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EFFECT OF ISOLATED PLANT GROWTH PROMOTING RHIZOBACTERIA ON GROWTH AND NUTRIENTS UPTAKE BY MAIZE IN ACIDIC AND ALKALINE SOIL CONDITIONS

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Abstract

Inoculation with plant growth-promoting rhizobacteria is imperative to improve the yield of crops on a sustainable basis. To evaluate the competence of PGPR as a biofertilizer and its effects on the growth, yield, and nutrient uptake of maize, a series of experiments were conducted during 2019-2021. Twenty rhizosphere samples were collected from maize field (ten from the acidic soil of Mansehra and ten from the alkaline soil of Islamabad) for isolation of PGPR, and forty PGPR strains (20 PGPR strains from acidic and 20 PGPR strains from alkaline) were isolated and characterized for multi-plant growth promoting traits. Six PGPR strains ($MS_6$4c, $MS_3$5b, $MS_5$4c from acidic and $IS_1$4b, $IS_3$3c, and $IS_5$4b from alkaline soils) were selected based on P solubilization and N fixation. The effect of these six PGPR strains was determined on the growth and nutrient uptake of maize in the pots experiment. Results revealed that most of the strains belong to *Bacillus* species, and all the strains have the capability of P solubilization efficiency, NH3 production, and enzyme and hormone production. In the pot experiment, an inoculation of PGPR strain $IS_3$3c in alkaline soil significantly ($p \leq 0.05$) increased total dry matter yield (94.11 g pot^{-1}) with a 65% increase over uninoculated treatment. The N uptake of 1.44 g pot^{-1} with 130% and P uptake of 0.153 g pot^{-1} with 134 % increase over control was recorded with strain $IS_3$3c inoculation in alkaline soil followed by strain $MS_6$4c inoculation in acidic soil with N 1.12 g pot^{-1} and $IS_1$4b inoculation in alkaline soil with P uptake of 0.132 g pot^{-1} respectively. Maximum AB-DTPA extractable soil NO_3-N of 19.94 mg kg^{-1} and P contents of 12.13 mg kg^{-1} were recorded in treatment where PGPR strain $IS_3$3c inoculated in alkaline soil followed by the soil NO_3-N content of 17.45 and P content of 10.56 mg kg^{-1} by the inoculation of PGPR strain $IS_5$4b in alkaline soil. The population density of bacteria in maize rhizosphere ranged from 51.4 to 79.6 x 10^7 cfu g^{-1} soil inoculated with different strains of PGPR. Research findings suggested crop-specific PGPR strains can efficiently colonize a crop's roots and improve crop yield through their multi-plant growth-promoting traits. Using PGPR strains as a biofertilizer can potentially improve crop yield and nutrient uptake sustainably and minimize the dependence on chemical fertilizers.

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Introduction

Nitrogen (N) is the primary nutrient involved in various plant processes (Araújo *et al*., 2012). Nitrogen is deficient worldwide, and about 50-75% of applied N is lost through leaching, denitrification, and volatilization (Alva *et al*., 2006). Nitrogen is not part of the parent material; 78% of elemental nitrogen is present in the atmosphere, which is unavailable to plants (Ashitha *et al*., 2021). Demand for N fertilizer will increase to 0.87 million metric tonnes by 2050. Due to these alarming situations, researchers worldwide are concerned about increasing the efficiency of N. by introducing different N biofertilizers (Pathak & Ladha, 2012). Using N biofertilizer reduces the N requirement from fertilizer in non-leguminous plants (Sreethu *et al*., 2024). N-fixing PGPR has a gene (if) that carries out the process of N-fixation. Atmospheric N_2 is converted into NH^{+4} inside bacterial cell cytoplasm and released outside by osmosis. The nitrogen-fixing ability of PGPR in non-leguminous crops reduces the dependency on N chemical fertilizers. (Mia & Shamsuddin, 2010).

Demand for P has been a burning issue for the last century due to the continuous mining of rock phosphate. About 83% of P deposits in Morocco, the USA, China, and South Africa are exploited with time due to the high demand for P fertilizer (Vaccari, 2009). If rock phosphate is utilized at this rate, there will be a P fertilizer crisis in the next 50 to 100 years. Plants take up about 20% of applied fertilizer, while the rest is either fixed in soil or lost by runoff. The availability of this residual P to plants is also deficient due to the low rate of diffusion of P to roots (Syers *et al*., 2008). The availability of P is also pH-dependent low in alkaline and acidic soils due to the formation of insoluble compounds of P with calcium, magnesium, aluminum, and Iron (Ahmad *et al*., 2018, Saentho *et al*., 2022).Residual P in the soil is the most neglected P source for plants. It is essential to recover the residual P in the soil (Cordell *et al*., 2009). Different species of P solubilizing bacteria are present in the rhizosphere that solubilize the insoluble P by releasing organic acids that lower the pH around the insoluble P form and release phosphate from the attached cation by solubilizing the bond or chelating cation with phosphate (Rawat *et al*., 2021).

Rhizobacteria, as a biofertilizer, can be used to reduce the use of inorganic fertilizers. In Pakistan, farmers use biofertilizer, which is less than 2% of the total fertilizers used on farms (Shand, 2007). It is essential to create awareness among farmers about eco-friendly agriculture. Plant growth-promoting rhizobacteria (PGPR) play an essential role in sustainable agriculture by boosting plant growth through direct and indirect mechanisms. (Bhattacharyya & Jha, 2012) Rhizobacteria regulate the nutrient balance by preventing its sequestration in the soil by solubilizing or chelating nutrients for easy uptake by the plant (Khati *et al*., 2017).

Maize (*Zea mays* L.) belonged to the family *Poaceae* and was first developed by individuals in antiquated Central America. It is presently the third most-grown grain after wheat and rice (Sahoo *et al*., 2021). The total area of Pakistan under maize cultivation is 1417.8 thousand hectares, with a production of 8939.8 thousand tons (Pakistan Bureau of Statistics, 2020-21). Maize is a nutritious crop; an enormous number of chemical fertilizers are used for N, P, and K to increase yield per unit area, adversely affecting soil health and the environment and is uneconomical. (Hogan, 2021, Zainab *et al*., 2021).

