

Assessment of phosphate-solubilizing rhizobacterial strains combined with rock phosphate and compost and their effect on the growth and yield of maize (*Zea mays* L.) under field conditions

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Abstract

Adequate phosphorus (P) supply is critical for optimum plant growth. However, soils enriched in total P content frequently do not fulfill the plant requirements due to the provided P, which may become unavailable in alkaline calcareous soils. The use of slow-release P fertilizers, such as rock phosphate (RP), compost, and plant growth-promoting rhizobacteria (PGPR) with P-solubilizing activity, can stimulate plant growth by solubilizing inaccessible P in the soil. A field study was conducted to estimate the efficacy of PGPR containing P-solubilizing and ACC-deaminase activity in soil amended with RP and compost on the production and growth of maize. Ten pre-isolated bacterial isolates were taken and tested for their effect on maize with individual and combined application of PSB, RP, and compost with a control. Overall, bacterial isolates (S5 and S6) containing P-solubilizing and ACC-deaminase activity combined with RP and compost significantly improved the maize crop development and production over the control. Finally, it is suggested that RP could be efficiently applied combined with compost and P-solubilizers.

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Introduction

Maize (*Zea mays* L.) crop is the 3rd key grain crop globally after wheat and rice. It is mostly cultivated for food, feed, and fodder (Ullah et al., 2010; Anwar et al., 2024). In Pakistan, with an agro-based economy, the maize crop was cultivated on 1,720 thousand hectares with a 4.1% increase

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compared to that of the last year, contributing 3% value added and 0.7% to GDP in 2022-23. However, its production increased by 6.9% from 9.525 MT to 10.18 MT in 2022, and was mainly due to improved yield and an increase in cultivated area (Anonymous, 2022-2023).

Phosphorus (P) is the most vital element among various nutrients required for the optimum growth of plants in agricultural crop production (Bielecki, 1973; Khan et al., 2023). It is the main component of cell ingredients such as ATP, and it plays a key role in various key biological processes like energy storage and transfer, photosynthesis, respiration, cell division, and cell enlargement (Khan et al., 2023). It is also required for initial root growth and development, and for the improvement of crop quality and seed development (Khan et al., 2010; Bossa et al., 2024). Moreover, adequate P stimulates early plant growth and hastens the maturity of crops (Busman, 1997; Nukte et al., 2010).

Globally, less soil P availability is a foremost restraint for better crop production (Gyaneshwar et al., 2002; Solangi et al., 2023). But, merely about 10-30% of the provided phosphorus-based mineral fertilizers can be recuperated by the crops sown after the fertilizer application, while the remaining become unavailable through physicochemical reactions in soil by fixing it (Drohan et al., 2019). The production of crops on the major portion of the world's arable land is inadequate by less availability of phosphorus (Vance et al., 2003; Walsh et al., 2023).

Phosphate-solubilizing bacteria (PSB) can solubilize labile P in soil and increase its availability to plants. These bacteria may release some enzymes like alkaline phosphatases and synthesize organic acids, alike malic acid, citric acid, lactic acid, and formic acid, that participate in the discharge of P from other intractable organic and inorganic soil P pools (Pan and Cai, 2023). Similarly, some PSBs can also release plant development regulators by encouraging the growth of roots, which in turn helps to enhance the uptake of P and further nutrients by plants (Rawat et al., 2021). The usage of PSBs in farming sustainably enhances crop yield and has a massive ability for handling problems with food safety worldwide, reducing ecological harms, and stimulating justifiable and resistant agricultural methods (Seifsahandi et al., 2019). Moreover, due to source scarcity, radical change in weather, and the necessity to lessen ecological dangers linked with the misuse of synthetic/ inorganic phosphatic fertilizers, PSBs have the ability to be sustainable bio-fertilizer substitutes in the sector of agriculture. Hence, phosphate-solubilizing rhizobacteria can establish an innovative field in both environmental and agricultural sciences (Ughamba et al., 2025).

