches, pods, leaves, leaf area, leaf weight, chlorophyll content, seed yield, and P concentration by 30–67%. Inoculation with *P. fluorescens* possessing only ACC-deaminase improved most traits by 17–40% and seed yield by 1.7%, though it slightly reduced P concentration. Dual-activity *P. fluorescens* improved growth traits by 33–63%, seed yield by 10%, and P concentration by 5%. Compared to single-activity inoculation, the dual-activity strain resulted in 1.2–6.0-fold increases in key traits, with lower P concentration increase (1.7-fold). Maximum interaction effects were observed in number of pods (3.5-fold), number of leaves (2.0-fold), seed yield (1.7-fold), and P concentration (1.7-fold). Overall, *P. fluorescens* strains with dual ACC-deaminase and P-solubilizing activity consistently outperformed single-activity strains and showed potential to enhance mustard growth and yield under both P-deficient and fertilized conditions. Future research may focus on multi-strain co-inoculation strategies and testing across diverse agroecological environments.

Keywords: Mustard growth, Phosphorus deficiency, *Pseudomonas fluorescens*, ACC-deaminase, Phosphate solubilizing rhizobacteria.

OPEN ACCESS

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Article History
Received: May 29, 2025
Accepted: October 10, 2025
Published: October 31, 2025

INTRODUCTION

Pakistani soils are reportedly facing severe land degradation, multiple soil related issues, with increasing nutrient mining and heavy metal toxicity (Zia-ul-hassan & Arshad, 2006; Bux et al., 2022, Ameer et al., 2023; Bux et al., 2023). Phosphorus

(P) is a vital nutrient for plant growth, but its deficiency significantly limits productivity (Ahmed et al., 2024a; Memon et al., 2024), particularly in P-deficient (< 10 mg kg^{-1} Olsen P) Pakistani soils. Moreover, P-use efficiency in Pakistan is low, often falling below 25% (Vishandas et al., 2006). The positive role of P in regulating gene expression linked to root architecture and stress tolerance has been witnessed, which enhances nutrient acquisition and crop resilience under sub-optimal conditions (Almeida et al., 2021; Rai et al., 2022). These findings underline the multidimensional importance of phosphorus in sustaining plant health and optimizing agricultural output. Crops in P-deficient soils demand substantial applications of expensive phosphatic fertilizers to achieve optimal yields (Afzal et al., 2014; Zia-ul-hassan et al., 2015, Zia-ul-hassan et al., 2016a,b; Sethar et al., 2025). However, this heavy reliance poses long-term sustainability concerns, especially given the non-renewable nature of global phosphate rock reserves (Vishandas et al., 2006).

Exploring alternative and sustainable P sources has become critically important. Recent studies have emphasized the potential of organic amendments, biofertilizers, and recycled P products in improving soil nutrient availability and crop uptake (Zhu et al., 2022; Ojo et al., 2023; Ahmed et al., 2024b; Zia-ul-hassan et al., 2024; Sethar et al., 2025). These approaches not only reduce dependency on mineral fertilizers but also promote circular nutrient management and environmental health. Ahmed et al. (2024b) reported P-use efficiency increases from 15% to over 40% when P-solubilizing biofertilizers were paired with synthetic P sources, while Khan et al. (2024) demonstrated that integrating P-solubilizing microorganisms boosted P uptake and yield in maize by nearly 35%. Integrating such microbial inoculants could thus be a practical and sustainable approach to improving P-use-efficiency in Pakistan's agriculture.

The P-solubilizing rhizobacteria can convert insoluble forms of P into plant-available forms by releasing low-molecular-weight organic acids such as gluconic and citric acids (Zia-ul-hassan et al., 2024; Sethar et al., 2025). These rhizobacteria, often classified as plant growth-promoting rhizobacteria (PGPR), are known to support root development and enhance nutrient uptake by stimulating phytohormone production either by the plant or the microbes themselves (Shaharoona et al., 2006;).

Under P-deficiency stress, these PGPR enhance plant growth by regulating ethylene levels through the enzyme ACC deaminase, which breaks down the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (Shaharoona et al., 2006). This mechanism helps plants cope with stress, improves gene expression, and promotes root elongation and biomass accumulation. PGPR also secrete enzymes that modulate plant growth regulators, ultimately improving nutrient acquisition and crop performance (Afzal et al., 2014; Zia-ul-hassan et al., 2015).

