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Effects of PGPR on phosphorus solubility in soil and on growth of Pea (*Pisum sativum* L.)

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ABSTRACT

We isolated 158 PGPR strains from the rhizosphere and rhizoplane of pea (*Pisum sativum*) plant and were characterized. Out of these, 7-test PGPR strains were selected for further studies, based on effects on growth, their capability to produce siderophores, phytohormone, nitrogenase activity and P-solubilization, respectively. These selected test PGPR produced a significant quantity of soluble phosphate (55-103 µg ml^{-1}), IAA (71.22- 91.21µg mL^{-1}) and all produced siderophores. Phytohormone (IAA) production by PGPR was measured by Electro-Spectrophotometer. The efficiency of phosphorus solubilizing bacteria (PSB) was measured in Pikovskaya broth medium. VOC emission analysis by SPME-GC-MS, showed that aldehydes, ketones and alcohols were the most abundant compounds in most rhizobacteria. Furthermore, biofertilizer was prepared from inoculation of these microorganisms and studied their effects in pot experiment on peas. All the inoculants showed positive effects on the growth and development of peas.

Key Words: Characterization, evaluation, growth, IAA, microorganisms, nitrogen, nutrients, pea, PGPR, phosphorus, *Pisum sativum,* pot culture, rhizoplane, rhizosphere, VOC

INTRODUCTION

Pea (*Pisum sativum* L.) is, an important legume crop, rich source of protein, improves the nitrogen status of soil and increases the yield of the succeeding crop in rotation. Its rhizosphere is rich in microbial population and particularly the plant growth-promoting rhizobacteria (PGPR). The use of native species of PGPR, isolated from the Pea rhizosphere to inoculate the pea seeds before sowing could increase the yield of *P. sativum*. The PGPR bacteria promotes the production of growth hormones (Auxins gibberellins etc.), anti-pathogenicity, nitrogen fixation and phosphate solubilization (2,3,4,5,6). For example, Auxins, Indole acetic acid (IAA improves the root growth by stimulating cell division or by influencing the ACC-deaminase activity of bacteria, root propagation (7), cell division and shoot growth (8). PGPRs induces the hormone production in plants (9,10) and some PGPRs known as phosphate solubilizing

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bacteria (PSB) enhances the phosphorus supply to plants. Besides the *Azospirillum* a well known PGPR improves the plant height, nutrients uptake, N content, leaf size, root length and plant biomass of cereals (11). Several mechanisms of action have been proposed for PGPR increasing nutrients level, nitrogen fixation, increasing beneficial symbioses, enhancing root surface area, and combination of multiple modes; IAA and soluble phosphate production is among those several mechanisms of action that a single PGPR may demonstrate *in situ* (41). PGPR indicates their potential to be used as commercial biofertilizer for the enhancement of growth and yield of many plants. Both *Pseudomonas* and *Azospirillum* as PGPR d could be used as biofertilizers to increase crop yields (12-14). However, no work has been done on the growth of vegetable crops. This is the first study to determine the microbial population in pea rhizosphere and to study the important characters of PGPR to increase pea yields.

MATERIALS AND METHODS

This Research was done in Soil Biology, Biochemistry and Soil Fertility Laboratories, Land Resources Research Institute (LRRI), National Agricultural Research Centre (NARC), Islamabad and Department of Botany, Hazara University Mansehra, Khyber Pakhtunkhwa. Pea (*Pisum sativum* L.) samples and rhizospheric soils were collected during September 2019-January, 2020 from the fields of District Swabi, KPK, Pakistan (34° 06' 60.00" N and 72° 27' 59.99" E, mean temperature: 22.2 °C, annual precipitation: 639 mm).

I. Sampling and Isolation of PGPR

Rhizosphere soil from pea fields was collected along with pea roots and nodules from different areas of District Swabi, Pakistan. Samples were brought to the laboratory in zipper bags to avoid external contamination and stored at 5 °C. The bacterial isolation was done by making the serial dilutions as per Khan *et al* (15). One g soil was suspended in 100 ml distilled water, mixed thoroughly and 1 ml of dilution was poured to 9 ml distilled water to make 10^{-2} dilution. Bacterial concentrations were further diluted to 10^{-5} for each sample. Luria Broth media containing plates were inoculated from each dilution under the laminar flow and incubated for 24 h at 28 °C.

II. Taxonomic characterization

(i). Gram Staining

Using the Gram staining standard method, the bacterial colonies were screened for Gram staining to know if the selected PGPR strains were positive or negative (1).

