

## INOCULATION OF MULTI-TRAIT PLANT GROWTH PROMOTING RHIZOBACTERIA FOR WHEAT GROWTH PROMOTION IN POTHOUSE CONDITIONS

Raina Singhmar, Vivek singh, Laxmi Bhatti, Sudha dalal, Deepak Kumar Malik\*

### Address(es):

Department of Biotechnology, University Institute of Engineering & Technology, Kurukshetra University, Kurukshetra-136119, Haryana, India.

\*Corresponding author: [dmalik2015@kuk.ac.in](mailto:dmalik2015@kuk.ac.in)

<https://doi.org/10.55251/jmbfs.10484>

### ARTICLE INFO

Received 17. 8. 2023  
Revised 22. 10. 2024  
Accepted 19. 11. 2024  
Published 1. 12. 2024

Regular article



### ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) are commonly used as inoculants to improve the growth and yield of wheat crops. Thus, the present study focused on the isolating and screening of effective isolates with multi-traits PGPR activities related to plant growth and development. Bacterial strains RA4, RA11 and RA26 were showing plant growth promoting activities like potassium solubilization, phosphate solubilization, indole acetic acid production (IAA), hydrogen cyanide production (HCN), ammonium production, zinc solubilization, anti-fungal activity. The antibiotic pattern showed that bacterial isolates RA11 and RA26 were resistant against azithromycin, ampicillin and gentamicin. Identification of multi trait PGPR was carried out by 16s rRNA sequencing. Bacterial strains RA4, RA11 and RA26 were showing 99.93 %, 100 % and 99.97 % similarity and identified as *Pseudomonas paraaeruginosa*, *Bacillus cereus* and *Pseudomonas aeruginosa* respectively. A pot house experiment was conducted in order to check the efficacy of multi-trait PGPR as inoculants for wheat growth. In our study, bacterial strain *Bacillus cereus* (RA11) was showing maximum plant growth promotion in the presence of the RDK of NPK in pot house conditions. Generally, results of this study revealed that use of bio fertilizers had an effective and significant role in the growth of plants.

**Keywords:** Multi-trait PGPR, Indole acetic acid production (IAA), HCN, Antibiotics, Wheat

### INTRODUCTION

More than 50 % of the world energy intake is provided by cereal crops such as wheat, rice and maize. Among them, wheat is the most important food in several developing countries. Chemical fertilizer requirements are very important for the cultivation of the wheat crop. The extensive use of chemical fertilizers in agriculture causes adverse effect on the environment and consumer health (Quim, 2020). Moreover, excessive use of agrochemicals for weed control has resulted into considerable pollution of water and soil (Dahiya et al., 2019). Therefore, microorganisms or their products could be used to develop cost effective, eco-friendly and sustainable weed management bio control practices (Seharawat et al., 2022; Phouret et al., 2022). Plant growth-promoting rhizobacteria (PGPR) as bio fertilizer are very effective in different environmental and climatic conditions (Gouda et al., 2018). The rhizospheric region has been directly influenced by the presence of plant roots (Pii et al., 2015). Rhizosphere supports active microbial population capable of beneficial, neutral and detrimental effects on the plants (Kumar et al., 2018).

Screening of effective PGPRs is very important to enhance the effective growth and yield of agricultural crops along with maintaining the sustainability of agro-ecosystems. PGPR are important for boosting plant biomass and minimize phytotoxic effects (Bhattacharyya et al., 2012). PGPR such as *Bacillus megaterium*, *Anabaena*, *Azolla*, *Bradyrhizobium*, *Bacillus polymyxa*, *Rhizobium* and *Sinorhizobium* etc enhanced the growth and yield of various crops (Olanrewaju et al., 2017). Generally, Plant Growth-Promoting Rhizobacteria (PGPR) support plant health in various ways, including protecting plants from pathogen attacks and enhancing the uptake of specific nutrients from the environment (Cakmakci et al., 2006 and Sagar et al., 2021). The plant growth promoting rhizobacteria (PGPR) can secrete multiple metabolites and enzymes that benefits plants growth (Zafar-ul-Hye et al., 2020).

Plant inoculation studies indicated that PGPR strains significantly increased shoot length, root length and root biomass. PGPR have great potential as bio fertilizers in enhancing the growth and nutrient content of various crops, including wheat, under both controlled pot house conditions and field conditions (Khan et al., 2022). The PGPR inoculations significantly decreased the pH, electrical conductivity and sodium adsorption ratio of rhizosphere soil over that of uninoculated soil (Ullah et al., 2022). The success of inoculation in crop can be influenced by various factors, including the plant genotype, the species of bacteria, and the agricultural practices (Souza et al., 2015 and Dal et al., 2017). However, studies regarding of bio inoculation process under pot house conditions are still few in the scientific literature (Naili et al., 2017). The aim of the present study was to

isolate the multi-trait plant growth promoting bacteria from pesticide contaminated soil and their evaluation for the plant growth promotion in wheat crop.

### MATERIAL AND METHODS

#### Isolation of bacterial strains from soil sample

Soil samples were collected from the various site of Mirzapur village of Kurukshetra district, Haryana. The bacterial strains were isolated from rhizospheric soil of wheat crop by using serial dilution method on nutrient agar plates. Morphologically different types of colonies were isolated and after purification by streaking method on nutrient agar plates, persevered in glycerol vials at -20 °C for further study.