Considering the significance of PGPR in crop production, this project was conducted to isolate, characterize, and determine the inoculation effect of indigenous PGPR on productivity and nutrient uptake by maize in acidic and alkaline soil conditions.

Material and Method

Collection of rhizosphere soil samples: Maize rhizosphere soil samples (20) were collected from different maize fields of acidic soil of Shinkiari, Mansehra, and alkaline soil condition of Islamabad area.

Isolation of bacteria from soil samples: Bacteria from rhizospheric soil samples were isolated using the serial dilution method; for isolation of rhizospheric bacteria, Luria Bertini (LB) media was prepared and autoclaved for 2 hours at 121ºC. The autoclaved LB was poured into the plates and allowed to cool. For spreading, 0.1 mL suspension was picked from each tube with the help of a micropipette and spread in four directions after these plates were incubated at 28ºC. (Maniatis *et al*., 1982).

Gram staining and cellular morphology: From the purified streak plates, a small number of bacteria was placed on a slide, two to three drops of water were poured and stirred with the help of a loop to dissolve bacterial colonies and dried to fix the smear. The bacteria smear was stained with a primary stain (crystal violet) for 1 minute, then washed with tap water carefully. The stained smear was then treated with gram iodine for 1 minute and washed with alcohol for 3 seconds to retain the purple color by shrinking and tightening the bacterial cells. The bacterial smear was then flooded with a second stain (safranin) for 1 minute and washed with tap water. The slides were then air-dried for microscopy. The bacteria with pink shading were Gram-negative, while purple was Gram-positive (Vincent, 1970).

Biochemical characterization of isolated bacteria: The isolated bacteria were biochemically characterized by different plant growth-promoting traits.

Phosphate solubilization: To detect phosphate solubilizing bacteria, each bacterial strain was spot inoculated on Pikovskaya's agar and incubated for 7 days at 28 ± 2ºC. After incubation, the halo zone around the colony was measured for phosphate solubilization using the method suggested by (Nautiyal, 1999).

Ammonia (NH_3) production: Inoculating broth bacterial strains checked ammonia production, as (Cappuccino & Sherman, 1992). A broth containing peptone water was incubated for 2 days at 30ºC. The appearance of a dark yellow color upon adding Nessler's reagent was taken positive for NH_3 production.

Indole acetic acid (IAA) production: The purified bacteria were tested for growth regulatory hormone indole acetic acid (IAA) production. A broth used for indole acetic acid-producing bacteria containing 10 g of peptone and 5 g of NaCl in 1 L of distilled water was prepared to determine IAA-producing bacteria. For this purpose, different bacterial strains were inoculated in their respective broth-containing tubes and incubated at 28 ± 2ºC for 5 days. After 5 days of incubation, 1 mL of Kovacs reagent was added to each tube, and its color was observed. (MacFaddin, 2000, Harley, 2005) described that the cherry red ring appeared in the presence of IAA-producing bacteria, while no green color was taken as the absence of IAA.

Pot experiment: A pot experiment was conducted to determine the effect of different isolated PGPR strains on maize's growth and nutrient uptake in acidic and alkaline soils. Soil samples were collected from different representative fields of Mansehra's acidic and Islamabad's alkaline soil conditions. Pots were filled with 10 kg sterilized acidic as well as alkaline soils. Three effective PGPR strains ($MS_6$4c, $MS_3$5b, $MS_5$4c) from acidic soil and three from alkaline soil ($IS_1$4b, $IS_3$3c, $IS_5$4b) were selected based on phosphate solubilization and N fixation. These potential PGPR strains were grown in their broth media for 3-7 days at 25ºC. Inoculums of respective PGPR were prepared by pouring broth of PGPR strains with 10^8 bacterial colony forming units (cfu) at 160 mL kg^{-1} in their respective carrier (1 kg mineral soil collected from northern areas). Maize seeds were soaked in sugar solution for 10-15 minutes and coated with an inoculum of respective PGPR with 10^8 bacterial colony forming units (cfu), and maize seeds were sown @ 5 seeds per pot and then thinned up to 3 plants per pot. Pots were arranged in two factors, using a completely randomized design (CRD) with three replications. Nutrients N, P, and K at the rate of 75, 45, and 30 kg ha^{-1} (half of the recommended dose) were applied as a basal dose in the form of urea, single super phosphate (SSP) and sulfate of potassium (SOP), respectively. Treatment combinations during study were, T1: Acidic soil (S- I) + N, P and K at the rate of 75-50-30 kg ha^{-1} as a basal dose, T2: Alkaline soil (S- II) + N, P and K at the rate of 75-50-30 kg ha^{-1} as a basal dose, T3: S - I + $MS_6$4c (Selected from acidic soil), T4: S - I + $MS_3$5b (Selected from acidic soil), T5: S - I + $MS_5$4c (Selected from acidic soil), T6: S - I + $IS_1$4b (Selected from alkaline soil), T7: S - I + $IS_3$3c (Selected from alkaline soil), T8: S - I + $IS_5$4b (Selected from alkaline soil), T9: S - II + $MS_6$4c, T10: S - II + $MS_3$5b, T11: S - II + $MS_5$4c, T12: S - II + $IS_1$4b, T13: S - II + $IS_3$3c, T14: S - II + $IS_5$4b.

Post-harvest soil and plant analysis: For chemical analysis, plant samples were ground and were determined

for plant N concentration (Bremner, 1996), P concentration (Westermann, 2005), and N and P uptake (Sharma *et al*., 2012). Soil samples were collected from each treatment after crop harvest, were air dried and sieved through a 2 mm sieve, and used for the analysis of Soil Texture (Koehler *et al*., 1984), N, P contents, and pH by using standard methods of (Kamphake *et al*., 1967, Soltanpour & Schwab, 1977, McLean, 1983) respectively.