(ii). Biochemical Characterization

A. Indole Acetic Acid (IAA) Assay: IAA production ability of different isolates was assessed by method of (16,17). In practice, 100 ml LB broth media containing 1mg/ml tryptophan was inoculated with bacterial isolates and incubated at 28±2 °C. One week after incubation, the bacterial culture was centrifuged for 10 min at 3000 rpm. Pelleted material was discarded and 2 ml supernatant was mixed with two drops of Orthophosphoric acid and 4 ml of Salkowski reagent (50 ml; 35 % Perchloric acid and

1 ml 0.5% $FeCl_3$). The development of pink colour indicated the IAA production. Absorbance was recorded at a 530 nm using spectrophotometer to quantify the hormone. IAA standard curve was made by recording the absorbance of standards.

B. Determination of Nitrogenase Activity by Colorimetric Method: The nitrogenase activity was estimated using the acetylene reduction assay (18). A single colony of each PGPR strain was inoculated in a semi-solid vial of N-Free Media (NFM) and incubated for 48 h at 30 ºC. The cotton plugs on vials were replaced with suba seals and 10 % v/v acetylene. One ml of acetylene per vial was injected. The vials were incubated for 24 h at 30 ºC. The production of Ethylene was measured by Spectrophotometer.

C. Siderophore Production Assay: Siderophore production ability of selected isolates was determined by blue agar assay. LB agar medium was mixed with chrome azurol-S (CAS) and isolates were spotted on it (19). After 72 h, isolates that changed the colour of the media from blue to orange were considered as siderophore producers.

D. Phosphorus Solubilization: The bacterial isolates were grown on the Pikovskaya medium. The media was amended with tricalcium phosphate as source of inorganic phosphate. Plates were inoculated with bacterial isolates and incubated at 28 °C. Microbe's ability to solubilize inorganic phosphorous was determined by measuring the solubilization index (SI) according to (19) equation given below:

$$\mathrm{SI} = \frac{\text{Colony diameter+Halo zone diameter}}{\text{Colony diameter}}$$

E. pH variations: Changes in pH of broth culture were recorded with pH meter for 7- days after incubation.

F. Quantification of solubilized phosphorus: Quantity of phosphorus solubilized by phosphate solubilizing bacteria was calculated by Phospho-molybdate blue color method (20). Bacterial culture was inoculated in 100 ml of Pikovskaya s' broth in 250 ml flasks. After inoculation, flasks were incubated at 24 ºC on rotary shaker @ 200rpm. 7 d after incubation, the cultures were centrifuged for 15 min at 10000 rpm. The supernatant was decanted, filtered and pH was recorded.
Two reagents, A and B were prepared by diluting the stock solutions. Phosphorus concentration of these solutions was determined by taking 1 ml of working solution and 4 ml of reagent-B and the total volume was made to 25 ml. one ml of this mixture was taken for Phosphorus concentration (P) of standard solutions was determined using spectrophotometer at 882 nm. This available phosphorus (P) showed the extent of solubilization of tri-calcium phosphate (21).

G. Analysis of PGPR Volatile organic compounds (VOCs): PGPR isolates were grown in 0.2 × MS medium and 2 ml samples were retained in a 4- ml vials for 48 h. Then the volatiles were collected using a blue SPME fiber (PDMS/DVB) (Supelco, Inc., Bellafonte, PA, U.S.A.) and desorbed at 180 °C for 30 s in the injector port of gas

chromatograph (Agilent 6850 Series II; Agilent, Foster City, CA, U.S.A.), equipped with a MS detector 5973 from Agilent and a free fatty acid–phase capillary column (HP-FFAP) (30 m × 0.25 mm I.D., film thickness of 0.25μm). The helium was used as the carrier gas (1 ml/min), detector temperature of 250 °C. The column was held for 5 min at 40 °C, and then programmed to rise at a rate of 3 °C/minute to a final temperature of 220 °C, which was maintained for 5 min. Three independent determinations were made for each strain. Source pressure was 7Pa, filament voltage was 70eVand a scan rate was employed of 1.9 scan s^{-1}. The compounds were identified by comparison with mass spectra from the library (NIST/EPA/NIH, "Chem Station" Agilent Technologies Rev. D.04.00 2002).