Recent studies confirm that inoculating seeds with ACC deaminase-producing PGPR not only boosts stress tolerance but also significantly improves crop yield (Zia-ul-hassan et al., 2024; Sethar et al., 2025). In Pakistan, biofertilizers based on phosphate-solubilizing microorganisms have been introduced as a sustainable solution to reduce reliance on expensive chemical fertilizers and their application in cereal-based systems has shown promising results in terms of improved nutrient use efficiency, soil health, and yield stability (Afzal et al., 2014; Zia-ul-hassan et al., 2024; Sethar et al., 2025).

Phosphate-solubilizing rhizobacteria improve cereal yields, especially in wheat and rice, by making soil phosphorus more available through the release of organic acids that lower rhizosphere pH (Afzal et al., 2014; Zia-ul-hassan et al., 2015; Singh et al., 2023). Besides P-solubilization, these microbes contribute to plant growth by producing phytohormones, fixing atmospheric nitrogen, and promoting root development (Zia-ul-hassan et al., 2016a,b; Mahmud et al., 2020).

Seed inoculation with P-solubilizing rhizobacteria has shown to enhance nutrient uptake, plant biomass, and grain yield, particularly under phosphorus-deficient or stress conditions (Zia-ul-hassan et al., 2024; Sethar et al., 2025). Their integration into cropping systems presents an eco-friendly strategy to reduce reliance on expensive synthetic fertilizers and improve sustainable crop production (Hassan et al., 2024).

This field study evaluated the impact of seed inoculation with Pseudomonas, possessing dual activities, i.e., ACC-deaminase and phosphate-solubilization, on mustard growth and yield under soil P deficiency. The aim was to assess whether these PGPR strains could reduce the need for phosphatic fertilizers and enhance crop performance under stress.

MATERIALS AND METHODS

Venue of Field Experiment

The field experiment was carried out at the Research Fields of the Oilseeds Research Institute, Agriculture Research Centre, Tandojam, Sindh, Pakistan.

Experimental Design and Treatment Details

The experiment was executed following a two-factor randomized complete block design with three replications, in plots measuring 3.0 m × 5.0 m. Factor A consisted of two P levels: P1 = no P fertilizer (control) and P2 = 50 kg ha^{-1} (recommended P dose). Factor B included three rhizobacterial treatments: RB1 = control (no inoculation), RB2 = seed inoculation with *Pseudomonas fluorescens* containing only ACC-deaminase activity, and RB3 = seed inoculation with *Pseudomonas fluorescens* biotype F having both ACC-deaminase and P-solubilizing activity.

Sowing of Mustard Crop

Pure seed of mustard variety S-9 was sown and managed using standard agronomic practices, followed throughout the crop growth period according to crop requirements.

Selection of Bacterial Strain

Two previously isolated strains of *Pseudomonas fluorescens* containing ACC-deaminase - one with and one without phosphate-solubilizing activity - were obtained from the Soil Microbiology and Biochemistry Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. These strains were selected based on their varying abilities to solubilize phosphate. These strains have already been isolated, characterized, and reported in our earlier studies (Shaharoona et al., 2006; Zia-ul-hassan et al., 2015; Zia-ul-hassan et al., 2016a,b), where their ACC-deaminase activity and phosphate-solubilizing potential were thoroughly described.

Preparation of Inocula

The inocula were prepared using general purpose media. The two selected rhizobacterial strains were cultured in broth and incubated at 28 ± 1 °C for 48 hours under constant shaking at 78 rpm. After incubation, the optical density of the cultures was measured to ensure a uniform bacterial population (10^7–10^8 CFU) at the time of seed inoculation (Shaharoona et al., 2006; Zia-ul-hassan et al., 2024).

Seed Inoculation

Peat and muck soils were finely ground, passed through a 2-mm mesh sieve, and sterilized in an autoclave at 121°C for 20 minutes. Ten milliliters of rhizobacterial inoculum were then thoroughly mixed with 50 grams each of sterilized peat and muck soil in a 1:1 weight ratio. This mixture was incubated at 28 ± 1°C for 24 hours before being used for coating the seeds. The inoculated seeds were air-dried overnight at room temperature in the laboratory (Shaharoona et al., 2006).