Bacteria population density: Roots, along with adhering soil, were uprooted from each treatment. Bacteria were isolated from rhizosphere soil, and the population density of bacteria was determined by procedure (James, 1978).

Statistical analysis

Two-factor CRD analyzed the recorded data at a 5% level of most minor significant difference by the producer of (Scientific, 1991) using software Statistic 8.1.

Results

Gram staining and cellular morphology: Cellular morphology of forty isolated strains from acidic and alkaline soils was observed for gram staining, which are given in (Tables 1 and 2). Most strains did not retain purple when washed with alcohol and were gram-negative. The isolated from both soils had rod shape followed by spherical shape bacteria. The standard types of bacteria in acidic and alkaline soil were diplobacillus and streptobacillus.

Biochemical characterization of isolated bacteria: The forty isolated bacterial strains from maize rhizosphere of acidic and alkaline soils were screened for phosphorus solubilizing efficiency (PSE), NH_3 Production, and Indole acetic acid production (Tables 3 and 4). Among the 20 isolates from acidic soil, 40% of the strains had a PSE greater than 100 compared to only 25% of the bacterial strains isolated from alkaline soil. In acidic soil, the maximum PSE of 171% was determined by the PGPR strain, $MS_6$4c, while $MS_2$5c and $MS_5$4a had minimum PSE of 29% and 25%, respectively. About 25% of the 20 isolated strains from alkaline soil had PSE greater than 100%, while 25% of the bacterial isolates had PSE greater than 50%. The strain $IS_3$3c isolated from alkaline soil had a very high PSE of 250%, and two isolated strains, $IS_4$4a and $IS_2$4b, had minimum PSE of 13 and 14, respectively. About 55% of the microbial strains isolated from acidic soil had medium production of ammonia, while 25% had weak ammonia production, and 20% of total isolates had no ammonia activity. About 45% of the isolates had high ammonia production, while 25% of the strains had medium production. About 25% of the strains had weak production ability of ammonia, while 5% of the bacterial isolates could not produce ammonia. Strains isolated from alkaline soil produced more indole acetic acid (lAA) than strains isolated from acidic soil. Among twenty isolated strains from acidic soil, 15% of the isolates were identified with high production ability of indole acetic acid (IAA), 20% had moderate or medium production ability, and 50% of the strains had weak production ability of IAA acid, and 15% had shown no IAA activity. Only 15% of the 20 bacterial isolates from alkaline soil had a high production ability of IAA. About 50% of the isolated strains had medium production of IAA, 20% of the strains had weak IAA production ability, and no IAA production was observed in 15% of the strains.

Table 1. Gram staining and PGPR cell morphology isolated from maize rhizosphere of acidic soil.

Soil series	Location	PGPR strains	Gram staining	Color	Shape of cell	Grouping
Mansehra	National Tea and High-Value Crop Research Center, Shinkiari	$MS_1$3a	–	Pink	Bacillus	Diplobacillus
		$MS_2$5a	–	Pink	Bacillus	Streptobacillus
		$MS_3$3C	–	Pink	Coccus	Diplococcus
		$MS_3$4C	–	Pink	Bacillus	Streptobacillus
		$MS_3$5a	+	Purple	Bacillus	Diplobacillus
		$MS_3$5b	–	Pink	Bacillus	Streptobacillus
		$MS_4$3a	–	Pink	Bacillus	Diplobacillus
		$MS_4$3b	–	Pink	Bacillus	Bacillus
		$MS_4$3c	–	Pink	Bacillus	Streptobacillus
		$MS_4$5a	–	Pink	Coccus	Coccus
		$MS_5$4a	+	Purple	Coccus	Staphylococcus
		$MS_5$4b	–	Pink	Bacillus	Diplobacillus
		$MS_5$4c	–	Pink	Bacillus	Diplobacillus
		$MS_5$5a	–	Pink	Bacillus	Streptobacillus
		$MS_5$5b	+	Purple	Bacillus	Diplobacillus
		$MS_5$5c	–	Pink	Bacillus	Streptobacillus
		$MS_6$3a	–	Pink	Bacillus	Diplobacillus
		$MS_6$4a	–	Pink	Bacillus	Diplobacillus
		$MS_6$4c	–	Pink	Bacillus	Diplobacillus
		$MS_6$5a	–	Pink	Coccus	Diplococcus

Maize growth parameters: The six most efficient strains, three from acidic and three from alkaline soils, were selected based on P solubilization and N fixation. The effect of strains on the growth and nutrient uptakes by maize grown in alkaline and acidic soils was evaluated by conducting a pot experiment. Data (Table 5) illustrated the effect of different PGPR strains on maize plant height and total dry matter. The effect of strains $IS_3$3c and $IS_5$4b of alkaline origin and $MS_6$4c and $MS_3$5b of acidic origin on plant height (101.70, 100.48, 97.41, and 92.97 cm, respectively) were statistically similar. Overall, soil types had a non-significant ($p \leq 0.05$) effect on maize plant height. In the interactive effect (Fig. 1a), a maximum maize height of 106 cm was in alkaline soil inoculated with $IS_3$3c. The lowest plant height, 76.4 cm, was recorded in uninoculated control of acidic soil, significantly different from all other treatment combinations except alkaline soil uninoculated control with 82.6 cm maize plant height.

Among the PGPR strains, $IS_3$3c inoculation produced the maximum total dry matter yield (85.57 g pot^{-1}), followed by $MS_6$4c (74.44 g pot^{-1}) and $IS_1$4b (72.92 g pot^{-1}), irrespective of soil type. Alkaline soil had significantly ($p \leq 0.05$) higher total dry matter yield (72.03 g pot^{-1}) mass than acid soil (68.53 g pot^{-1}). In Interaction (Fig. 1b), the inoculation of $IS_3$3c in alkaline soil maximum total dry matter yield (94.11 g pot^{-1}) followed by $IS_1$4b (83.19 g pot^{-1}) in alkaline soil condition and $MS_6$4c (78.22 g pot^{-1}) in acidic soil. The PGPRs isolated from acidic and alkaline soils had performed better for total dry matter yield under their respective soil type than control with no PGPR inoculation in acidic and alkaline soils, respectively.