Ethylene, a crop development hormone, facilitates several responses in plants (Arshad and Frankerberger, 2002; Khan et al., 2024). It is stimulatory at low levels while becoming inhibitory at high levels and can be damaging by causing premature senescence and shorter roots (Iqbal et al., 2017; Khan et al., 2024). Generally, plants synthesize ethylene in low concentrations to perform various growth attributes such as germination of seed, fruit, and leaf senescence, fruit ripening, and several stresses, including both abiotic and biotic (Yang and Hoffman, 1984; Fatma et al., 2022). 1-aminocyclopropane-1-carboxylate (ACC, originator of ethylene) is used as the nitrogen (N) source by certain microorganisms such as rhizobacteria; these microorganisms contain ACC-deaminase that encourages the growth of roots significantly by converting ACC into NH_3 and α -ketobutyrate (Glick et al., 1998; Shahid et al., 2023). Its possible inhibitory effects are reduced under low endogenous ethylene levels due to decreased ACC levels. Thus, applying particular seeds or root inoculants may inhibit endogenous ethylene synthesis, which in turn triggers physiological reactions (Glick et al., 1998). Alternatively, application of low-grade rock phosphate (RP) along with PSB to improve its solubilization is gaining more attention. Shaharoona et al. (2006) screened nine rhizobacteria with ACC-deaminase for their ability to promote *Zea mays* growth in an axenic environment and reported a significant improvement in growth attributes, particularly root and shoot growth. Thus, inoculants might be inoculated containing PSB (with and without ACC-deaminase) to resolve high ethylene and lower P solubility in soil. Siddiqui et al. (2016) suggested that the growth and yield of wheat were significantly improved by PGPR containing P-solubilizing and/or ACC-deaminase, along with RP and compost. The PGPR working as solubilizers and/ or mobilizers may contribute to reducing the harmful effects of chemical fertilizers on the environment (Siddiqui et al., 2024).

Composting is a method of recycling nutrients along with organic and inorganic fertilizers, which enhances soil quality by increasing the stability of aggregates that leads to improved soil structure, thereby promoting plant growth (Tate, 1987; Ouelid Lhaj et al., 2025). Moreover, Balda and Giri (2023) have also reported that organic wastes after composting could be used as organic fertilizer, which can support and develop the soil fertility and soil structure, ameliorate deficiency of macro- and micro-nutrients, and overall improve soil productivity and crop yield on a sustainable basis. Scientists believed that biofertilizers may not replace inorganic fertilizers. However, a combination of organic, inorganic, and biofertilizers can improve plant growth more promisingly compared to their

individual application (Gao et al., 2020). Long ago, Nevens and Reheul (2003) investigated the effects of compost on silage maize and found that improved N uptake and concentration occurred in the maize crop in sandy loam soil texture. Moreover, it is also emphasized that RP, as a cheaper P source, might be efficient to use if applied with compost and PSB (Siddiqui et al., 2016; Aslam et al., 2024). Thus, the principal objective of this planned research trial was to identify the effectiveness of soil bacteria with and without the activity of ACC-deaminase, compost, and rock phosphate for enhancing the development and production of *Zea mays* under field conditions.

Materials and Methods

A field trial was conducted during the Kharif season to assess the effectiveness of rhizobacteria containing PSB, combined with RP and compost, for enhancing crop growth and production attributes of *Zea mays* in the research area of the University of Agriculture, Faisalabad (UAF). Ten pre-isolated strains of PGPR (S1-S10) containing PSB were obtained from the Laboratory of Soil Microbiology, Institute of Soil & Environmental Sciences (ISES), UAF, Punjab, Pakistan.

Inocula preparation

The inocula of selected strains with PSB were made by the DF minimal salt medium in a conical flask (of 250 mL volume) and reared on a shaking orbital incubator with 100 rpm for 48 h at 28 ± 1 °C. After incubation, the optical density (OP) of the suspension was measured at 535 nm and achieved a value of 0.5 by dilution with distilled water to retain a constant cell density, i.e., 10^8 - 10^9 CFU/mL.

Seed inoculation

Maize seeds were inoculated with the inoculum (200 mL per kg seeds) by mixing through disinfected peat (w/w seed to peat ratio 1.25:1). A sugar solution of 10% was mixed with inoculated peat for seed dressing with the inoculum. While the seeds for the control treatment were mixed with the disinfected peat having disinfected broth through sugar solution (10%) and placed nightly at ambient laboratory conditions.