Fertilization

The recommended P fertilizer dose (50 kg P_2O_5 ha^{-1}) was applied using triple super phosphate (containing 51% P_2O_5). In addition, the crop received 100 kg ha^{-1} of nitrogen (N) through urea (46% N) and 25 kg ha^{-1} of potassium (K) using sulfate of potash (SOP, 50% K_2O). All of the P (as per treatment), full K, and one-third N were broadcast onto the soil and mixed thoroughly before sowing. The remaining two-thirds of the N was applied in two equal splits - one at the first irrigation and the other at the second.

Soil Sampling, Processing and Analysis

Before sowing, a composite soil sample was collected from the experimental site and analysed for selected soil properties, viz. texture, electrical conductivity (EC), pH, organic matter, and Olsen-P (Ryan et al., 2001). The analysis showed that the soil was clayey in texture (42% clay, 33% silt, and 25% sand), alkaline in reaction (pH 8.1), and non-saline (EC 1.3 dS m^{-1}). The soil was also low in fertility, with organic matter at 0.58% and Olsen-P at 5.2 mg kg^{-1}.

Agronomic and Other Practices

Irrigation was applied as per the crop's water requirements. All other standard agronomic practices recommended for mustard cultivation were properly implemented, during the crop's life cycle.

Growth and Yield Parameters

At crop maturity, ten representative plants were sampled from each experimental unit to record key growth and yield attributes. These included plant height (cm), stem diameter (mm), number of branches per plant, number of pods per plant, number of leaves per plant, total leaf area per plant, leaf weight (g per plant), chlorophyll content (SPAD value), and seed yield (g per plant).

Plant Analysis

Phosphorus concentration of mustard plants, obtained from each experimental unit, was determined following the single acid digestion method (Zia-ul-hassan et al., 2014).

Statistical Analysis

The analysis of variance (ANOVA) was done using Statistix software version 8.1. Treatment means were separated by employing Tukey's Honestly Significant Difference (HSD) test ($\alpha = 0.05$).

RESULTS

The P application had a significant effect on all observed growth traits. Similarly, the rhizobacterial strains significantly influenced most traits, except plant height and P concentration. The interaction between P levels and rhizobacterial strains was statistically significant for the number of pods, number of leaves, P concentration, and seed yield (Table 1).

Table 1. P-values from analysis of variance of various parameters of mustard as affected by different phosphorus levels and rhizobacterial strains

Parameter	Phosphorus Dose (P)	Rhizobacterial Strains (R)	P × R
Plant height (cm)	0.0004	0.1852	0.7791
Stem diameter (mm)	0.0085	0.0143	0.7299
Number of branches (plant^{-1})	0.0160	0.0037	0.2632
Number of pods (plant^{-1})	0.0055	0.0000	0.0002
Number of leaves (plant^{-1})	0.0026	0.0000	0.0007
Leaf area (cm^2 plant^{-1})	0.0005	0.0000	0.0563
Leaf weight (g plant^{-1})	0.0004	0.0000	0.0533
Chlorophyll content (SPAD)	0.0065	0.0003	0.2579
Seed yield (g plant^{-1})	0.0099	0.0293	0.0152
Phosphorus concentration (%)	0.0007	0.1741	0.0010

Plant Height (cm)

The P application had a significant impact on mustard plant height. Plants receiving the recommended P dose reached an average height of 148 cm, which was about 60% taller than those in the control group (92 cm). However, the rhizobacterial strains had no significant effect on plant height, and there was no significant interaction between P levels and rhizobacterial treatments (Table 2).

Table 2. Plant height (cm plant^{-1}) of mustard as affected by different phosphorus levels and rhizobacterial strains

Rhizobacteria	Phosphorus dose (kg ha^{-1})		Rhizobacteria mean
	00	50	
Control	89	142	116
P. fluorescence (ACC)†	93	147	120
P. fluorescence (ACC + PS)‡	95	154	124
Phosphorus dose mean	92B	148A	
	Phosphorus Dose (P)	**Rhizobacteria (R)**	**P × R**
F-value	2703.47**	NS	NS
$HSD_{0.05}$	4.5677	-	-

†: *Pseudomonas fluorescence* with single activity, i.e. ACC-deaminase activity
‡: *Pseudomonas fluorescence* with dual activities, i.e. ACC-deaminase activity and phosphate solubilizing activity
**: significant at alpha 0.01. NS: Non-significant.