Table 2. Gram staining and PGPR cell morphology isolated from maize rhizosphere of alkaline soil.

Soil series	Location	PGPR strains	Gram staining	Color	Shape of cell	Grouping
Islamabad	National Agricultural Research Center	$IS_1$4a	–	Pink	Coccus	Diplococcus
		$IS_1$4b	+	Purple	Bacillus	Streptobacillus
		$IS_1$4c	–	Pink	Virgo	Vibrio
		$IS_1$5b	–	Pink	Bacillus	Streptobacillus
		$IS_2$4a	–	Pink	Bacillus	Diplobacillus
		$IS_2$4b	+	Purple	Bacillus	Diplobacillus
		$IS_2$5c	–	Pink	Bacillus	Streptobacillus
		$IS_3$3b	–	Pink	Coccus	Coccus
		$IS_3$3c	–	Pink	Bacillus	Diplobacillus
		$IS_3$4a	–	Pink	Bacillus	Streptobacillus
		$IS_3$5a	–	Pink	Bacillus	Streptobacillus
		$IS_4$1a	–	Pink	Bacillus	Diplobacillus
		$IS_4$1b	–	Pink	Coccus	Streptococcus
		$IS_4$1c	+	Purple	Bacillus	Diplobacillus
		$IS_4$1d	–	Piink	Bacillus	Streptobacillus
		$IS_4$4a	–	Piink	Bacillus	Diplobacillus
		$IS_4$4b	–	Piink	Bacillus	Streptobacillus
		$IS_5$4a	–	Piink	Bacillus	Diplobacillus
		$IS_5$4b	–	Piink	Bacillus	Streptobacillus
		$IS_5$4c	+	Purple	Bacillus	Diplobacillus

Table 3. Biochemical Characterization of PGPR isolated from maize rhizosphere of acidic soil.

Soil type	Location	Strains	P solubilization efficiency (%)	NH_3 Production	Indole acetic acid
Mansehra	National Tea and High-Value Crop Research Center, Shinkiari	$MS_1$3a	120	++	-
		$MS_2$5a	83	+	-
		$MS_3$3C	60	+	+++
		$MS_3$4C	29	++	+
		$MS_3$5a	75	++	+
		$MS_3$5b	167	++	++
		$MS_4$3a	40	-	+
		$MS_4$3b	71	++	+
		$MS_4$3c	125	++	+
		$MS_4$5a	113	+	-
		$MS_5$4a	25	+	+
		$MS_5$4b	60	-	+++
		$MS_5$4c	150	++	++
		$MS_5$5a	100	++	+
		$MS_5$5b	120	+	+
		$MS_5$5c	80	-	+
		$MS_6$3a	100	-	+
		$MS_6$4a	111	++	++
		$MS_6$4c	171	++	+++
		$MS_6$5a	60	++	++

(-) = Negative, (+) = Low, (++) = Moderate, (+++) = High

Table 4. Biochemical Characterization of PGPR isolated from maize rhizosphere of alkaline soil.

Soil type	Location	Strains	P solubilization efficiency (%)	NH_3 Production	Indole acetic acid
Islamabad	National Agricultural research center	$IS_1$4a	50	+++	-
		$IS_1$4b	175	+++	++
		$IS_1$4c	33	-	++
		$IS_1$5b	100	+	-
		$IS_2$4a	33	++	-
		$IS_2$4b	14	+	++
		$IS_2$5c	20	+++	++
		$IS_3$3b	75	+++	+
		$IS_3$3c	250	++	+++
		$IS_3$4a	80	+	+++
		$IS_3$5a	60	++	++
		$IS_4$1a	50	++	+
		$IS_4$1b	40	+++	+
		$IS_4$1c	75	+	++
		$IS_4$1d	33	+	+++
		$IS_4$4a	13	+++	++
		$IS_4$4b	150	++	++
		$IS_5$4a	133	+++	+
		$IS_5$4b	225	+++	++
		$IS_5$4c	50	+++	++

(-) = Negative, (+) = Low, (++) = Moderate, (+++) = High

Table 5. Plant height, dry matter, as influenced by different PGPR strains in acidic and alkaline soils.

PGPR strains	Plant height (cm)	Dry matter (g pot^{-1})
Control	79.56 d*	56.41 d*
$MS_6$4c	97.41 abc	74.44 b
$MS_3$5b	92.97 abc	64.53 c
$MS_5$4c	90.33 c	69.31 bc
$IS_1$4b	91.79 bc	72.92 b
$IS_3$3c	101.70 a	85.57 a
$IS_5$4b	100.48 ab	68.78 bc
LSD $_{(0.05)}$	9.6421	5.7696
Soil types		
Acidic soil	93.18	68.53 b
Alkaline soil	93.75	72.03 a
LSD $_{(0.05)}$	NS	3.0840
Interaction		
P*S	*	*

* Significant at p<0.05, PGPR (P): Plant growth promoting rhizobacteria, Soil types (S), means followed by same alphabets are statistically similar