Formulation of compost and rock phosphate

A locally manufactured equipment consisting of a composter, crusher, grinder, and dryer was utilized to synthesize compost from fruit and vegetable-based organic waste. Firstly, undesired material (such as plastic bags, glass, and stones) was removed from the waste and then air-dried. To extract moisture, the material was crushed using a composter crusher. The ground material was then oven-dried for one day at 65 °C and added to the processing unit, i.e., composter, to transform organic leftovers into compost along with water (300-350 mL per kg compost) to ensure proper moisture levels during the composting process. After six days of incubation under controlled conditions, the composted material was ground into granules and stored in gunny bags before usage. Value-added PR-enriched compost was prepared according to the method described by Ahmad et al. (2008). Ratios of C: N, C: K, & C: P of the compost were 10.9, 14.8, and 35.8, respectively.

Field trial

An experiment was demonstrated by using the inoculated maize variety, i.e., Sahiwal 2002. The said seeds of the inoculated maize variety were grown in the field area as per the treatment plan. Similarly, a composite soil sample was taken from the said research field, dried (air), ground, sieved through a 2 mm sieve, and examined for physico-chemical properties of soil (Table 1). Recommended urea and K_2SO_4 were applied as a source of N and K. The following research treatment strategy was followed:

F0 = Control (NK, without PSBs, RP + Compost)

F1 = NK + RP

F2 = NK + RP + Compost

The experiment was replicated thrice, and a randomized block design (RCBD) with factorial design was used. The canal water was used to irrigate the field. After 120 days, crop growth and production parameters were recorded according to standard procedure. The efficacy of inoculation was investigated with and without RP, PSBs, and compost.

Table 1: Physicochemical attributes of the field trial

Properties of soil	Value	Reference
Physical properties of soil		
Textural class	Sandy clay loam	Bouyoucos (1962)
Sand (g/kg)	560 ± 4.61	
Clay (g/kg)	245 ± 5.49	
Silt (g/kg)	195 ± 3.84	
Chemical properties of soil		
ECe (µS/cm)	2584 ± 38.6	
pH	7.62 ± 0.03	
Soil organic matter (g/kg)	8.74 ± 0.15	Walkley and Black (1934)
Available soil K (mg/kg)	169.3 ± 7.21	US Salinity Laboratory Staff (1954)
Total soil N (mg/kg)	463.8 ± 13.6	Jackson (1958)
NaHCO ₃ Extractable-P (mg/kg)	8.78 ± 0.15	Watanabe and Olsen (1965)

Values are means of three replicates, followed by (±) standard error of means

Characterization of bacterial isolates

The bacterial strains were characterized for phosphate solubilization, root colonizing ability, production of IAA, ACC-deaminase, and chitinase activity. ACC-deaminase activity in the cell was checked as per the technique described by Duan et al. (2009). Bacterial isolates were grown in 20 µg/mL tetracycline (TY) for 2 to 3 days at 28 ± 1 °C and washed twice with 0.1 M tris-hydrochloride adjusted at pH 7.5 and resuspended in modified M9 minimal medium with 5 mM ACC-deaminase. The media was incubated for 40 hours at 28 °C in a shaker. Later, the ACC-deaminase activity was assessed by α-ketobutyrate formed from ACC breakdown (Penrose and Glick, 2001). The technique of Mehta and Nautiyal (2011) was applied to investigate the ability of isolates for the solubilization of inorganic phosphate. Phosphate growth media was used, i.e., NBRI-PBB (National Botanical Research Institute Phosphate Bromophenol Blue). At 0.5 optimal density, 10⁷-10⁸ CFU/mL was obtained in three-day-old broth cultures. Petri plates were inoculated with a loopful of each culture and incubated for seven days at 28 ± 1 °C. After a week, colonies presenting clear zones around them were categorized as positive for phosphate solubilization and others as negative. Root colonization potential was checked in an axenic environment (Simons et al., 1996). The chitinase activity was calculated according to the process outlined by Cattelan et al. (1999) (Table 2).