Stem Diameter (mm)

The P application significantly improved stem diameter in mustard. At the recommended P dose, stem diameter reached 15.7 mm - about 44% higher than the control (10.9 mm). Rhizobacterial inoculation also had a significant effect. The smallest diameter (11.0 mm) was observed in the non-inoculated control. Inoculation with *Pseudomonas fluorescens* containing only ACC-deaminase increased diameter to 14.0 mm (27% higher), while the dual-activity strain (ACC-deaminase + P-solubilization) resulted in the highest diameter of 14.8 mm (35% more than control). This indicates a 1.1-fold improvement with the dual-activity strain over the single-activity one. However, as shown in Table 1, the P × R interaction was not significant for stem diameter (Table 3).

Table 3. Stem diameter (mm) of mustard as affected by different phosphorus levels and rhizobacterial strains

Rhizobacteria	Phosphorus Dose (kg ha^{-1})		Rhizobacteria mean
	00	50	
Control	9.0	13.0	11.0B
P. fluorescence (ACC)†	11.7	16.3	14.0A
P. fluorescence (ACC + PS)‡	12.0	17.7	14.8A
Phosphorus dose mean	10.9B	15.7A	
	Phosphorus Dose (P)	**Rhizobacteria (R)**	**P × R**
F-value	115.56**	7.57*	NS
$HSD_{0.05}$	1.9153	2.9563	-

†: *Pseudomonas fluorescence* with single activity, i.e. ACC-deaminase activity
‡: *Pseudomonas fluorescence* with dual activities, i.e. ACC-deaminase activity and phosphate solubilizing activity
* and **: significant at alpha 0.05 and 0.01, respectively. NS: Non-significant.

Number of Branches (plant^{-1})

The P application significantly increased the number of branches per mustard plant (Table 4). At the recommended P dose, plants produced 16.7 branches - about 29% more than the control (12.9). Rhizobacterial inoculation also had a significant effect. The lowest branch number (12.7) was recorded in the uninoculated control, while inoculation with *Pseudomonas fluorescens* containing only ACC-deaminase raised it to 14.8 branches (17% higher). The highest number (16.8) was observed with the dual-activity strain (ACC-deaminase + P-solubilization), which was 33% greater than the control and 1.1 times higher than the single-activity strain. However, the P × R interaction was not significant for this trait (Table 1).

Table 4. Number of branches (plant^{-1}) of mustard as affected by different phosphorus levels and rhizobacterial strains

Rhizobacteria	Phosphorus dose (kg ha^{-1})		Rhizobacteria mean
	00	50	
Control	10.0	15.3	12.7B
P. fluorescence (ACC)†	13.7	16.0	14.8AB
P. fluorescence (ACC + PS)‡	15.0	18.7	16.8A
Phosphorus dose mean	12.9B	16.7A	
	Phosphorus dose (P)	**Rhizobacteria (R)**	**P × R**
F-value	60.84*	12.18**	NS
$HSD_{0.05}$	2.0872	2.4086	-

†: *Pseudomonas fluorescence* with single activity, i.e. ACC-deaminase activity
‡: *Pseudomonas fluorescence* with dual activities, i.e. ACC-deaminase activity and phosphate solubilizing activity
* and **: significant at alpha 0.05 and 0.01, respectively. NS: Non-significant.

Number of Pods (plant^{-1})

The P fertilization significantly increased the number of mustard pods. Plants receiving the recommended P dose produced 246 pods - 53% more than those in the control (160). Rhizobacterial treatments also showed significant effects. The fewest pods (152) were recorded in the uninoculated control, while inoculation with *Pseudomonas fluorescens* containing only ACC-deaminase increased pod count to 208 (37% more). The highest number (248) was achieved using the dual-activity strain (ACC-deaminase + P-solubilization), representing a 63% increase over control and 1.2 times more than the single-activity strain. The P × R interaction was also significant, with the maximum number of pods (267) observed in plants receiving both the recommended P dose and dual-activity inoculation. This was 3.5 times higher than the lowest pod count (77), seen in the uninoculated and unfertilized treatment (Table 5).