#### Screening for plant growth promoting activity

##### IAA production

Indole acetic acid production (IAA) production of isolated bacterial strains were checked in liquid medium supplemented with 0.5 mg/ml L-tryptophan and salkowski reagent (0.5 M FeCl<sub>3</sub> in 35 % perchloric acid and incubated at 37 °C (Rana et al., 2011). The quantitative estimation of IAA, was expressed as µg IAA produced per unit of optical density.

##### Phosphate solubilization

Isolated bacterial strains were spot inoculated on Pikovskaya's agar plates and incubated at 37 °C for 7 days. Bacterial colonies shows clear zone around considered as phosphate solubilizer (Sharma et al., 2011). Quantitative analysis of phosphate solubilization was determined spectrophotometrically at 600 nm by adding chloromolybdic acid and chlorostannous acid (Patel et al., 2015).

##### Zinc solubilization

Bacterial strains were spot inoculated on modified agar plates containing 0.1% zinc carbonate and incubated at 37 °C for 5 days. Bacterial colonies shows clear zone considered as zinc solubilizer (Kumar et al., 2014).

**Potassium solubilization**

Bacterial strains were spot inoculated on Aleksandrow agar medium followed by incubation at 35 °C for 7 days. Bacterial colonies show a clear zone considered as potassium solubilizer. In quantitative analysis of potassium solubilization, available potassium present in supernatant after membrane filtration was estimated by flame photometry (Baba et al., 2021).

**HCN production**

To check the hydrogen cyanide production, isolated bacterial strains were streaked on King's B agar medium amended with 4.4 g glycine. The Whatman filter papers soaked in picric acid (0.05 % solution in 2 % sodium carbonate) were placed in the lid of petri plates. The plates were sealed air-tight with parafilm and incubated at 30 °C for 48 hours (Joseph et al., 2007).

**Anti-fungal activity against *Fusarium oxysporum***

The anti-fungal activity of isolated bacterial strains was determined against *Fusarium oxysporum* with slight modifications. The growth suspension of *Fusarium oxysporum* was spread on the surface of potato dextrose agar (PDA) plates. After spread of *Fusarium oxysporum* suspension on PDA plates, wells of 6mm were punctured and filled with bacterial culture. Anti-fungal activity of bacterial strains will be assessed on the basis of inhibition zone size after 4 days of incubation at 28 °C (Bano and Musarrat, 2013).

**Ammonium production**

Bacterial strains inoculated peptone broth. After incubation add 1mL Nessler's reagent was mixed and formation of yellow to brown precipitate showed the presence of ammonium (Deb et al., 2023).

**Protease activity**

Isolated bacterial strains were spot inoculated on skimmed milk agar plates and after incubation at 35 °C for 48 hr., clear zone around bacterial colonies indicate the protease activity (Tulini et al., 2016).

**Antibiotic resistance pattern of isolated bacterial strains**

Antibiotic (azithromycin, ampicillin, gentamicin, ciprofloxacin and clarithromycin) resistant pattern of isolated bacterial strains was determined by using disc diffusion method on nutrient agar media (Dibah et al., 2014; Gurunget al., 2019).

**Identification of multi trait PGPR strains**

Identification of multi trait PGPR bacterial strains was carried out by 16s rRNA sequencing at NCIM, Pune.

**Efficacy of multi-trait PGPR as inoculants for wheat growth promotion under pot house conditions**

Seeds of *Triticum aestivum* (HD-2851) were taken from kurukshetra local seed market. Before sowing, the seeds were sterilized with 70 % ethanol for 1 min then rinsed with autoclaved distilled water twice. After washing, seeds were treated with 5 % sodium hypochlorite solution for 10 minutes (Bahari et al., 2019). After sterilization, seeds were treated with multitrait PGPR as shown in table 1. After sowing the treated seeds, pots were kept in pothouse. The wheat plants were uprooted at tillering (50 days) and grain development stage (100 days) to check the root length, fresh and dry weight of roots, shoot length, fresh and dry weight of shoots, plant biomass, and panicle weight (Thakur et al., 2023). The data was statistically analyzed by using factorial randomized block designs (RBD). ANOVA was used to detect the statistical significance of data. Duncan's multiple range test was applied to check the statistical significance of data. The standard error of Mean ( $\pm$  SEM) was calculated with a significance threshold at .05 or  $P \leq .05$  levels.

**Table 1** The following treatments were applied to *Triticum aestivum* under pot house conditions

Treatment	Inoculation	Chemical fertilizer
T1	Seed without inoculation (Control)	No fertilizer applied under pot house conditions
T2	Seed inoculated with bacterial strain RA4	No fertilizer applied under pot house conditions
T3	Seed inoculated with bacterial strain RA11	No fertilizer applied under pot house conditions
T4	Seed inoculated with bacterial strain RA26	No fertilizer applied under pot house conditions
T5	Seed without inoculation (Control)	RDK of NPK under pot house conditions
T6	Seed inoculated with bacterial strain RA4	RDK of NPK under pot house conditions
T7	Seed inoculated with bacterial strain RA11	RDK of NPK under pot house conditions
T8	Seed inoculated with bacterial strain RA26	RDK of NPK under pot house conditions

**RESULTS AND DISCUSSION**

**Isolation and screening of isolated bacterial strains for PGPR activity**

A total of 30 morphologically different bacterial strains were isolated and labelled as (RA1 to RA30). Bacterial isolates, (RA4, RA11 and RA26) were showing multi PGPR trait like (phosphate solubilization, zinc solubilization, potassium solubilization, indole acetic acid production, hydrogen cyanide production, ammonium production, antifungal activity) as shown in table 2. The antibiotics-

resistant pattern of isolated bacterial strains was shown in table 3. The bacterial strain RA11 and RA26 were resistance against azithromycin, ampicillin and gentamicin. The qualitative estimation of phosphate solubilization, potassium solubilization and indole acetic acid production by bacterial isolates RA4, RA11 and RA26 was shown in table 4. The maximum phosphate solubilization (31.71  $\mu\text{g/ml}$ ) was shown by strain RA4. The maximum potassium solubilization (15.03  $\mu\text{g/ml}$ ) and IAA production (30.9  $\mu\text{g/ml}$ ) was shown by strain RA11.