Table 2: Characterization of plant growth-promoting activities in selected bacterial isolates

Bacterial isolate	Colony color	ACCd activity (α-keto nmol mg ⁻¹ protein h ⁻¹)	Indole-acetic acid (mg L ⁻¹)		Phosphate solubilization	Chitinase activity	Root colonization (CFU g ⁻¹ root)
			Without TRP	With TRP			
S1	Cream	369.4 ± 12.7	9.13 ± 1.31	14.32 ± 1.78	ND	++	6.43 × 10 ⁵
S2	Brown	342.9 ± 10.9	7.39 ± 1.23	13.70 ± 2.01	ND	+	4.86 × 10 ⁵
S3	White	ND	6.47 ± 1.20	10.56 ± 1.33	++	+	7.12 × 10 ⁵
S4	Pink	ND	7.12 ± 0.97	11.08 ± 1.24	+	++	6.37 × 10 ⁵
S5	Pink	392.7 ± 11.3	6.41 ± 1.12	11.39 ± 1.39	+++	++	6.29 × 10 ⁶
S6	Pink	364.2 ± 13.1	6.90 ± 1.09	10.87 ± 1.34	++	++	5.98 × 10 ⁶
S7	Pink	361.1 ± 11.1	6.80 ± 1.19	10.47 ± 1.54	++	++	5.87 × 10 ⁶
S8	Pink	363.2 ± 13.4	6.40 ± 1.21	11.07 ± 1.24	++	++	6.03 × 10 ⁶
S9	Pink	362.3 ± 12.8	6.18 ± 1.24	11.37 ± 1.44	++	++	6.11 × 10 ⁶
S10	Pink	363.2 ± 13.9	6.27 ± 1.07	10.67 ± 1.37	++	++	5.94 × 10 ⁶

Data are means of three replicates trailed by (±) standard error of means. ACCD: 1-aminocyclopropane-1-carboxylate deaminase. α-keto: α-ketobutyrate, single positive means halo size < 2 mm, double positive means halo size > 2 mm, while triple positive means halo size > 3, ND: Not detected

Statistical analysis of the data

All research data were subjected to one-way ANOVA (analysis of variance) using the MSTAT-C. With the use of Duncan's multiple range test, all treatment means were compared using the LSD (Least Significant Difference) for significant or non-significant differences at *P* < 0.05.

Results and Discussion

All growth attributes of the maize crop were significantly improved by the integrated application of PSB combined with RP and compost compared to the control. In case of F0, there was a significant increase in plant height (23.05%), total biomass (35.72%), fresh straw weight (32.55%), oven-dried

straw weight (41.54%), fresh ear weight (59.66%), cob weight (24.48%), cob length (33.37%), number of rows/ cob (25.29%), 100 grain weight (32.36%), and grain yield (33.44%), respectively as compared to those of the control treatment (Figs. 1-10). In case of F1, there was a significant enhancement in plant height (23.25%), total biomass (41%), fresh straw weight (37.02%), oven-dried straw weight (47.39%), fresh ear weight (62.52%), cob weight (25.48%), cob length (35.96%), number of rows/ cob (29.18%), 100 grain weight (38.64%), and grain yield (40.84%), respectively as compared to those of the control treatment (Figs 1-10). In this trial in F2, there was also a marked improvement in plant height (44.48%), total biomass (111%), fresh straw weight (127%), oven-dried straw weight (172%), fresh ear weight (101%), cob weight (83.48%), cob length (51.25%), number of rows/cob (44.29%), 100 grain weight (120.55%), and grain yield (94.70%), respectively regarding those of the control treatment (Figs. 1-10).

Ten strains, S1, S2, S3, S4, S5, S6, S7, S8, S9, and S10 alongside with control (S0) were tested. There was an upsurge of 23.05%, 23.25% and 48.44% in plant height in the control, NK + RP, and NK + RP + Compost, respectively, as compared to the control (uninoculated).

Likewise, marked enhancements of 35.72%, 41.0% and 111% in total biomass in the control treatment, NK + RP, and NK + RP + Compost, as compared to the control (uninoculated), were noted. There was an upsurge in fresh straw weight, i.e., 32.55%, 37.02% and 127% respectively in control, NK + RP, and NK + RP + Compost, as compared to the control (uninoculated). Moreover, significant increases were recorded, such as 41.54%, 47.39% and 172.2% in oven-dried straw weight in the control, NK + RP, and NK + RP + Compost, respectively, as compared to the control (uninoculated). In addition, growth in fresh ear weight was 59.66%, 62.52% and 101% in the control, NK + RP, and NK + RP + Compost, respectively, as compared to the control (uninoculated). Alike, the rise in grain yield was 33.44%, 40.84% and 94.70%, respectively, in the control, NK + RP, and NK + RP + Compost over the uninoculated control. Similar outcomes were seen in fresh cob weight, 100 grain yield, cob length, and number of rows/cob, which displayed that inoculation of maize seeds with different phosphate-solubilizing bacteria with ACC deaminase meaningfully enhanced all parameters over the uninoculated control (**Figures 1-10**).