Pot Experiment

To determine the effects of PGPR on seeds germination and seedlings growth of peas, pot culture was conducted in a growth chamber (25 °C temperature, 50 % humidity, light for 16 h). There were 8-experimental treatments viz., 7-PGPR strains [Control (uninoculated), T1 (RS.P1-B),T2 (RP.P1-E), T3 (RS.P2-I),T4 (RS.P3-D), T5 (RP.P3-L), T6 (RS.P4-B), T7 (RS.P5-F)]. The treatments were replicated thrice in a complete randomized design. Pea seeds were surface sterilized with 5 % H_2SO_4 for 1 min and thereafter seeds were washed 2- 3 times with double distilled water. The PGPR strains were applied on pea seeds by coating the seeds in plastic bags congaing inoculants. Seeds in control, were not inoculated. Three seeds were sown per pot (9 cm dia, 7 cm depth) having 700 g autoclaved soil (autoclaved at 121°C for 30 min). The pots were kept in growth chamber for one month. Seeds germination was recorded on 6th day. Fifty ml of autoclaved distilled water was applied daily to each pot during this period. Plants were harvested after 1 month and growth parameters (root/shoot length, number of leaves and root/shoot dry weight) were recorded.

Statistical Analysis

Experimental Data were subjected to analysis of variance (ANOVA) using software STATISTIX 8.1 and means obtained were compared by Least Significance Difference (LSD) test at 5 % level of significance.

RESULTS AND DISCUSSION

AGAR BIOASSAY

Isolation and Morphological Characterization of PGPR Isolates

A total of 158 PGPR strains were isolated and were characterized by cell morphology and colony morphology, Gram reaction, colony size, shape, and colour. Only 7 strains (RP.P1-E; RS.P1-B; RS.P2-I; RP.P3-L; RS.P3-D; RS.P4-B and RP.P5-F) were selected for further study based on their phosphate-solubilizing ability, hormone production, nitrogenase activity, and siderophores production.

The colour of the colony varied from off-white to yellowish; whereas RS.P2-I was orange in color (Table 1). In most cases, the shape/form of the colony was circular, punctiform, and irregular (Table 1) Colony elevations of the isolates were mostly umbonate, while, RS.P2-I had convex elevation, RP.P3-L was umbilicate and RS.P3-D and RS.P4-B were

flat in elevation (Table 1). Colony margins were mostly observed from entire to lobate and RP.P3-L arisen at the margin (Table 1). Out of 7 isolates 4 (RS.P1-B, RP.P1-E, RS.P3D, and RP.P5F) were observed as Gram +ive while 3 isolates (RS.P2-I, RP.P3-L and RS.P4-B) were Gram–ive (Table 1). All isolates were tested microscopically and different cell shapes were observed from bacillus to coccus except PS.P4-B which was spiral (Table 1).

Table 1. Morphological characteristic of 7-lest PGPR strains

Isolates	Gram Staining	Cell Morphology	Shape of colony	Elevation of colony	Margin	Colony Colour
RS.P1-B	+	Coccus	Irregular	Umbonate	Lobate	White
RP.P1-E	+	Rod shaped	Punctiform	Umbonate	Lobate	White
RS.P2-I	-	Coccus	Punctiform	Convex	Entire	Orange
RS.P3-D	+	Rod shaped	Punctiform	Flat	Entire	White
RP.P3-L	-	Coccus	Circular	Umbilicate	Erose	White
RS.P4-B	-	Spirillum	Circular	Flat	Entire	Yellowish
RS.P5-F	+	Coccus	Punctiform	Umbonate	Entire	Yellowish

Note: PGPR=Plant Growth Promoting Rhizobacteria, RS=Rhizosphere, RP=Rhizoplane, P=Pea

Biochemical Characterization of PGPR

Quantification of Indole Acetic Acid (IAA): All tested PGPR strains were IAA producers (Table 2). The concentration of Indole Acetic Acid was calculated spectrophotometrically. All 7-tested strains showed high quantity of IAA ranging from 91.21µg/ml to 74.21µg/ml (Table 2). Two strains (RP.P1-E, E-RP.P3-L) produced the highest quantities of IAA, while, RS.P5-F produced the lowest quantity. These results are in agreement with earlier studies (22,23,24,25). Among all the isolates RP.P1-E and RP.P3-L produced the maximum concentration 91.21*u*g/ml of plant hormone IAA (Table 2) while RS.P4-B produced the lowest (74.21µg/ml) IAA (Fig. 1).

Table 2. Biochemical characteristics of 7- lest PGPR strains

PGPR Strains	IAA Concentrations (µg/ml)	µmoles C_2H_2/h.	Siderophores production	Available Phosphorus (µg ml^{-1})
RS.P1-B	74.76	7.3	+++	61
RP.P1-E	91.21	9.2	+++	55
RS.P2-I	79.15	4.5	+++	75
RS.P3-D	74.76	7.8	-	70
RP.P3-L	91.21	8.3	+++	85
RS.P4-B	74.21	5.6	+++	98
RS.P5-F	71.22	4.2	+++	103

Note: PGPR=Plant Growth Promoting Rhizobacteria, RS=Rhizosphere, RP=Rhizoplane, P=Pea, IAA=Indole Acetic Acid

Nitrogenase Activity: Nitrogenase activity was determined by colorimetric method using acetylene reduction assay (18). The production of ethylene by the PGPR isolates ranged from 3.1*µ*moles C_2H_2/hr to 12.3 µmoles C_2H_2/h (Table 2). Maximum ethylene was produced by RP.P1-E (9.2µmoles C_2H_2/h) following RP.P3-L (8.3µmoles C_2H_2/h).