**Table 2** Screening of isolated bacterial strains for their Plant growth promoting activity

Bacterial strains	Protease activity	IAA production	Phosphate solubilization	Potassium solubilization	Zinc solubilization	HCN production	Ammonium production	Anti-fungal activity
RA1	-	-	+	+	-	-	++	+
RA2	+	-	-	-	-	++	++	-
RA3	-	+	-	-	-	+++	-	-
RA4	-	++	+++	+	+	++	+	-
RA5	-	+	-	-	-	-	-	+
RA6	+	-	+	-	-	-	-	+
RA7	-	-	-	-	-	-	-	+
RA8	-	+	-	-	-	++	-	-
RA9	-	-	++	-	-	+	-	+
RA10	-	-	-	-	-	+	++	-
RA11	+	+++	+++	++	+++	+	++	+
RA12	-	-	-	-	-	-	-	-
RA13	-	-	-	-	-	-	+++	-
RA14	-	+++	-	+	+++	++	-	+
RA15	-	-	-	-	-	-	+	-
RA16	+	-	++	-	-	+	++	-
RA17	-	-	-	-	-	-	+	-
RA18	-	-	++	-	-	-	-	+
RA19	-	-	-	+	++	+	+++	+

RA20	-	-	-	-	-	-	-	-
RA21	-	-	-	-	-	-	-	+
RA22	-	++	+	-	-	+	-	-
RA23	-	-	-	-	-	-	++	-
RA24	-	+	-	-	-	-	-	+
RA25	-	+	-	-	-	+	-	-
RA26	-	+	+++	+	+	+	+	+
RA27	-	+	-	-	-	+	+++	+
RA28	-	-	-	-	++	-	+	-
RA29	-	-	-	-	-	-	+	-
RA30	-	+	-	-	-	-	-	-

Note: -, Negative; +, Slightly positive; ++, Moderate positive; +++, Maximum positive activity

Table 3 Antibiotics resistance pattern of isolated bacterial strains

Bacterial Isolates	Azithromycin	Ampicillin	Gentamicin	Ciprofloxacin	Clarithromycin
RA2, RA4, RA5, RA9, RA16, RA19, RA27	-	-	-	-	-
RA28	-	-	-	+	-
RA14	-	-	-	+	+
RA11, RA26	+	+	+	-	-

Note: -, sensitive against antibiotics; +, resistance against antibiotic

Table 4 Quantitative analysis of IAA, phosphate solubilization and potassium solubilization by selected plant growth promoting bacterial strains

Isolated strains	IAA production (µg/ mL)	Phosphate solubilization (µg/ mL)	Potassium solubilization (µg/ mL)
RA4	22.7	31.71	12.76
RA11	30.9	14.67	15.03
RA26	23.5	17.3	12.09

Identification of PGPR strains RA4, RA11 and RA26

The identification of multi trait PGPR strains was done by 16s rRNA sequencing. The 16S rRNA sequence of strains RA4, RA11 and RA26 was compared with other sequences by using BLAST analysis. The 16s rRNA sequence of strains RA4, RA11 and RA26 was showing 99.93 %, 100 % and 99.97 % similarity and identified as *Pseudomonas paraaeruginosa*, *Bacillus cereus* and *Pseudomonas aeruginosa* respectively. The phylogenetic tree of strains RA4, RA11 and RA26 were constructed by Neighbour-Joining method as shown in fig.1, 2 and 3 respectively.

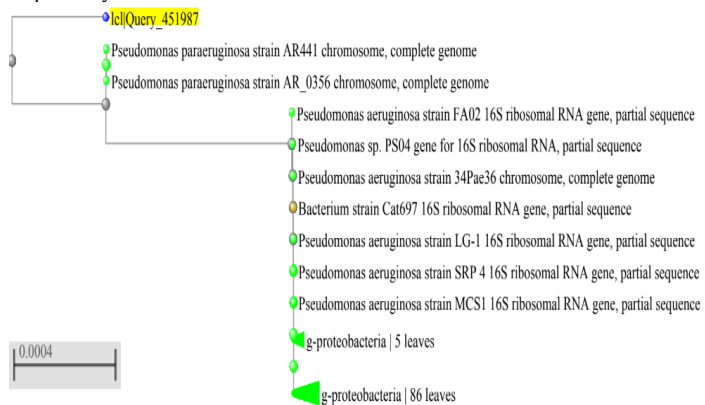


Figure 1 Phylogenetic tree of bacterial strain RA4 constructed by MEGA7



Figure 2 Phylogenetic tree of bacterial strain RA11 constructed by MEGA7

Figure 2 Phylogenetic tree of bacterial strain RA11 constructed by MEGA7

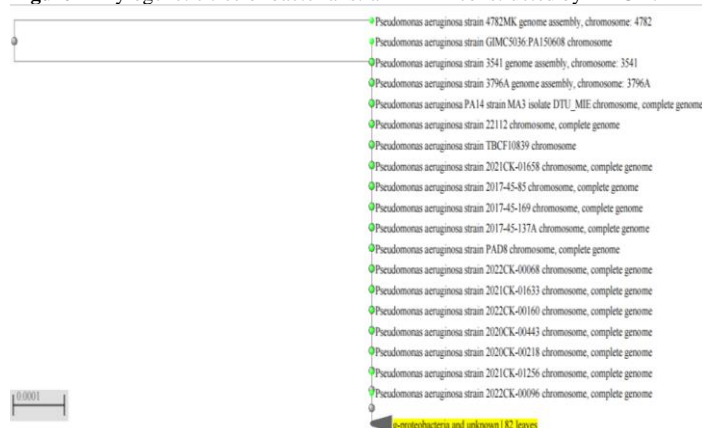


Figure 3 Phylogenetic tree of bacterial strain RA26 constructed by MEGA7

Plant growth promotion of *Triticum aestivum* multi-trait PGPR under pot house condition