Post harvest soil properties: The different PGPR strain (Table 6) effect on soil pH was insignificant. The effect of soil types on soil pH was significant. ($p \leq 0.05$). A maximum pH of 7.70 was recorded in alkaline soil, which is not due to PGPR inoculation but is inherent. The interactive effect of soil types and PGPR strains on pH was non-significant (Fig. 2a). The different types of PGPR caused a significant variation ($p \leq 0.05$) in soil NO_3-N (Table 6) after harvesting maize. The strain $IS_3$3c had a maximum mean NO_3-N content of 17.06 mg $kg^{-1,}$ followed by $IS_5$4b and $MS_6$4c, which had a mean NO_3-N content of 15.57 and 15.12 mg $kg^{-1,}$ respectively. Alkaline soil has a significant ($p \leq 0.05$) effect on soil NO_3-N content (14.22 mg kg^{-1}) than acidic soil (13.41 mg kg^{-1}). The maximum NO_3-N (Fig. 2b) was determined in the interactive effect of $IS_3$3c (19.94 mg kg^{-1}) under alkaline soil conditions that significantly varied from all the other treatment combinations under acidic and alkaline soils. The alkaline and acidic soils inoculated with different PGPRs had higher NO_3-N than treatments without PGPR, i.e., acidic and alkaline soil control (NO_3-N, 8.56 and 9.28 mg kg^{-1}). Among the PGPRs, data in Table 6 showed that $IS_3$3c had the more excellent residual available soil P contents (10.19 mg kg^{-1}) followed by $IS_5$4b, which had P content (8.85 mg kg^{-1}) irrespective of soil type, indicating the effectiveness of these strains for enhancing P bioavailability in soils at different soil pH. The effect of soil types on P availability in soil was nonsignificant. In interaction, maximum available P (12.1 mg kg^{-1}) (Fig. 2c) after harvesting of maize was determined in the treatment having $IS_3$3c under alkaline soil conditions, suggesting sufficient available P even at a post-harvest stage in soil followed by the inoculation of $IS_5$4b under alkaline soil with (10.5 mg kg^{-1}). The lowest available residual P (3.03 and 3.17 mg kg^{-1}) was determined in the treatments where no inoculation of PGPR was carried out in both acidic and alkaline soils, respectively, than in soils treated with PGPRs.

Concentration and uptake of N and P by maize: The effect of PGPR isolates except $MS_3$5b on the N concentration (Table 7) in maize plants was statistically at par but significantly ($p \leq 0.05$) higher than control (1.08%). A

non-significant variation occurred for N concentration in maize for soil types. The interactive effect of $IS_3$3c inoculation in alkaline soil had a maximum maize N concentration (1.53%), statistically at par with isolates $IS_1$4b and $IS_5$4b had N concentrations of 1.50% and 1.49% (Fig. 3a), respectively, under alkaline soil and $MS_6$4c in acidic soil. The maize N contents of all PGPRs inoculated in acidic and alkaline soils were significantly higher than controls with no inoculation of PGPRs under acidic and alkaline soils. Maximum mean maize P concentration (Table 7) of 0.145% was recorded with the strain $IS_3$3c inoculation followed by $IS_1$4b (0.139%), $IS_5$4b (0.138%) and $MS_6$4c (0.137%) irrespective of soil types. The alkaline soil had a significantly higher P concentration (0.139%) in maize than in acidic soil. The interactive effect of PGPRs and soil types was non-significant (Fig. 3b). Results (Table 7) illustrated that $IS_3$3c inoculation had significantly ($p\leq0.05$) increased N uptake by maize (1.19 g pot^{-1}) followed by $MS_6$4c (1.01 g pot^{-1}) irrespective of soil type. Soil type, i.e., acidic or alkaline, significantly affected N uptake ($p\leq0.05$). Alkaline soil had greater N uptake (0.97 g pot^{-1}) than acidic soil (0.85 g pot^{-1}). The interactive effect of the isolated PGPRs and soil type was significant ($p\leq0.05$) for N uptake. The maximum N uptake (1.44 g pot^{-1}) (Fig. 3c) was from the $IS_3$3c strain, followed by $IS_1$4b (1.25 g pot^{-1}) under alkaline soil and $MS_6$4c (1.12 g pot^{-1}) under acidic soil. The lowest N uptake of 0.59 and 0.63 g pot^{-1} by maize was determined in both acidic and alkaline soils, respectively, without any PGPR inoculation. The PGPR strains significantly ($p\leq0.05$) affect the P uptake of maize plants. The strain $IS_3$3c had a maximum P uptake of 0.125 g $pot^{-1,}$ irrespective of soil type. The alkaline soil had a higher P uptake of 0.103 g pot-1 than the acidic soil, with a P uptake of 0.086 g pot^{-1}. In interactive effect, the maximum P uptake of 0.153 g pot^{-1} (Fig. 3d) by maize was in alkaline soil inoculated with $IS_3$3c, followed by $IS_1$4b, which had a P uptake of 0.132 g pot^{-1} under alkaline soil conditions. The lowest P uptake (0.056 g pot^{-1}) was calculated in the control of acidic soil where no PGPR was inoculated and was statistically similar to the alkaline soil control, which had an N uptake of 0.066 g pot^{-1}.

Table 6. Post harvest soil pH, NO_3-N and AB-DTPA extractable P contents and population density of bacteria as influenced by different PGPR strains in acidic and alkaline soil conditions.

PGPR strains	Soil pH	NO_3-N	AB-DTPA extractable P contents	Bacteria population density
			mg kg^{-1})	CFU g^{-1} soil
Control	7.03	8.92 e*	3.10 f*	24.0 f*
$MS_6$4c	6.95	15.12 b	8.16 bc	63.8 b
$MS_3$5b	6.98	12.66 d	5.44 e	54.4 e
$MS_5$4c	6.96	13.16 cd	6.20 de	56.9 d
$IS_1$4b	6.94	14.23 bc	7.00 cd	63.3 b
$IS_3$3c	6.90	17.06 a	10.19 a	71.1 a
$IS_5$4b	6.97	15.57 b	8.85 ab	60.5 c
LSD (0.05)	NS	1.4784	1.4489	2.4203
Soil types				
Acidic soil	6.23 b	13.41 b	6.67	54.4 b
Alkaline soil	7.70 a	14.22 a	7.31	58.1 a
LSD (0.05)	0.0615	0.7902	NS	1.2937
Interaction				
P*S	NS	*	*	*

* Significant at $p<0.05$, PGPR (P): Plant growth promoting rhizobacteria, Soil types (S), means followed by same alphabets are statistically similar