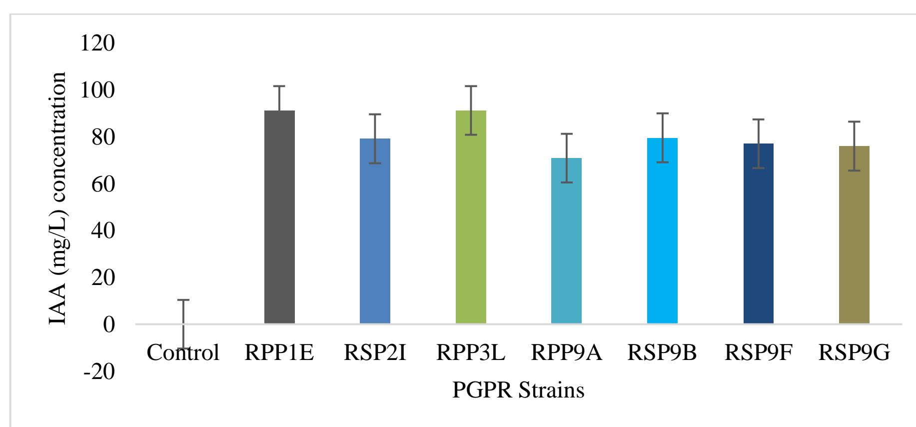

Figure 1. Indole Acetic Acid Concentration of PGPR Strains

Siderophores Production: Siderophore, a bio-molecule of low molecular weight, helps the plants to absorb iron and is an antagonist against plant pathogenic microbes (35,36,37). Hence, these play an important role in biocontrol of some soil-borne plant diseases and in plant iron nutrition. They chelate iron and transport into bacterial cells. Of the 7- PGPR strains, 6- strains showed positive results for siderophore production but one isolate RS.P3-D did not produce any siderophore (Table 2). These investigations are in line with Sajid *et al* (35) who studied siderophore production of some rhizospheric bacteria.

Phosphate Solubilization Index (SI): The phosphate SI activity was determined using Pikovskaya media. Tricalcium phosphate, used as an insoluble phosphorus source, was solubilized by the P-solubilizing bacteria, which produced a transparent hollow zone around the colony. All 7- isolates were phosphate solubilizers and showed high solubilizing index from 3.16 to 2.6. The PGPR isolates RP.P5-F (Fig. 2) produced the highest solubilization index (3.16) followed by RP.P3-L and RS.P4-B (3.07). The lowest solubilization index (2.6) was of PGPR RS.P2-I (Fig.3).

Similar to Vivas *et al.*, (38) who observed that out of 266 strains of PGPR, 100 % solubilized the insoluble phosphates, while 95 % and 85 % produced IAA and siderophores, respectively. The majority of rhizospheric isolates regularly produced IAA and siderophores and solubilized the tricalcium phosphate (38). The amount of IAA and solubilized phosphate by the isolated strains in this study is comparable to the results reported earlier (39). However, the phosphate solubilization rate was low compared to Xiang, *et al*., (40). Plant growth-promoting activities of PGPR have been reported in many studies (32,33,41). Production of IAA and soluble phosphate are common advantages of PGPR and microbes with these attributes are widespread in the rhizosphere. Nonetheless, an isolate producing considerable amount of IAA and solubilized the phosphate in the laboratory may not exhibit these attributes under field conditions and conversely, some PGPR may not produce soluble phosphate or IAA in the laboratory but stimulated the growth and yield of host plant in the field.