At tillering (50 days), in treatment T3 (RA11 + no fertilizer) root length, fresh weight of roots, dry weight of roots, shoot length, fresh weight of shoot, dry weight of shoot and plant biomass was 13.9 cm, 1.03 gm, 0.84 gm, 52.2 cm, 1.48 gm, 1.38 gm and 1.82 gm, which increased (25%, 56%, 48%, 27%, 62%, 67% and 45% respectively as compared to treatment T1 (seed + No fertilizer) as shown in table 5. In treatment T7 (RA11 + RD of NPK) root length, fresh weight of roots, dry weight of roots, shoot length, fresh weight of shoot, dry weight of shoot and plant biomass of *Triticum aestivum* was 23.8 cm, 1.27 gm, 1.01 gm, 55.2 cm, 2.30 gm, 2.17 gm and 3.71 gm, which increased (47 %, 29 %, 23 %, 26 %, 60 %, 67% and 53 % respectively as compared to treatment T5 (seed +Recommended dose of fertilizer) as shown in table 5.

**Table 5** Effect of multi-traits PGPR strains inoculation on wheat crop under the pot house conditions at tillering stage

Treatment	Root length	Fresh weight of root	Dry weight of root	Shoot length	Fresh weight of shoot	Dry weight of shoot	Plant biomass
T1	10.4 ± 0.51 <sub>f</sub>	0.456 ± 0.027 <sup>c</sup>	0.432 ± 0.011 <sup>b</sup>	38.4 ± 1.63 <sup>e</sup>	0.55 ± 0.004 <sup>d</sup>	0.45 ± 0.004 <sup>d</sup>	1.002 ± 0.02 <sup>d</sup>
T2	11.7 ± 0.66 <sub>3<sup>f</sup></sub>	0.532 ± 0.034 <sup>b<sub>c</sub></sup>	0.475 ± 0.024 <sup>b</sup>	50.8 ± 1.772 <sup>b</sup>	0.768 ± 0.083 <sup>cd</sup>	0.768 ± 0.083 <sup>cd</sup>	1.412 ± 0.02 <sub>2<sup>d</sup></sub>
T3	13.9 ± 0.33 <sub>2<sup>d</sup></sub>	1.034 ± 0.205 <sup>a</sup>	0.845 ± 0.077 <sup>ab</sup>	52.4 ± 0.98 <sup>b</sup>	1.485 ± 0.311 <sup>b</sup>	1.385 ± 0.311 <sup>b</sup>	1.82 ± 0.024 <sup>b</sup>
T4	12.9 ± 0.71 <sub>4<sup>e</sup></sub>	0.924 ± 0.053 <sub>ab</sub>	0.838 ± 0.111 <sup>ab</sup>	51.2 ± 1.319 <sup>b</sup>	1.178 ± 0.091 <sup>bc</sup>	1.178 ± 0.091 <sup>bc</sup>	1.48 ± 0.019 <sup>bc</sup>
T5	12.6 ± 0.87 <sub>2<sup>e</sup></sub>	0.898 ± 0.069 <sup>a<sub>b</sub></sup>	0.778 ± 0.127 <sup>ab</sup>	40.8 ± 0.8 <sup>d</sup>	0.90 ± 0.074 <sup>cd</sup>	0.7 ± 0.074 <sup>cd</sup>	1.722 ± 0.053 <sup>cd</sup>
T6	15.4 ± 0.67 <sub>8<sup>c</sup></sub>	0.934 ± 0.049 <sup>a<sub>b</sub></sup>	0.888 ± 0.096 <sup>ab</sup>	52.4 ± 0.678 <sup>c</sup>	1.76 ± 0.066 <sup>bcd</sup>	1.60 ± 0.066 <sup>bcd</sup>	2.168 ± 0.018 <sup>bc</sup>
T7	17.2 ± 1.59 <sub>4<sup>a</sup></sub>	1.078 ± 0.05 <sup>a</sup>	1.015 ± 0.052 <sup>a</sup>	55.2 ± 2.177 <sup>a</sup>	2.375 ± 0.401 <sup>a</sup>	2.175 ± 0.401 <sup>a</sup>	3.716 ± 0.027 <sup>a</sup>
T8	19.4 ± 0.6 <sup>b</sup>	0.968 ± 0.029 <sup>a</sup>	0.863 ± 0.038 <sup>ab</sup>	51 ± 1.304 <sup>b</sup>	2.11 ± 0.078 <sup>bcd</sup>	1.11 ± 0.078 <sup>bcd</sup>	2.32 ± 0.019 <sup>bc</sup>
C.V.	2.2	22.1	10.3	6.57	13.8	13.8	2.83
p-value	0.00024	0.00003	0.0000	0.0000	0.0000	0.00001	0.0000