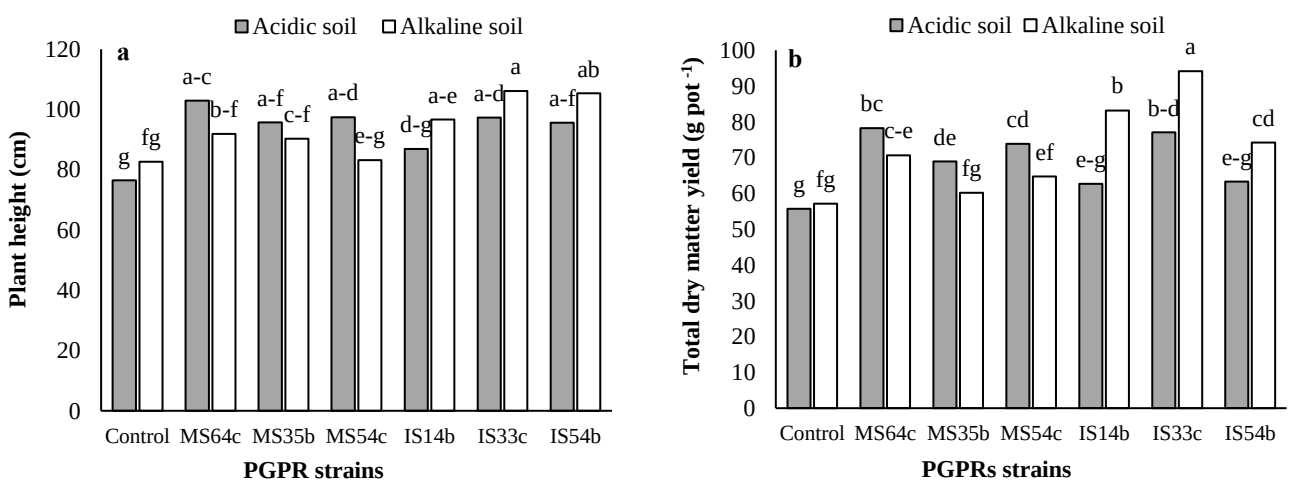

Fig. 1. Interactive effect of PGPR strains inoculation in acidic and alkaline soils on plant height and total dry matter yield.

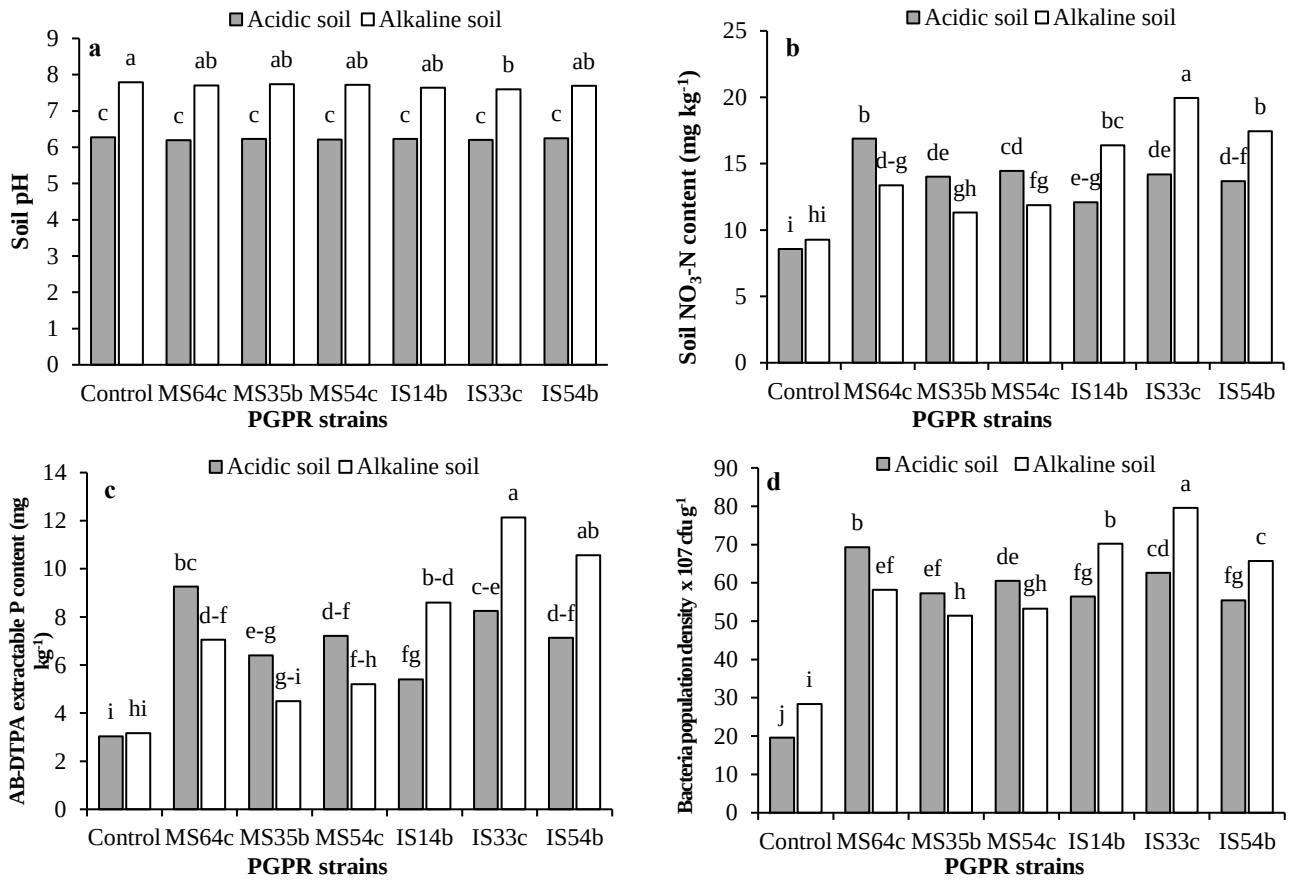

Fig. 2. Interactive effect of PGPR strains inoculation in acidic and alkaline soils on soil pH, soil NO_3-N content, AB-DTPA extractable P content, and bacteria population density in soil.