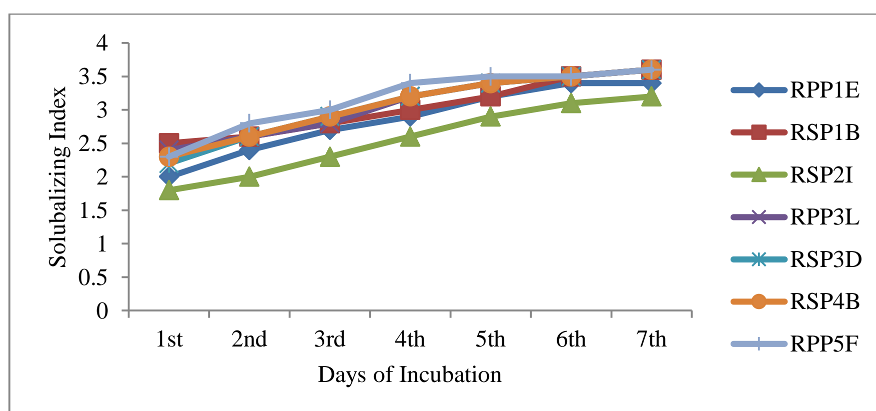

Figure 2. Solubilization index of PGPR isolates on Pikovskaya media

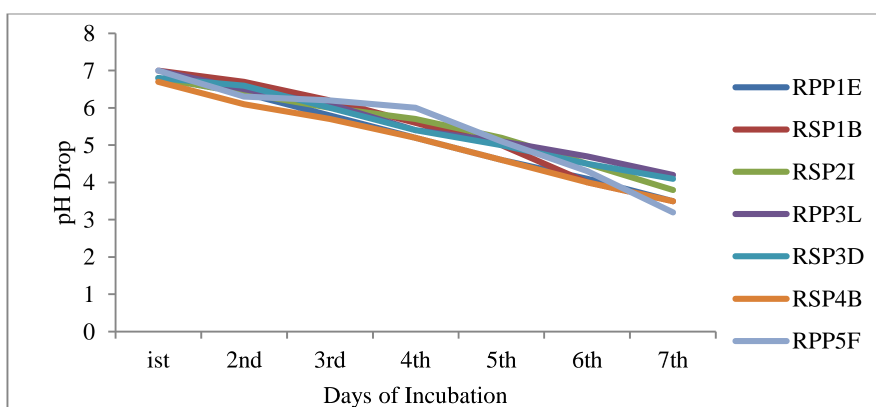

Figure 3. Effects of PGPR isolates on pH during seven days of incubation

pH and Phosphate solubilization ability

The PGPR isolates significantly decreased the pH to 4.2 to 3.2 from initial pH of 6.8-7.0 (Fig. 3). The maximum decrease in pH was observed in RP.P5-F, which was 3.2, followed by RP.P1-E, RS.P1-B, and RS.P4-B which were 3.5. Minimum pH was decreased by RP.P3-L, which was 4.2 (Fig. 4).

The phosphate solubilization by PGPR isolates was expressed as available P (µg ml^{-1}) in broth culture (Fig. 5). Maximum P was solubilized by RP.P5-F (103 µg ml^{-1}) followed by RS.P4-B (98 µg ml^{-1}) and RP.P3-L (85 µg ml^{-1}). The lowest level of P was solubilized by RP.P1-E which was 55 $µgml^{-1}$ (Table 2).

The P-solubilization and pH change are caused by the production of organic acids (26). The organic acids are mostly cations (Ca, Fe, Al) chelators, which bind the cation, attach with P and reduce the pH, making P accessible to plants (27).

The solubilization was determined by incubation of selected PGPR colonies for 7 days. The diameter of the cleared zone indicated the solubilization index, which ranged from 2.56 - 3.16 µg.ml-1, which are in line with Patten & Glick (28). In broth culture, the

pH decreased from 7.0 to 3.2 due to Phosphorus Solubilizing Bacteria (Fig. 3), many researchers have earlier reported decline in pH (29,30,31). In PGPR, all the tested strains solubilized the phosphate. In this study, all the tested PGPR strains solubilized the phosphate from 55-103 µg ml^{-1} by PGPR (Fig. 4). The current studies support early findings (32,33,34).