Table 5. Number of pods (plant^{-1}) of mustard as affected by different phosphorus levels and rhizobacterial strains

Rhizobacteria	Phosphorus dose (kg ha^{-1})		Rhizobacteria mean
	00	50	
Control	77d	227bc	152
P. fluorescence (ACC)†	173c	243ab	208
P. fluorescence (ACC + PS)‡	230ab	267a	248
Phosphorus dose mean	160	246	
	Phosphorus dose (P)	**Rhizobacteria (R)**	**P × R**
F-value	181.45**	79.61**	28.5**
$HSD_{0.05}$	27.406	21.833	48.251

†: *Pseudomonas fluorescence* with single activity, i.e. ACC-deaminase activity
‡: *Pseudomonas fluorescence* with dual activities, i.e. ACC-deaminase activity and phosphate solubilizing activity
**: significant at alpha 0.01.

Number of Leaves (plant^{-1})

The P application significantly improved the number of leaves in mustard. Plants treated with the recommended P dose produced 167 leaves, which is 41% higher than the control (118). Rhizobacterial treatments also had a significant impact. The lowest leaf count (120) occurred in the uninoculated control, while inoculation with *Pseudomonas fluorescens* containing only ACC-deaminase raised the count to 151 (25% more). The highest number of leaves (156) was observed with dual-activity strains (ACC-deaminase + P-solubilization), showing a 30% increase over the control and ~1.0-1.1 times more than the single-activity treatment. A significant interaction (P × R) was also noted, where the combination of recommended P and dual-activity inoculation produced 174 leaves - twice the number (87) found in the no-P, no-inoculation control (Table 6).

Table 6. Number of leaves (plant^{-1}) of mustard as affected by different phosphorus levels and rhizobacterial strains

Rhizobacteria	Phosphorus dose (kg ha^{-1})		Rhizobacteria mean
	00	50	
Control	87d	153b	120
P. fluorescence (ACC)†	128c	173a	151
P. fluorescence (ACC + PS)‡	139bc	174a	156
Phosphorus dose mean	118	167	
	Phosphorus dose (P)	**Rhizobacteria (R)**	**P × R**
F-value	387.98**	125.22**	20.93**
$HSD_{0.05}$	10.696	6.944	16.9365

†: *Pseudomonas fluorescence* with single activity, i.e. ACC-deaminase activity
‡: *Pseudomonas fluorescence* with dual activities, i.e. ACC-deaminase activity and phosphate solubilizing activity
**: significant at alpha 0.01.

Leaf Area (cm^2 plant^{-1})

The P application significantly improved the leaf area of mustard plants. With the recommended P dose, leaf area reached 1370 cm² per plant - 67% higher than the control (819 cm²). Rhizobacterial treatments also had a notable effect. The smallest leaf area (843 cm²) was seen in the uninoculated control, while inoculation with *Pseudomonas fluorescens* containing only ACC-deaminase increased it to 1181 cm², a 40% rise. The highest leaf area (1260 cm²) was observed when mustard was inoculated with strains having both ACC-deaminase and P-solubilizing activity - 50% greater than control and 1.1 times more than single-activity strains. However, the interaction between P levels and rhizobacterial strains (P × R) was found to be non-significant for this trait (Table 7).

Table 7. Leaf area (cm^2 $plant^{-1}$) of mustard as affected by different phosphorus levels and rhizobacterial strains

Rhizobacteria	Phosphorus dose (kg ha^{-1})		Rhizobacteria mean
	00	50	
Control	515	1171	843B
P. fluorescence (ACC)†	916	1445	1181A
P. fluorescence (ACC + PS)‡	1026	1494	1260A
Phosphorus dose mean	819B	1370A	
	Phosphorus dose (P)	**Rhizobacteria (R)**	**P × R**
F-value	2091.4**	90.34**	NS
$HSD_{0.05}$	51.901	94.008	-

†: *Pseudomonas fluorescence* with single activity, i.e. ACC-deaminase activity
‡: *Pseudomonas fluorescence* with dual activities, i.e. ACC-deaminase activity and phosphate solubilizing activity
**: significant at alpha 0.01. NS: Non-significant.