Note: CV= coefficient of variation and p-value = probability

At grain development stage (100 days), in treatment T3 (RA11 + No fertilizer) root length, fresh weight of roots, dry weight of roots, shoot length, fresh weight of shoot, dry weight of shoot, plant biomass and panicle weight was 18.5 cm, 1.19 gm, 1.05 gm, 57.7 cm, 1.70 gm, 1.40 gm, 2.18 gm and 1.4 gm which increased (27 %, 54 %, 52 %, 29 %, 58 %, 59 % 30% and 29 % respectively as compared to treatment T1 (seed + No fertilizer) as shown in table 6. In treatment T7 (RA11 + Recommended dose of fertilizer) root length, fresh weight of roots, dry weight of

roots, shoot length, fresh weight of shoot, dry weight of shoot plant, biomass and panicle weight was 27 cm, 1.53 gm, 1.47 gm, 59.7 cm, 2.68 gm, 2.48 gm, 4.57 gm and 1.9 gm, which increased (38 %, 34 %, 38 %, 20 %, 45 %, 46 %, 38% and 19 % respectively as compared to treatment T5 (seed + Recommended dose of fertilizer) as shown in table 6.

**Table 6** Effect of inoculation with plant growth-promoting (PGP) traits on wheat crop under the pot house trial at grain developmental stage

Treatment	Root length	Fresh weight of roots	Dry weight of roots	Shoot length	Fresh weight of shoots	Dry weight of shoots	Plant biomass	Panicle weight
T1	13.5 ± 0.5 <sup>f</sup>	0.54 ± 0.018 <sup>c</sup>	0.505 ± 0.018 <sup>c</sup>	40.75 ± 0.854 <sup>e</sup>	0.705 ± 0.03 <sup>f</sup>	0.685 ± 0.03 <sup>f</sup>	1.52 ± 0.018 <sup>e</sup>	1.002 ± 0.22 <sup>d</sup>
T2	16.75 ± 1.181 <sup>e</sup>	0.783 ± 0.075 <sup>c</sup>	0.627 ± 0.013 <sup>c</sup>	51 ± 2.345 <sup>c</sup>	1.023 ± 0.032 <sup>ef</sup>	1.023 ± 0.032 <sup>ef</sup>	1.953 ± 0.019 <sup>f</sup>	1.312 ± 0.022 <sup>d</sup>
T3	17.5 ± 0.5 <sup>d</sup>	1.195 ± 0.069 <sup>b</sup>	1.05 ± 0.027 <sup>b</sup>	57.75 ± 1.109 <sup>ab</sup>	1.708 ± 0.124 <sup>cd</sup>	1.408 ± 0.124 <sup>cd</sup>	3.185 ± 0.031 <sup>d</sup>	1.42 ± 0.024 <sup>b</sup>
T4	18.5 ± 0.5 <sup>de</sup>	0.953 ± 0.024 <sup>bc</sup>	0.923 ± 0.046 <sup>b</sup>	53.75 ± 1.931 <sup>c</sup>	1.26 ± 0.084 <sup>de</sup>	1.26 ± 0.084 <sup>de</sup>	1.593 ± 0.032 <sup>e</sup>	1.348 ± 0.019 <sup>c</sup>
T5	16.5 ± 1.19 <sup>e</sup>	1.005 ± 0.137 <sup>ac</sup>	0.91 ± 0.044 <sup>b</sup>	47.25 ± 2.287 <sup>d</sup>	1.458 ± 0.101 <sup>de</sup>	1.358 ± 0.101 <sup>de</sup>	2.805 ± 0.038 <sup>e</sup>	1.722 ± 0.053 <sup>cd</sup>
T6	21.5 ± 1.658 <sup>c</sup>	1.268 ± 0.228 <sup>b</sup>	1.125 ± 0.05 <sup>b</sup>	58.5 ± 1.848 <sup>b</sup>	2.108 ± 0.094 <sup>bc</sup>	2.108 ± 0.094 <sup>bc</sup>	3.518 ± 0.023 <sup>c</sup>	1.898 ± 0.018 <sup>bc</sup>
T7	27 ± 1.732 <sup>a</sup>	1.535 ± 0.12 <sup>a</sup>	1.47 ± 0.021 <sup>a</sup>	59.75 ± 3.198 <sup>a</sup>	2.68 ± 0.079 <sup>a</sup>	2.48 ± 0.079 <sup>a</sup>	4.57 ± 0.026 <sup>a</sup>	1.971 ± 0.027 <sup>a</sup>
T8	25 ± 1.08 <sup>b</sup>	1.15 ± 0.068 <sup>ab</sup>	1.01 ± 0.024 <sup>b</sup>	57.25 ± 1.25 <sup>b</sup>	2.218 ± 0.154 <sup>b</sup>	2.218 ± 0.154 <sup>b</sup>	3.783 ± 0.09 <sup>b</sup>	1.829 ± 0.019 <sup>bc</sup>
C.V.	3.365	21.532	7.234	7.538	11.634	11.637	1.784	13.326
p-value	0.0000	0.0000	0.00013	0.00001	0.0000	0.0000	0.0000	0.0000

Note: CV= coefficient of variation and p-value = probability

**Statistical analysis**

The data of pothouse experiment was compared with two variables i.e., 50 and 100 days as shown in table 5 and 6. The Coefficient variation (CV) and SE(m) value of root length, fresh weight of roots, dry weight of roots, shoot length, fresh weight of shoot, dry weight of shoot and plant biomass was low at 50 and 100 days of incubation. The CV and SE(m) of panicle weight at 100 days was 13.3% and 0.02 respectively. Plant growth-promoting rhizobacteria (PGPR) play a crucial role in plants growth, stress tolerance and disease prevention (Kong et al., 2022). Out of 30, only 3 bacterial strains RA4, RA11 and RA26 were showing multi-trait PGPR activity.