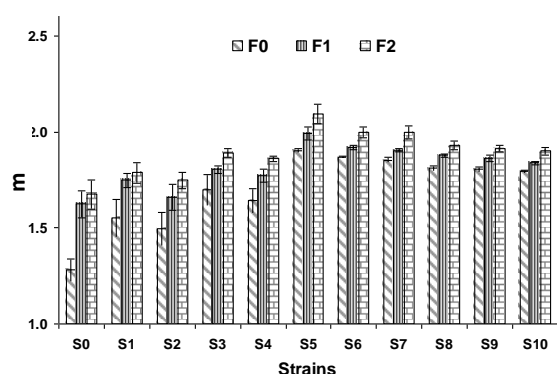


Figure 1: Effect of PSB, RP, and Compost on plant height of the maize crop

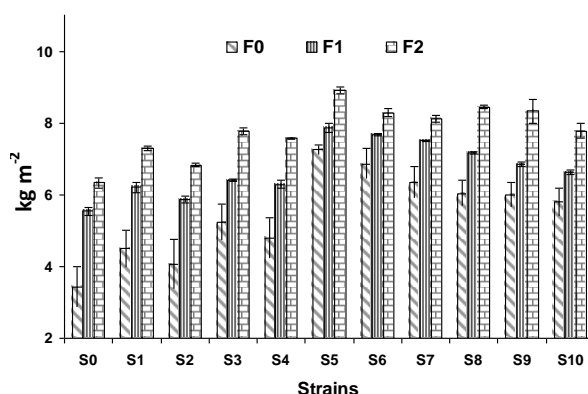


Figure 2: Effect of PSB, RP, and Compost on total plant biomass of the maize crop