Leaf Weight (g $plant^{-1}$)

The P application significantly influenced mustard leaf weight. Plants receiving the recommended P dose had a leaf weight of 274 g per plant - 67% higher than the control (164 g). Rhizobacterial inoculation also had a significant impact. The lowest leaf weight (169 g) was observed in the uninoculated control, while inoculation with *Pseudomonas fluorescens* containing only ACC-deaminase increased it to 236 g, a 40% rise. The highest leaf weight (252 g) resulted from inoculation with strains possessing both ACC-deaminase and P-solubilizing abilities - 49% higher than control and 1.1 times more than single-activity inoculants. The interaction between P level and rhizobacteria (P × R) was non-significant for this parameter (Table 8).

Table 8. Leaf weight (g $plant^{-1}$) of mustard as affected by different phosphorus levels and rhizobacterial strains

Rhizobacteria	Phosphorus dose (kg ha^{-1})		Rhizobacteria mean
	00	50	
Control	103	234	169B
P. fluorescence (ACC)†	183	289	236A
P. fluorescence (ACC + PS)‡	205	299	252A
Phosphorus dose mean	164B	274A	
	Phosphorus dose (P)	**Rhizobacteria (R)**	**P × R**
F-value	2281.84**	92.47**	NS
$HSD_{0.05}$	9.9637	18.592	-

†: *Pseudomonas fluorescence* with single activity, i.e. ACC-deaminase activity
‡: *Pseudomonas fluorescence* with dual activities, i.e. ACC-deaminase activity and phosphate solubilizing activity
**: significant at alpha 0.01. NS: Non-significant.

Chlorophyll Content (SPAD)

The P application significantly enhanced mustard chlorophyll content. Plants receiving the recommended P dose had a SPAD value of 54, which was 33% higher than the control (43). Rhizobacterial inoculation also showed a notable effect. The lowest chlorophyll content (38) occurred in uninoculated control plants. Inoculation with *Pseudomonas fluorescens* containing only ACC-deaminase raised it to 43 (13.5% more), while dual-activity inoculation increased it to 49 (27% higher than control). Compared to single-activity strains, dual-activity inoculation resulted in nearly twice the improvement compared with single-activity strains. The interaction between P level and rhizobacteria (P × R) was statistically non-significant (Table 9).

Table 9. Chlorophyll content (SPAD) of mustard as affected by different phosphorus levels and rhizobacterial strains

Rhizobacteria	Phosphorus dose (kg ha-1)		Rhizobacteria mean
	00	50	
Control	31	46	38C
P. fluorescence (ACC)†	38	48	43B
P. fluorescence (ACC + PS)‡	43	54	49A
Phosphorus dose mean	37B	49A	
	Phosphorus dose (P)	**Rhizobacteria (R)**	**P × R**
F-value	153.16**	26.57**	NS
HSD0.05	4.2559	4.0434	-

†: Pseudomonas fluorescence with single activity, i.e. ACC-deaminase activity
‡: Pseudomonas fluorescence with dual activities, i.e. ACC-deaminase activity and phosphate solubilizing activity
**: significant at alpha 0.01. NS: Non-significant.

Seed Yield (g plant^{-1})

The P application significantly increased mustard seed yield. Plants receiving the recommended P dose produced 14.6 g plant^{-1}, which was 48% higher than the control (9.9 g plant^{-1}). Rhizobacterial inoculation also impacted yield. The lowest yield (11.8 g plant^{-1}) was from uninoculated plants, while inoculation with *Pseudomonas fluorescens* containing only ACC-deaminase gave a slight increase to 12.0 g plant^{-1} (1.7%). The highest yield (13.0 g plant^{-1}) came from seeds treated with dual-activity strains, a 10.2% improvement over the control. Yield was ~1.1 times greater with dual-activity strains than with ACC-deaminase alone. A significant interaction between P level and bacterial strain was observed, with the maximum yield (14.7 g plant^{-1}) achieved when both P and dual-activity rhizobacteria were applied - 1.7 times higher than the lowest yield (8.7 g plant^{-1}) in the no-P, no-inoculation treatment (Table 10).