Phosphorus solubilization by PGPR plays a crucial role in various physiological and biochemical processes within plants including energy transfer, photosynthesis, respiration and cell division (Peng et al., 2023). The synthesis of IAA by PGPR plays a significant role in plant growth promotion (Modi et al., 2017). Improving potassium availability through PGPR-mediated solubilization can enhance crop yields and reduce the need for synthetic fertilizers, thereby contributing to more sustainable and environmentally friendly agricultural practices (Bhagyalaxmi et al., 2017).

Zinc is essential for photosynthesis, cell membrane integrity, protein synthesis, pollen development and plant disease resistance (Nadeem et al., 2019). It also increases the levels of antioxidant enzymes and chlorophyll in plant tissues (Kang et al., 2017). HCN productions by PGPR protects the plants from diseases (Mehmood et al., 2023). Some PGPR strains are capable of producing ammonium through various mechanisms such as nitrogen fixation and mineralization of organic matter. This ammonium production can contribute to improved nutrient uptake and overall plant health (Etesami et al., 2020).

The multi-trait PGPR were inoculated to check their efficiency for wheat growth promotion under pot house condition. In absence and presence of recommended

dose of fertilizer at both tillering stage and developmental stage, bacterial strain RA11 was promoting root length, fresh weight of root, dry weight of root, shoot length, fresh weight of shoot, dry weight of shoots and plant bio-mass compare to control (T1 and T5).

In presence of recommended dose of fertilizer at developmental stage, bacterial strain RA11 was promoting root length (31 %), fresh weight of root (22 %), dry weight of root (28 %), shoot length (3 %), fresh weight of shoot (36 %), dry weight of shoots (43 %), plant biomass (52 %) and panicle weight (8 %) as compare to RA11 without fertilizer. In absence and presence of recommended dose of fertilizer bacterial strain RA11 was promoting *Triticum aestivum* plant growth as compared to bacterial strains RA4 and RA26 at both 50 and 100 days of inoculation.

The P value, significant difference in mean and small value of CV and SE(m) at both stages indicates the consistency, reproducibility, sample representativeness and statistically significant data of root length, fresh weight of roots, dry weight of roots, shoot length, fresh weight of roots, dry weight of shoots, plant biomass and panicle weight.

Bacterial strains RA11 was promoting the plant growth might be due to increases in the availability of the soil nutrients, particularly N, P and iron. Bacterial inoculant in the presence of chemical fertilisers significantly influenced soil enzymes and microbial biomass carbon relative to the uninoculated control (Kumar et al., 2022). The multi-trait PGPR isolates effectively improved wheat crop productivity and maintained soil fertility under controlled conditions in a pot experiment (Rana et al., 2011).

**Conclusion**

PGPR could be recommended as biofertilizer in agriculture to prevent the deterioration of the environment. Out of 3 multitrait PGPR, strain *Bacillus cereus* (RA11) was showing maximum plant growth promotion of *Triticum aestivum* at both 50 and 100 days of inoculation under pot house conditions. As a result of multiple PGPR traits (phosphate solubilization, zinc solubilization, potassium

solubilization, IAA production, HCN production, ammonium production, antifungal activity) of RA11, it prove significant improvement inefficiency of wheat crop and conservation of soil fertility.

**Acknowledgement:** We are obliged to Department of Biotechnology, U.I.E.T. for providing Laboratory facility to carry out the project.

**Conflict of interest:** There is no conflict of interest.

**Financial Assistance:** The project was carried out in Department Laboratory and no financial assistance was obtained from anywhere.