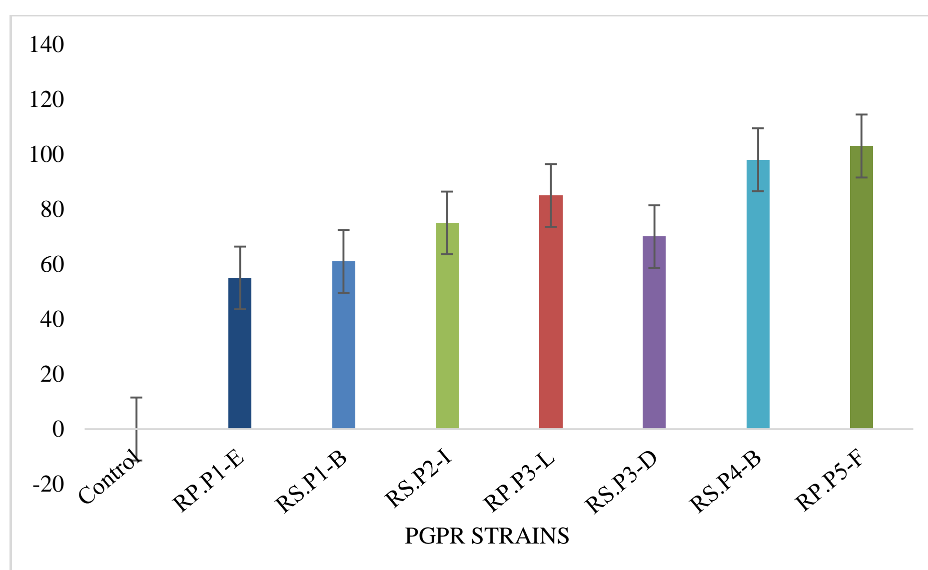

Figure 4. Available Phosphorus (µg ml-1) in broth culture

Determination of VOCs profiles from bacterial strains:

To determine VOCs we used SPME-GC-MS for 7-test bacterial strains (RP.P1-E; RS.P1-B; RS.P2-I; RP.P3-L; RS.P3-D; RS.P4-B and RP.P5-F). Strains RS.P1-B and RS.P2-I had moderate and significant effects on pea growth, while, RP.P3-L, RS.P3-D and RP.P5-F, strongly altered the plant root-system architecture. Among the detected VOCs, the aldehydes, ketones and alcohols were most abundant (Table 3). A total of 19 different VOCs were identified in all 7-test PGPR strains acetophenone, tridecanal and tetradecanal, were most abundant volatiles in all isolates. The isolate RS.P2-I released many compounds. The isolates RP.P1-E and RS.P2-I, which strongly stimulated the lateral root length, produced higher concentration of aldehydes Tetradecanal and Tridecanal (47 and 54%, respectively). On the other hand, the isolate RP.P3-L, which increased the primary root length, lateral root number and total biomass had similar contents of aldehydes and ketones (45 and 42 %, respectively), while the strain RS.P4-B and RP.P5-F that also increased primary root length contained different proportions of aldehydes (50 %) and ketones (39 %). The strains RS.P4-B and RP.P5-F, with modest effects on growth showed the highest content of alcohols (12 %). This analysis suggested that the effects observed in roots by the different bacterial strains were proportional to the type and amount of compounds produced by the bacteria.

Table 3. Volatile organic compounds produced by 7- lest PGPR identified by SPME-GC-MS

Compound	RT (min)	Rhizospheric isolates						
		RP.P1-E	RS.P1-B	RS.P2-I	RP.P3-L	RS.P3-D	RS.P4-B	RP.P5-F
		Normalized values of volatile compound (%)[a]						
1-Butanol	8.18	0 a	0 a	0 a	4.61 b	5.82 b	0.58 a	0.59 a
6-Methyl 2-heptanol	18.31	1.32 a	0.88 a	0.9 a	1.31 a	0.91 a	2.02 b	2.05 b
Butyrolactone	28.54	0 a	0 a	2.17 b	0 a	0 a	0 a	0 a
1-Octen-3-ol	21.43	0 a	1.45 b	0 a	0 a	0 a	0 a	0 a
Benzaldehyde	24.34	14.47 d	0 a	11.26cd	8.84bc	3.83 ab	24.09 e	8.60 bc
Tetradecanal	39.18	16.31 a	15.82 a	14.38 a	14.34 a	18.83 a	9.51 a	10.11 a
Acetophenone	29.35	16.27 ab	9.80 a	9.31 a	20.43 b	20.02 b	22.78 b	19.70 b
2-Nonenal	20.35	1.43bc	0 a	1.56bc	2.02bc	2.23 c	0.99 ab	0.87 ab
4-Decanone	39.70	1.56 a	1.61 a	0.91 a	1.35 a	1.31 a	1.65 a	1.71 a
Tridecanal	35.52	19.44 a	13.70 a	17.91 a	18.77 a	17.89 a	15.13 a	14.31 a
Cyclododecane	47.39	1.63 a	1.47 a	2.41 a	2.44 a	2.41 a	1.45 a	1.47 a
6-Undecanone	39.79	1.08 a	1.66 a	0.95 a	0.97 a	0.67 a	1.38 a	1.66 a
5-Tridecanone	39.94	1.72 a	3.16 a	1.76 a	1.94 a	2.14 a	1.66 a	1.76 a
Cyclodecane	40.91	1.68 a	6.85 b	1.54 a	2.87 a	3.71 a	3.01 a	1.68 a
3-Tetradecanone	41.25	1.25 a	1.27 a	1.12 a	1.40 a	1.12 a	1.53 a	1.40 a
1-Tridecanol	44.22	2.21 a	1.72 a	2.56 a	2.95 a	3.47 a	2.70 a	2.21 a
2-Pentadecanol	45.87	0.65 ab	0.60 ab	1.09 b	0.70 ab	0.75 ab	0.51 a	1.09 b
2-Pentadecanone	42.49	1.03 a	1.43 ab	3.28 b	1.56 ab	1.18 ab	1.60 ab	3.28 b
9-Octadecanone	46.30	4.45bc	1.05 a	6.36 c	4.37abc	4.49bc	2.57 ab	4.45bc