Table 10. Seed yield (g plant^{-1}) of mustard as affected by different phosphorus levels and rhizobacterial strains

Rhizobacteria	Phosphorus dose (kg ha^{-1})		Rhizobacteria mean
	00	50	
Control	8.7c	14.9a	11.8
P. fluorescence (ACC)†	9.7bc	14.3a	12.0
P. fluorescence (ACC + PS)‡	11.3ab	14.7a	13.0
Phosphorus dose mean	9.9	14.6	
	Phosphorus dose (P)	**Rhizobacteria (R)**	**P × R**
F-value	99.39**	5.66*	7.39*
$HSD_{0.05}$	2.0556	1.0897	2.9831

†: *Pseudomonas fluorescence* with single activity, i.e. ACC-deaminase activity
‡: *Pseudomonas fluorescence* with dual activities, i.e. ACC-deaminase activity and phosphate solubilizing activity
* and **: significant at alpha 0.05 and 0.01, respectively.

Phosphorus Concentration (%)

The P application significantly increased P concentration in mustard. At the recommended P dose, plants had a P concentration of 0.29%, which was 42% higher than the control (0.21%). Rhizobacterial inoculation also influenced P uptake. The lowest concentration (0.24%) was found with ACC-deaminase-only strains, followed by 0.25% in uninoculated plants - both statistically similar. The highest P concentration (0.26%) was recorded with dual-activity rhizobacteria, though also statistically comparable to the other treatments. A significant interaction between P levels and bacterial strains was observed, with the maximum P concentration (0.31%) noted when full P was applied without inoculation -1.72 times higher than the lowest value (0.18%) in the no-P, no-inoculation treatment (Table 11).

Table 11. Phosphorus concentration (%) of mustard as affected by different phosphorus levels and rhizobacterial strains

Rhizobacteria	Phosphorus dose (kg ha^{-1})		Rhizobacteria mean
	00	50	
Control	0.18c	0.31a	0.25
P. fluorescence (ACC)†	0.20bc	0.28a	0.24
P. fluorescence (ACC + PS)‡	0.23b	0.28a	0.26
Phosphorus dose mean	0.21	0.29	
	Phosphorus dose (P)	**Rhizobacteria (R)**	**P × R**
F-value	1482.25**	NS	18.27**
$HSD_{0.05}$	0.0258	-	0.0331

†: *Pseudomonas fluorescence* with single activity, i.e. ACC-deaminase activity
‡: *Pseudomonas fluorescence* with dual activities, i.e. ACC-deaminase activity and phosphate solubilizing activity
**: significant at alpha 0.01. NS: Non-significant.

DISCUSSION

Phosphorus (P) is a vital nutrient for plant growth. However, its deficiency, specially in P-deficient soils with very low P-use efficiency can be a limiting factor for sustainable crop production, such as in Pakistan (Vishandas et al., 2006; Ahmed et al., 2024a). Soils with P-deficiency are globally widespread and appear to be one of the biggest issues of modern-time agriculture. Coupled with the scorching prices of phosphatic fertilizers, their adulteration, and black marketing, they are playing havoc with the sustainability of agriculture (Vishandas et al., 2006). For the above-mentioned reasons, any interventions which enhance soil P availability not only lower the input cost of P fertilizers but also promote plant growth and crop yields.

It has been advocated that seed inoculation with ACC deaminase-rhizobacteria can enhance plant stress tolerance and crop yield (Afzal et al., 2014; Zia-ul-hassan et al., 2016a,b). In Pakistan, biofertilizers based on P-solubilizing microorganisms have been introduced as a sustainable solution to reduce reliance on expensive chemical fertilizers (Afzal et al., 2014). Their application in cereal-based systems has shown promising results in terms of improved nutrient use efficiency, soil health, and yield stability (Zia-ul-hassan et al., 2024, Sethar et al., 2025).

The findings of present study endorse the results of previous studies. We found that adequate P nutrition significantly improved most growth traits, seed yield, and P accumulation in mustard (Table 2 to11). This aligns with earlier findings that P is relatively immobile and often bound in soil, limiting its availability to plants (Sharma et al., 2011; Afzal et al., 2014; Ahmed et al., 2024a). Recent research confirms that efficient P uptake is essential for optimizing yield-related traits like plant height, leaf development, and pod formation (Suhail et al., 2023).