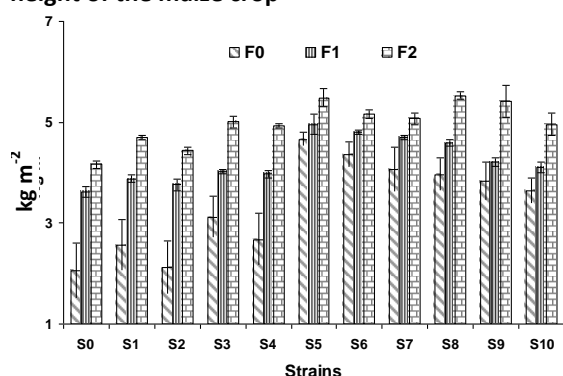


Figure 3: Effect of PSB, RP, and Compost on fresh straw weight of the maize crop

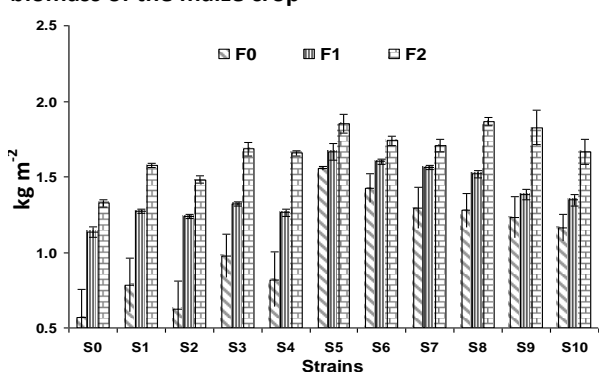


Figure 4: Effect of PSB, RP, and Compost on dry straw weight of the maize crop

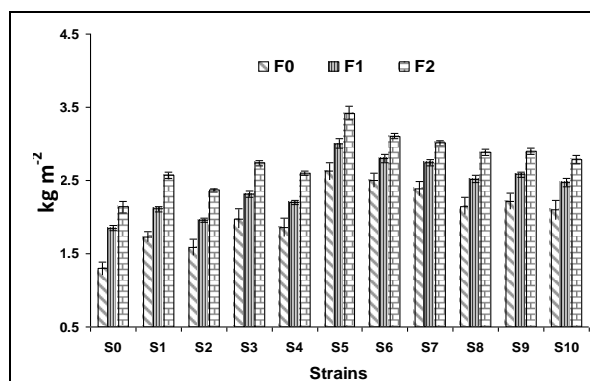


Figure 5: Effect of PSB, RP, and Compost on fresh ear weight of the maize crop

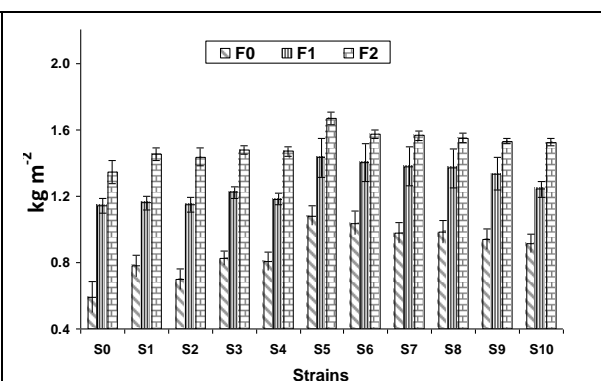


Figure 6: Effect of PSB, RP, and Compost on cob weight of the maize crop

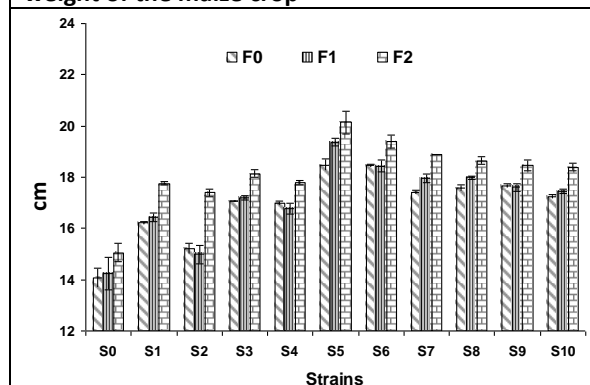


Figure 7: Effect of PSB, RP, and Compost on cob length of the maize crop

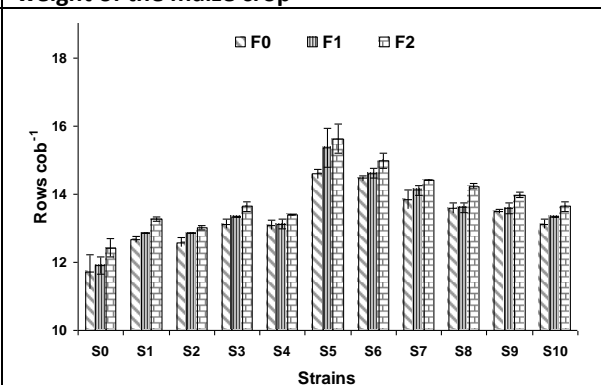


Figure 8: Effect of PSB, RP, and Compost on the number of rows/cob of the maize crop

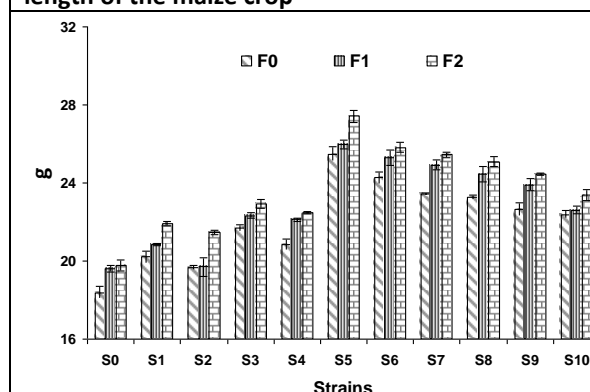


Figure 9: Effect of PSB, RP, and Compost on hundred-grain weight of the maize crop