[a] Normalized values of volatile compound = (peak area of volatile compound) / (total peak area of all volatile compounds) The values represent the average of three replicates The statistical analysis was conducted independently for each data set; different letters indicate statistically significant differences according to the LSD test ($P \leq 0.05$)

POT EXPERIMENT

Effects of PGPR on pea growth:

The effects of PGPR on pea plants' growth in pots were measured as: root/shoot length, number of leaves per plant, root/ shoot dry weight.

(i). Root/Shoot Length (cm): All 7-test bacterial strains significantly increased the Root/Shoot length than un-inoculated control (Plate 1,2). Maximum shoot length was observed in T6 (22.3 cm) inoculated with RP.P5-F followed by T7 treated with RP.P5-F which increased the length up to 21.0 cm. Minimum shoot length (9.5) was recorded in un-inoculated control (Table 4, Figure 5). The shoot length of T6 inoculated with (RP.P5-F) was highly significant over T2 (RS.P1-B). T1 (RP.P1-E), T3 (RS.P2-I), T4 (RP.P4-L), T5 (RS.P3-D) and T7 (RP.P5-F) were statistically same (Plate 1).

The highest root length was observed in T5 (RP.P3-L) followed by T3 (RS.P1-B); 11.5 cm (Plate 2). The root length of all treated plants was significantly higher than the control. Among the treatments, T5 (RS.P3-D) root length was highly significant over T2 (RS.P1-B), T4 (RP.P3-L) and T7 (RP.P5-F). While, T1 (RP.P1-E), T3 (RS.P2-I) and T6 (RS.P4-B) lengths were similar but significantly higher than control.

Table 4. Stimulatory effects of PGPR strains on Pea plants growth

Treatments	Root length (cm)	Shoot length (cm)	Root dry weight (g)	Shoot dry weight (g)	No of leaves per plant
Control	4.67C	9.500 C	0.133B	0.267C	8.000C
T1 (RP.P1-E)	10.67AB	20.33AB	0.433A	0.633AB	16.67AB
T2 (RS.P1-B)	10.50B	18.00B	0.400AB	0.700AB	18.00AB
T3 (RS.P2-I)	11.50AB	18.67AB	0.433A	0.500BC	16.00B
T4 (RP.P3-L)	10.33 B	19.67AB	0.367AB	0.633AB	19.00AB
T5 (RS.P3-D)	12.83A	18.67AB	0.367AB	0.767AB	20.00A
T6 (RS.P4-B)	11.17AB	22.33A	0.500A	0.733AB	18.67AB
T7 (RP.P5-F)	10.33B	21.00B	0.500A	0.833A	18.33AB
LSD value (5%)	**2.2692**	**3.9145**	**0.2946**	**0.3165**	**3.9660**