## REFERENCES

- Baba, Z. A., Hamid, B., Sheikh, T. A., Alotaibi, S. H., El Enshasy, H. A., Ansari, M. J., & Sayyed, R. Z. (2021). *Psychrotolerant Mesorhizobium sp.* isolated from temperate and cold desert regions solubilizes potassium and produces multiple plant growth promoting metabolites. *Molecules*, 26(19), 5. <https://doi.org/10.3390/molecules26195758>
- Bahari, Z., Sazegari, S., Niazi, A., & Afsharifar, A. (2019). The application of an Agrobacterium-mediated in planta transformation system in a Catharanthus roseus medicinal plant. *Czech Journal of Genetics and Plant Breeding*, 56(1), 34-41. <https://doi.org/10.17221/153/2018-CJGPB>
- Bano, N., & Musarrat, J. (2003). Characterization of a new *Pseudomonas aeruginosa* strain NJ-15 as a potential biocontrol agent. *Current microbiology*, 46(5), 0324-0328. <https://doi.org/10.1007/s00284-002-3857-8>
- Bagyalakshmi, B., Ponnuragan, P., & Balamurugan, A. (2017). Potassium solubilization, plant growth promoting substances by potassium solubilizing bacteria (KSB) from southern Indian Tea plantation soil. *Biocatalysis and Agricultural Biotechnology*, 12, 116-124. <https://doi.org/10.1016/j.bcab.2017.09.011>
- Bhattacharyya, P. N., & Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology*, 28, 1327-1350. <https://doi.org/10.1007/s11274-011-0979-9>
- Cakmakci R, Donmez F, Ayдын A and Sahin F (2006). Growth promotion of plants by plant growth promoting rhizobacteria under greenhouse and two different field soils conditions. *Soil Biol Biochem*, 38, 1482-1487. <https://doi.org/10.1016/j.soilbio.2005.09.019>
- Dahiya, A., Sharma, R., Sindhu, S., & Sindhu, S. S. (2019). Resource partitioning in the rhizosphere by inoculated *Bacillus spp.* towards growth stimulation of wheat and suppression of wild oat (*Avena fatua* L.) weed *Physiology and Molecular Biology of Plants*, 25, 1483-1495. <https://doi.org/10.1007/s12298-019-00710-3>
- Dal Cortivo, C., Barion, G., Visioli, G., Mattarozzi, M., Mosca, G., & Vamerli, T. (2017). Increased root growth and nitrogen accumulation in common wheat following PGPR inoculation: Assessment of plant-microbe interactions by ESEM. *Agriculture, Ecosystems & Environment*, 247, 396-408. <https://doi.org/10.1016/j.agee.2017.07.006>
- Deb, L., Dutta, P., Mandal, M. K., & Singh, S. B. (2023). Antimicrobial Traits of *Beauveria bassiana* Against Rhizoctonia solani, the Causal Agent of Sheath Blight of Rice Under Field Conditions. *Plant Disease*, PDIS-04. <https://doi.org/10.1094/PDIS-04-22-0806-RE>
- Dibah, S., Arzanlou, M., Jannati, E., & Shapouri, R. (2014). Prevalence and antimicrobial resistance pattern of methicillin resistant *Staphylococcus aureus* (MRSA) strains isolated from clinical specimens in Ardabil, Iran. *Iranian journal of microbiology*, 6(3), 163-8. PMID: 25870749; PMCID: PMC4393492
- Etesami, H., & Adl, S. M. (2020). Plant growth-promoting rhizobacteria (PGPR) and their action mechanisms in availability of nutrients to plants. *Phyto-Microbiome in stress regulation*, 147-203. [https://doi.org/10.1007/978-981-15-2576-6\\_9](https://doi.org/10.1007/978-981-15-2576-6_9)
- Gouda, S., Kerry, R. G., Das, G., Paramithiotis, S., Shin, H. S., & Patra, J. K. (2018). Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. *Microbiological research*, 206, 131-140. <https://doi.org/10.1016/j.micres.2017.08.016>
- Gurung, R. R., Maharjan, P., & Chhetri, G. G. (2020). Antibiotic resistance pattern of *Staphylococcus aureus* with reference to MRSA isolates from pediatric patients. *Future science OA*, 6(4), FSO464. <https://doi.org/10.21203/rs.2.10785/v1>
- Joseph, B., Patra, R. R., & Lawrence, R. (2007). Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). *International Journal of Plant Production*, 1(2), 141-152. <https://doi.org/10.9734/bmrj/2015/14496>
- Khan, M. Y., Nadeem, S. M., Sohaib, M., Waqas, M. R., Alotaibi, F., Ali, L., ... & Al-Barakah, F. N. (2022). Potential of plant growth promoting bacterial consortium for improving the growth and yield of wheat under saline conditions. *Frontiers in microbiology*, 13, 958522. <https://doi.org/10.3389/fmicb.2022.958522>
- Kang, J. S., Singh, H., Singh, G., Kang, H., Kalra, V. P., & Kaur, J. (2017). Abiotic stress and its amelioration in cereals and pulses: a review. *Int. J. Curr. Microbiol. Appl. Sci*, 6(3), 1019-1045. <http://dx.doi.org/10.20546/ijcmas.2017.603.120>
- Kong, Z., & Liu, H. (2022). Modification of rhizosphere microbial communities: A possible mechanism of plant growth promoting rhizobacteria enhancing plant growth and fitness. *Frontiers in Plant Science*, 13, 920813. <https://doi.org/10.3389/fpls.2022.920813>
- Kumar, A., B.R. Maurya, and R. Raghuvanshi. (2014). Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.). *Biocat. Agric. Biotechnol.* 3:121–128. <https://doi.org/10.1016/j.bcab.2014.08.003>
- Kumar, A., Singh, V. K., Tripathi, V., Singh, P. P., & Singh, A. K. (2018). Plant growth-promoting rhizobacteria (PGPR): perspective in agriculture under biotic and abiotic stress. In *Crop improvement through microbial biotechnology* (pp. 333-342). <https://doi.org/10.1016/B978-0-444-63987-5.00016-5>
- Kumar, S., Sindhu, S. S., & Kumar, R. (2022). Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. *Current Research in Microbial Sciences*, 3, 100094. <https://doi.org/10.1016/j.crmicr.2021.100094>
- Mehmood, N., Saeed, M., Zafarullah, S., Hyder, S., Rizvi, Z. F., Gondal, A. S., ... & Kupe, M. (2023). Multifaceted Impacts of Plant-Beneficial *Pseudomonas spp.* in Managing Various Plant Diseases and Crop Yield Improvement. *ACS omega*, 8(25), 22296-22315. <https://doi.org/10.1021/acsomega.3c00870>
- Modi, K.; Patel, P. (2017) Isolation and characterization of plant growth promoting rhizobacteria associated with *Saccharum officinarum* L. *Curr. Syn. Syst. Biol.* 5, 132. <https://doi.org/10.3390/app13127105>
- Nadeem, F., & Farooq, M. (2019). Application of micronutrients in rice-wheat cropping system of South Asia. *Rice Science*, 26(6), 356-371. <https://doi.org/10.1016/j.rsci.2019.02.002>
- Naili, F., Neifar, M., Elhidri, D., Cherif, H., Bejaoui, B., Aroua, M., ... & Cherif, A. (2018). Optimization of the effect of PGPR-based biofertilizer on wheat growth and yield. *Biom. Biostat. Int. J.*, 7, 226-232. <https://doi.org/10.15406/bbij.2018.07.00213>
- Olanrewaju, O. S., Glick, B. R., & Babalola, O. O. (2017). Mechanisms of action of plant growth promoting bacteria. *World Journal of Microbiology and Biotechnology*, 33, 1-16. <https://doi.org/10.1007/s11274-017-2364-9>
- Patel, R. R., Patel, D. D., Thakor, P., Patel, B., & Thakkar, V. R. (2015). Alleviation of salt stress in germination of *Vigna radiata* L. by two halotolerant *Bacilli sp.* isolated from saline habitats of Gujarat. *Plant Growth Regulation*, 76(1), 51-60. <https://doi.org/10.1007/s10725-014-0008-8>
- Peng, S. H. T., Chee, K. H., Saud, H. M., Yusop, M. R., & Tan, G. H. (2023). Potential Novel Plant Growth Promoting Rhizobacteria for Bio-Organic Fertilizer Production in the Oil Palm (*Elaeis guineensis* Jacq.) in Malaysia. *Applied Sciences*, 13(12), 7105. <https://doi.org/10.3390/app13127105>
- Phour, M., & Sindhu, S. S. (2022). Mitigating abiotic stress: microbiome engineering for improving agricultural production and environmental sustainability. *Planta*, 256(5), 85. <https://doi.org/10.1007/s00425-022-03997-x>
- Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S., & Crecchio, C. (2015). Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. *Biology and fertility of soils*, 51, 403-415. <https://doi.org/10.1007/s00374-015-0996-1>
- Qaim, M. (2020). Role of new plant breeding technologies for food security and sustainable agricultural development. *Applied Economic Perspectives and Policy*, 42(2), 129-150. <https://doi.org/10.1002/aep.13044>
- Rana, A., Saharan, B., Nain, L., Prasanna, R., & Shivay, Y. S. (2011). Enhancing micronutrient uptake and yield of wheat through bacterial PGPR consortia. *Soil Science and Plant Nutrition*, 58(5), 573-582. <https://doi.org/10.1080/00380768.2012.716750>
- Nadeem, F., & Farooq, M. (2019). Application of micronutrients in rice-wheat cropping system of South Asia. *Rice Science*, 26(6), 356-371. <https://doi.org/10.1016/j.rsci.2019.02.002>
- Sagar, A., Rathore, P., Ramteke, P. W., Ramakrishna, W., Reddy, M. S., & Pecoraro, L. (2021). Plant growth promoting rhizobacteria, arbuscular mycorrhizal fungi and their synergistic interactions to counteract the negative effects of saline soil on agriculture: Key macromolecules and mechanisms. *Microorganisms*, 9(7), 1491. <https://doi.org/10.3390/microorganisms9071491>
- Sehrawat, A., Sindhu, S. S., & Glick, B. R. (2022). Hydrogen cyanide production by soil bacteria: Biological control of pests and promotion of plant growth in sustainable agriculture. *Pedosphere*, 32(1), 15-38. [https://doi.org/10.1016/S1002-0160\(21\)60058-9](https://doi.org/10.1016/S1002-0160(21)60058-9)
- Sharma, M.; Mishra, V.; Rau, N.; Sharma, R.S. (2011). Increased iron-stress resilience of maize through inoculation of siderophore-producing *Arthrobacter globiformis* from mine. *J. Basic Microbiol.* 56, 719–735. <https://doi.org/10.1002/jobm.201500450>
- Sood, G., Kaushal, R., Chauhan, A., & Gupta, S. (2018). Indigenous plant-growth-promoting rhizobacteria and chemical fertilisers: impact on wheat (*Triticum aestivum*) productivity and soil properties in North Western Himalayan region. *Crop and Pasture Science*, 69(5), 460-468. <https://doi.org/10.1071/CP18016>
- Souza, R. D., Ambrosini, A., & Passaglia, L. M. (2015). Plant growth-promoting bacteria as inoculants in agricultural soils. *Genetics and molecular biology*, 38, 401-419. <https://doi.org/10.1590/S1415-475738420150053>
- Thakur, R., Srivastava, S., & Yadav, S. (2023). Multitrait *Pseudomonas sp.* isolated from the rhizosphere of *Bergenia ciliata* acts as a growth-promoting