The dual-activity Pseudomonas fluorescens strain - combining ACC-deaminase and phosphate-solubilizing functions - enhanced growth and nutrient uptake more effectively than the single-trait strain, as reported in some early studies also (Afzal et al., 2014, Zia-ul-hassan et al., 2015, Zia-ul-hassan et al., 2016a,b). For instance, stem diameter increased by 44–35%, pod count by 53–63%, and leaf traits by 41–67% under dual treatment (Table 2 to 8). These findings resonate with Suhail et al. (2023), who demonstrated similar biomass and P-uptake improvements in mustard under drought conditions using PSB. Likewise, Frontiers et al. (2023) reported significant increases in wheat growth and chlorophyll when dual-action PSB were used, reinforcing the additive benefits of combining microbial traits.

Seed inoculation with PSB also supports root growth and stress tolerance through ACC-deaminase activity, which reduces plant ethylene levels. Pseudomonas strains carrying ACC-deaminase have been shown to regulate ethylene and auxin pathways, improving root architecture especially under stress (Shaharoona et al., 2006; Naqqash et al., 2023). Enhanced root systems improve soil nutrient exploration, consistent with our results showing higher leaf area, weight, and chlorophyll in dual inoculated plants under P deficiency (Table 7 to 9).

We also observed that dual inoculation with adequate P application resulted in significantly greater yield parameters - up to 3.5-fold higher pod count and 1.7-fold higher seed yield - compared to unfertilized controls (Table 5 & 10). This mirrors findings by Mahmud et al. (2020), who found similar grain yield improvements in cereals using ACC-deaminase–producing PSB, especially when combined under optimal nutrient supply. Such integrated nutrient-microbe

practices can reduce chemical fertilizer reliance by up to 30-40% while maintaining or improving crop output (Hassan et al., 2024).

The significant P × R interaction observed for seed yield indicates that microbial inoculation and P nutrition acted synergistically. Dual-activity Pseudomonas fluorescens strains likely enhanced root architecture and P mobilization, which ultimately increased applied P fertilizer-use-efficiency. These findings endorse early results that P-solubilizing microbes can transform soil P into plant-available forms, thereby boosting nutrient uptake and yield when combined with mineral fertilization (Silva et al., 2023; Pang et al., 2024). Such synergistic effects highlight the potential of integrated biofertilizer–fertilizer strategies for improving P-use efficiency in sustainable crop production.

Despite direct assays of rhizobacterial colonization and enzyme activity were not performed in this field study, we previously exploited both these strains and characterized them for their dual ACC-deaminase and phosphate-solubilizing potential (Shaharoona et al., 2006; Zia-ul-hassan et al., 2024), which provides clear mechanistic support for the observed plant responses.

CONCLUSIONS

In crux, we conclude that dual-activity rhizobacteria markedly improved mustard growth and yield, with the strongest benefits observed when combined with phosphorus fertilization. The significant P × microbe interactions underline their role in enhancing phosphorus-use efficiency. These bioinoculants provide a sustainable alternative to costly fertilizers, and future work should focus on multi-strain inoculation and testing across diverse soils to broaden their applicability.

ACKNOWLEDGEMENTS

This humble research work is dedicated to the loving memories of Late Prof. Dr. Muhammad Arshad (T.I.), Institute of Soil & Environmental Sciences, University of Agriculture Faisalabad, Punjab, Pakistan whose sincere help enabled the corresponding first author to start Soil Microbiology (Organic and Biofertilizer) Research at the Plant Nutrition Lab., Department of Soil Science, Sindh Agriculture University, Tandojam, Sindh, Pakistan, one and a half decades ago.

AUTHOR CONTRIBUTIONS

ZHN conceived the idea, done seed inoculation, supervised research work, edited all drafts of manuscript, and submitted research paper for publication as corresponding author; JAA conducted field study and collected data; NAT performed soil and plant analyses and statistical analysis; HJK provided logistic support and technical help during field study; FNM, IHK and IAJ reviewed literature, helped in writing introduction and discussion sections, formatted final draft for submission to journal. All authors checked and endorsed final draft of the manuscript.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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