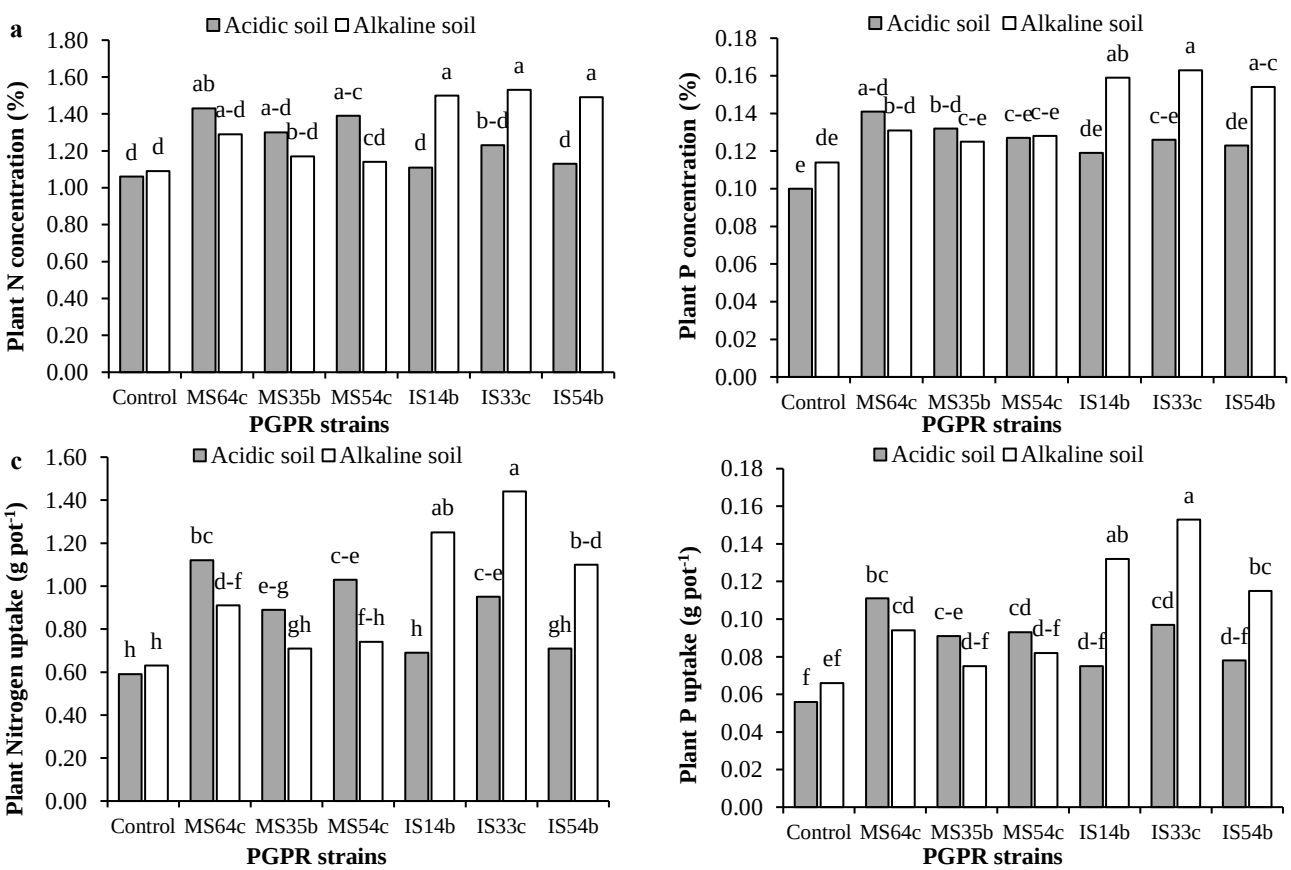

Fig. 3. Interactive effect of PGPR strains inoculation in acidic and alkaline soils on Plant N, P concentrations and uptakes.

Population density of bacteria in the maize rhizosphere: Data in Table 5 showed that different PGPR strains had a significant ($p \leq 0.05$) effect on the population density of bacteria compared to uninoculated soil. The maximum bacteria density of 71.1 x 10^7 cfu g^{-1} soil (Table 6) was recorded with strain $IS_3$3c, followed by strains $MS_6$4c and $IS_1$4b with bacteria densities of 63.8 and 63.3 x 10^7 cfu g^{-1} soil. The minimum bacteria density of 24.0 x 10^7 cfu g^{-1} soil was recorded with control. The effect of soil type on bacteria population density was significant ($p \leq 0.05$). The maximum bacteria population density of 58.1 x 10^7 cfu g^{-1} soil was recorded with alkaline soil. The interactive effect of PGPR and soil types was significant ($p \leq 0.05$). The population density of bacteria varied from 19.6 to 79.6 x 10^7 cfu g^{-1} soil. The $IS_3$3c inoculation in alkaline soil had a maximum bacteria population density of 79.6 x 10^7 cfu g^{-1} soil (Fig. 2d), followed by $IS_1$4b inoculation in alkaline soil, $MS_6$4c inoculation in acidic soil with bacteria population density of 70.2 and 69.3 x 10^7 cfu g^{-1} soil, respectively. Overall, the population density of alkaline soil is higher than acidic soil.

Table 7. Nitrogen, P concentration, and uptake by maize plants as influenced by different PGPR strains in acidic and alkaline soil conditions.