- bioinoculant for plants. *Frontiers in Sustainable Food Systems*, 7, 1097587. <https://doi.org/10.3389/fsufs.2023.1097587>
- Tulini, F. L., Hymery, N., Haertlé, T., Le Blay, G., & De Martinis, E. C. (2016). Screening for antimicrobial and proteolytic activities of lactic acid bacteria isolated from cow, buffalo and goat milk and cheeses marketed in the southeast region of Brazil. *Journal of Dairy Research*, 83(1), 115-124. <https://doi.org/10.1017/S0022029915000606>
- Ullah, S., Bano, A., Ullah, A., Shahid, M. A., & Khan, N. (2022). A comparative study of plant growth promoting rhizobacteria (PGPR) and sowing methods on nutrient availability in wheat and rhizosphere soil under salinity stress. *Rhizosphere*, 23, 100571. <https://doi.org/10.1016/j.rhisph.2022.100571>
- Zafar-ul-Hye, M., Naeem, M., Danish, S., Khan, M. J., Fahad, S., Datta, R., ... & El-Esawi, M. A. (2020). Effect of cadmium-tolerant rhizobacteria on growth attributes and chlorophyll contents of bitter melon under cadmium toxicity. *Plants*, 9(10), 1386. <https://doi.org/10.3390/plants9101386>