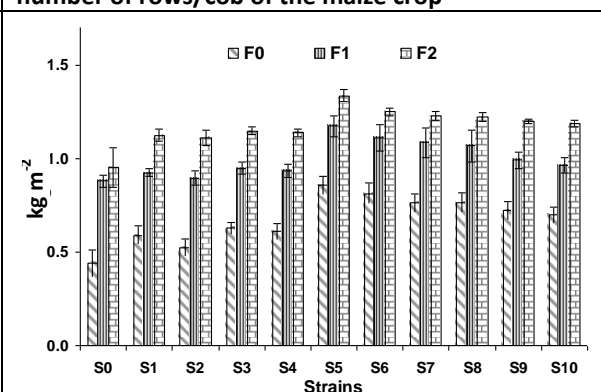


Figure 10: Effect of PSB, RP, and Compost on grain yield of the maize crop

Phosphorus-lacking roots created twice as much ethylene (DW basis) as P-sufficient plants. Phosphorus-deficient treatment inhibited shoot and root development but increased ethylene production in roots, as previously reported (Borch et al., 1999). Inoculation with PSB may have reduced endogenous inhibitory ranks of ethylene in growing seeds and roots, resulting in improved development due to their ACC-deaminase and P-solubilizing activities. Similar findings have been documented in various studies (Chabot et al., 1996; Glick et al., 1998; Mayak et al., 1999; Wang et al., 2000; Shaharouna et al., 2003).

Rhizobacteria use ACC as a nitrogen source via deaminase activity, converting it into alpha-ketobutyric acid and ammonia rather than producing ethylene (Arshad and Frankenberger, 2002). To confirm the theory that reduced ethylene stages were accountable aimed at enhanced root length, Penrose and Glick (2001) found that roots from treated seeds with *P. putida* pRKACC and *Enterobacter cloacae* CAL3, which transmit a gene of ACC-delaminase on a wide plasmid host range, were 28% and 73% higher than those of the roots from the control plants when exposed to magnesium sulphate, respectively. The isolates engaged in solubilization of P, as well as greater foraging of soluble P,

can improve crop development by increasing the accessibility of additional trace elements, boosting the efficacy of biological nitrogen fixation, and producing PGPR (Elahaisoufi et al., 2022).

Based on the results of this study, it is evident that PSBs having ACC-deaminase play a significant role in increasing the development and harvest of *Zea mays* crop; however, many plant development-promoting characters might have been tangled in complete development elevation of *Zea mays* by relevant PGPR strains and development-stimulating action due to the combination of PGPR with compost and rock phosphate. They could not merely protect the chemical fertilizer but might also cause improved development and harvest by enhancing nutrient status in the standing crop. Moreover, PSBs having ACC deaminase can significantly improve the development and production of *Zea mays*.

Conclusion

The current study was conducted to improve the development and production of the maize crop by the unified implementation of RP and compost by inoculation with PSB with and without ACC deaminase. Among ten isolates, S5 and S6 containing P-solubilizing and ACC-deaminase activities integrated with RP and compost significantly enhanced all development and production attributes of the maize crop, particularly plant height, total biomass, cob weight and length, and grain yield over the control. It is concluded that the PGPR with multiple traits is more effective than those with fewer traits. Moreover, RP could be efficiently applied with compost and P-solubilizers. The outcomes suggest that PGPR can be used to develop an effective biofertilizer as a supplement to inorganic fertilizers. Finally, the results concluded that maize crop inoculation with PSB having ACC deaminase integrated with slow-release fertilizers like RP and compost can effectively improve growth and yield and may lead to the development of sustainable soil-plant systems and farming.

Author(s), Editor(s) and Publisher's declarations

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Contribution of authors

Conceived the idea: AR, FB, ARS, SN, AS, MAP, SAJ, SJ, MB. Conduct of research, AR, ARS. Data analysis: AR, FB, ARS, SN, AS, MAP. Preparation of first draft: AR, ARS, SN, AS, MAP, SAJ, SJ, MB, FB, MAQ. Revision of the manuscript and reading of the proof: All authors.

Permissions and ethical compliance

This study does not involve human/animal subjects, and thus no ethical approval is required.

Handling of bio-hazardous materials

The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination.

Supplementary material

No supplementary material is included with this manuscript.

Conflict of interest

The authors declare no conflict of interest.

Availability of primary data and materials

As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

Authors' consent

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