PGPR Strains	N concentration	P concentration	N uptake	P uptake
	(%)		(g pot-^{1})	
Control	1.08 b*	0.107 b*	0.61 d	0.061 d*
$MS_6$4c	1.36 a	0.137 a	1.01 b	0.103 b
$MS_3$5b	1.24 ab	0.129 ab	0.79 c	0.083 c
$MS_5$4c	1.26 a	0.127 ab	0.88 bc	0.088 bc
$IS_1$4b	1.30 a	0.139 a	0.97 b	0.104 b
$IS_3$3c	1.38 a	0.145 a	1.19 a	0.125 a
$IS_5$4b	1.31 a	0.138 a	0.91 bc	0.097 bc
LSD $_{(0.05)}$	0.1815	0.0216	0.1400	0.0192
Soil types				
Acidic soil	1.23	0.125 b	0.85 b	0.086 b
Alkaline soil	1.32	0.139 a	0.97 a	0.103 a
LSD $_{(0.05)}$	NS	0.0115	0.0749	0.0103
Interaction				
P*S	*	NS	*	*

* Significant at $p<0.05$, PGPR (P): Plant growth promoting rhizobacteria, Soil types (S), means followed by same alphabets are statistically similar

Discussion

Plant growth-promoting rhizobacteria (PGPR) isolated from maize rhizosphere of acidic and alkaline soil were different in shape, phytohormone production, NH_3 production, and nutrient solubilization and belong to different groups. The rhizosphere is a microclimate around the roots of plants, which have nutrients for plants and microorganisms. The successful colonisation of microorganisms is essential for the successful exchange of nutrients and transmission of signals among plants and microorganisms (Smith *et al*., 2017). The standard types of bacteria in acidic and alkaline soil were diplobacillus and streptobacillus**.** The roots of different crops excrete specific exudates that attract and repel specific communities of microorganisms in the rhizosphere. The microbial community associated with plants is also affected by heavy metal accumulation in soil, drought, temperature, salinity, and pH (Schlemper *et al*., 2017). Soil bacterial communities' structure and biomass in the rhizosphere are a function of root exudates, plant genotype, plant growth stage, and soil type (Wang *et al*., 2015).

The findings of this study showed that inoculation of PGPRs in acidic and alkaline soils increased maize height, total dry matter, N, P concentration and uptake by maize, soil N and P contents, and bacterial population density over uninoculated treatments. Results showed that rhizobacteria improved maize height and total dry matter attributes in the pot experiment. The diverse type of PGPR was isolated from the root rhizosphere, which improved the growth of maize (Arruda *et al*., 2013). The PGPR inoculation increased maize height total dry matter by increasing the assimilation of nutrients in the root and its uptake by the root. The phytohormone indole acidic acid-producing PGPR increased the number and area of metaxylem vessels by differentiating more perivascular cells to metaxylem vessels that facilitate the transportation of more nutrients from the root to the upper part of the plant (Calzavara *et al*., 2018, Nozari *et al*., 2021). The phytohormones producing rhizobacteria increased the vegetative growth of vegetables and cereal crops by a complex mechanism (Kuan *et al*., 2016). The Rhizobacteria improved the growth of maize in nutrient-deficient and optimum soil by adopting the adverse conditions and successfully colonized the rhizosphere of maize by reducing the effect of negative factors (Mubeen *et al*., 2021).

Rhizobacteria inoculation increased the bioavailability of soil N and P in a pot over uninoculated through its multi-plant growth-promoting traits. The inoculated PGPR improved the N availability in the soil by increasing the urease enzyme in the soil. (Ng *et al*., 2022, Rana *et al*., 2023) reported that free-living microorganisms carry out cereal N fixation.

Different PGPR strains inoculated in acidic and alkaline soil had different abilities to solubilize the unavailable form of P. Different PGPRs produce different types and concentrations of organic acids to solubilize unavailable forms of phosphorus in different types of soils (Gerke, 2021, Rawat *et al*., 2021) reported that the carboxylic acid is converted to a carboxylate anion inside the cell of microbes (pH 7) and released in soil as carboxylate anion by PGPRs that decreased P sorption by reducing positive site on exchange surfaces. Carboxylate anion released by PGPRs also increases P availability by chelating metal ions (Fe, Al, and Ca) of Iron phosphate, aluminum phosphate, and calcium phosphate.

The PGPR with multi-plant growth-promoting traits increased N and P concentration by increasing uptake of N and solubilizing the residual P in soil by releasing different types of organic acids that either solubilized or chelates the cation in Iron phosphate, aluminum phosphate, and calcium phosphate (Pereira *et al*., 2020). The PGPR increased the N uptake of maize by BNF and delayed remobilization of N and contributed 30.5 to 25.5% of plant total nitrogen (Kuan *et al*., 2016). PGPR also released IAA, which increased the length of the primary root and the number of root hairs to explore more nutrients and make them available to plants (Keswani *et al*., 2020).

Results showed that plant growth promoting rhizosphere inoculation increased the population density of indigenous microbes in acidic and alkaline soil over uninoculated treatments. The PGPR inoculation synergistically affects indigenous bacteria by providing soil organic carbon and nutrients. The population density of bacteria is greatly influenced by the types of plants and soil (Bakker *et al*., 2015). Overall, the population density of alkaline soil is higher than acidic soil. The population density of bacteria decreased in acidic soil due to the phytotoxicity of micronutrients that adversely affect the root and bacteria (Gahoonia, 1993). Soil pH, nutrient availability, and release of exudates by plants' roots select specific microflora to establish in the rhizosphere (Yadav, 2020).

Conclusion

The native PGPR strains isolated from acidic and alkaline maize rhizosphere soils mostly belonged to *Bacillu*s species capable of solubilizing insoluble forms of phosphorus, fixed nitrogen, releasing different enzymes, and growth-promoting hormone, indole acetic acid in the soil. Inoculation of PGPR strains ($IS_3$3c, $IS_1$4b in alkaline and $MS_6$4c in acidic soils) significantly improved maize growth, post-harvest soil NO_3-N and P contents and uptake by maize plant and have a synergistic effect on the indigenous bacteria population in acidic and alkaline soils.

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