RS=Rhizosphere, RP=Rhizoplane, P=Pea, T=Treatment

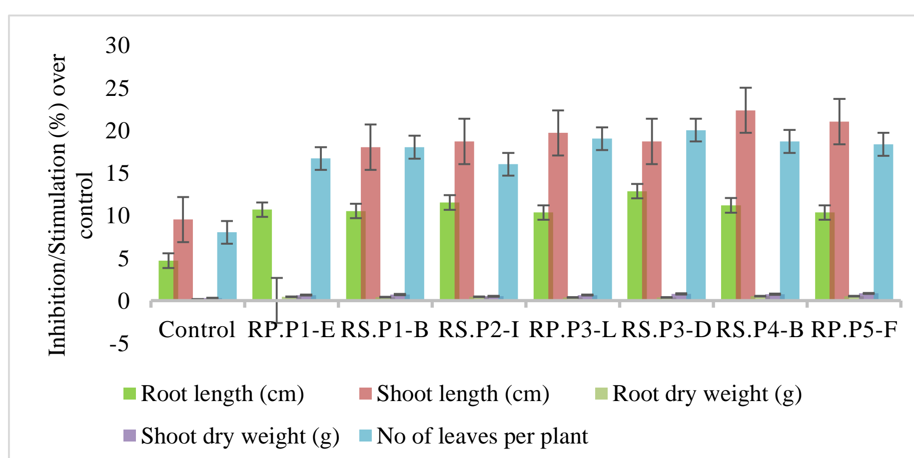

Figure 5. Stimulatory/Inhibitory effects of PGPR strains on Pea plants growth

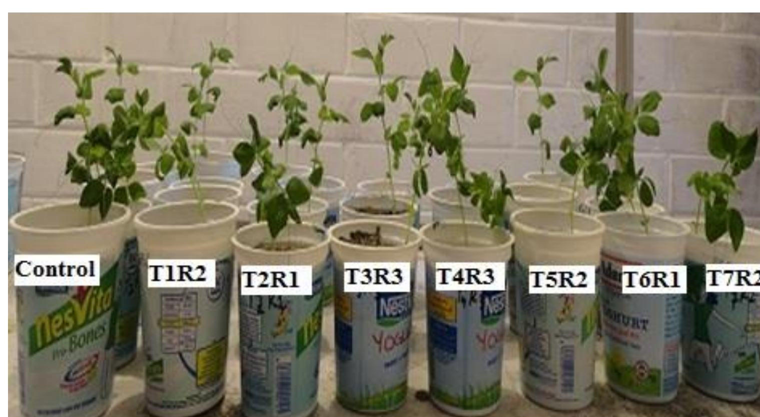

Plate 1. Effects of PGPR treatments on plants growth in Pot Experiment

Plate 2. Effects of PGPR treatments on roots development

(ii). Number of Leaves/Plant: All treated plants produced significantly more number of leaves than control (Table 4). The highest number of leaves were found in plants inoculated with RS.P3-D (20 leaves/Plant) followed by RP.P3-L inoculated plants with 19 leaves/Plant (Plate 1).

(iii). Root/Shoot Dry Weight (mg/plant): PGPR inoculation significantly improved the biomass as indicated by Root/Shoot dry weight (Table 4, Figure 5). The maximum shoot dry weight (0.83 g/plant) was observed in RP.P5-F inoculated plants, while, minimum with RS.P2-I inoculated plants. The root dry weight of treated plant was significantly higher than control. The maximum root dry weight (0.50 g/plant) was increased by the isolate RS.P4-B and RP.P5-F as compared with un-inoculated control (0.13 g/plant). The root-system besides providing anchorage, plays an important role in nutrients and water uptake from soil, and is the site of synthesis of many metabolites such as auxins and cytokinins, which play an important role in plant growth and development (42,43). The root-system shows significant flexibility in its morphology and physiology in response to fluctuations in environmental conditions. In the current study, we found that plant growth promotion properties of 7-test PGPR isolated from the pea rhizosphere were found associated with root-system architecture and nodulation in pea.

VOCs in live orgasims are effective mediators of chemical communication acting as attractant, repulsive or threatening signals. Microbial species can release volatiles for different purposes such as communication and defence (44). Due to their volatility properties, the VOCs emitted by associated microorganisms such as PGPR affect the root system. The bacterial VOCs are involved both in plant growth promotion and in root architecture remodelling.

The results of our pot experiment of peas were similar to findings of Zheng *et al* (43), who found that PGPRs influenced the growth and development of wheat by increasing the shoot/root dry weights. All 7-test PGPR strains improved the root and shoot dry biomass by 100 % and 70 %, respectively. Our results are in agreement with earlier results (44), who observed 70 % increase in peas root/shoot dry biomass by PGPRs

inoculation than control (uninoculated). In this study, PGPR significantly increased the roots, plant height and shoot/root dry biomass.

CONCLUSIONS

The 7-test PGPR strains produced indole acetic acid and the RPP1-E and RPP3-L produced highest quantity (91.21 mg/L), when the precursor L-tryptophan was added to the culture medium. All 7-test PGPR strains also produced a significant quantity of siderophores and solubilized the inorganic phosphate and the RPP5-F solubilized highest quantity (103mg/L) of inorganic phosphate. In pot experiment, the PGPR strains increased the growth (root dry weight, shoot dry weight and root surface area) of pea plants. Moreover, the selected PGPR isolates have the ability to enhance the growth of pea by increasing the root length in pot experiment by increasing. Further research in developing mutants of strain with higher production of IAA and siderophores, phosphate solubilization or ACC activity may help in elucidating the mechanism of PGPR isolates in promoting the pea growth in lab and field conditions. This would help in developing a potential inoculant for use in agriculture.

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CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

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