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Research Article
Screening Multifunctional Plant Growth Promoting Rhizobacteria Strains for Enhancing Seed Germination in Wheat (*Triticum aestivum* L.)

[1]Jay Kishor Prasad, [1]Sandeep Kumar Gupta and [2]Richa Raghuwanshi

[1]Department of Botany, Institute of Science, Banaras Hindu University, 221005 Varanasi, Uttar Pradesh, India
[2]Department of Botany, Mahila Mahavidyalaya, Banaras Hindu University, 221005 Varanasi, Uttar Pradesh, India

Abstract

Background and Objective: Food security to the increasing population has become a challenge under shrinking agricultural lands and global climate changes. Increasing wheat production by Plant Growth Promoting Rhizobacteria (PGPR) can be a sustainable approach to meet the demand. The present study was aimed to screen an efficient PGPR for enhancing seed germination in wheat. **Methodology:** For this concern, rhizospheric soil samples were collected from 17 sites from 3 district of India and the isolated bacteria were tested for their plant growth promoting attributes like phosphate solubilization, Indole Acetic Acid (IAA) and Hydrogen Cyanide (HCN) production, catalase test and growth performance under altered pH and temperature. Selected PGPR's were then tested on wheat for their effect on seed germination and vigour index. **Results:** Of the 26 bacterial isolates screened for their growth promoting attributes, four isolates performed extremely well. High level of phosphate solubilization and IAA production (215.55 μg mL^{-1}) was observed by the selected isolates. Fluctuation in pH and temperature affected the bacterial growth, however considerable growth was observed upto 40°C and at pH 5 and 7. On inoculation of isolates Ac21 and Ac26, the percent seed germination of wheat got augmented by two fold and vigour index observed was much higher compared to earlier reports. **Conclusion:** The bacterial isolates Ac21 and Ac26 possessing a number of plant growth promoting attributes can be promising in accelerating seed germination and plant vigour.

Key words: PGPR, seed germination, seed priming, vigour index

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Corresponding Author: Richa Raghuwanshi, Department of Botany, Mahila Mahavidyalaya, Banaras Hindu University, 221005 Varanasi, Uttar Pradesh, India

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The PGPR are a group of free-living saprophytic soil bacteria that aggressively colonize plant roots and benefit plants by growth promotion. It is well established that, in the rhizosphere, only 1-2% of bacteria promote plant growth[1]. The soil attached to the root system act as a hot spot of microbial abundance and activity due to the presence of root exudates and bacterial deposits. Inoculation of crop plants with PGPR at an early stage of development improves biomass production through their effect on root system, which either directly or indirectly enhances plant growth[1,2]. The PGPR facilitate plant growth directly either by production of plant hormones, nitrogen fixation, production of IAA, solubilization of inorganic phosphate and mineralization of organic phosphate or indirectly through antagonism against phytopathogenic microorganism by the production of siderophores, synthesis of antibiotics, enzyme or fungicidal compounds or competition with harmful microorganisms[3-5]. Applying microbial inoculants helps to reduce the use of chemical pesticides and inorganic fertilizers and hence is a promising agricultural approach that can play a vital role in crop protection, growth promotion and biological disease control and sustain soil fertility. There is a little information about physical methods of seed treatment. Seed priming involves exposing quiescent seed to a solution or matricum of low water potential that permits partial seed hydration without seed germinations[6]. This seed treatment can improve seed germination and seedling emergence particularly under adverse seedbed conditions, such as low temperature, reduced water availability[7] or salinity[8]. In recent years, interest concerning the use of physical methods of seed priming due to their effects on plant growth has increased[9-12]. Seed priming with potential strain of PGPR increases seed germination and facilitates healthy seedling[13]. Priming initiates the physiological process of germination and help in the proliferation of PGPR in the spermosphere[14]. Wheat (*Triticum aestivum* L.) is the most important and extensively cultivated food crop in the world. It is cultivated globally over an area of about 215.48 million hectares with an annual production of nearly 670.87 million tonnes[15]. Wheat is the second major staple cereal crop after rice in India. Increasing wheat production under shrinking agricultural lands and changing climatic conditions has become a challenge. The chemical fertilizer added to meet the field demands has raised up to183 million tones which has added hazards to human and soil health. Improving plant growth and vigour during early stages of germination is a good idea in their better establishment and robust growth under adverse condition too. The PGPR are therefore a promising sustainable approach to meet the current yield demands, maintaining soil health. The present study was therefore undertaken to screen efficient PGPR's from rhizospheric soil and to evaluate its effect on germination characteristics of wheat crops by seed priming technique.

MATERIALS AND METHODS

Isolation and preliminary screening of the bacterial isolates: Soil samples were collected from the rhizospheric soil of *Acacia* species, wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) plants growing in two different states viz Uttar Pradesh (UP) and Madhya Pradesh (MP) of India and were stored at 4°C for further study. The soil of the collection sites were silty loam in nature with good permeability. The sites of collection were Rajeev Gandhi South Campus (RGSC, Mirzapur, UP), Agricultural farm (AF, BHU, UP), Chaundauli district (UP) and Riva district (MP) as indicated in Table 1. Bacteria were isolated from rhizospheric soil samples by serial dilution plate technique. Appropriate dilution was spread on Yeast Extract Mannitol Agar (YEMA) media containing per liter of distilled water: Yeast extract 1.00 g, mannitol 10.00 g, KH_2PO_4 0.50 g, $MgSO_4.7H_2O$ 0.20 g, NaCl 0.10 g and agar 15.00 g, pH 6.8-7.0. Plates were incubated at 28°C for 24-48 h. Colonies were picked from these plates and maintained as pure cultures in YEMA media with periodic transfer to fresh media and stock for further use. All the isolates were morphologically characterized based on colony morphology and Gram staining.

Screening for plant growth promoting activities: A total of 26 isolates obtained from rhizospheric soil samples were screened for different plant growth promoting attributes.

Phosphate solubilizing activity: Inorganic phosphate solubilizing activity was measured by plate assay as described by Pikovskaya[16] on Pikovskaya's Agar Medium (PVK) containing per liter of distilled water, yeast extract 0.50 g, dextrose 10.00 g, $Ca_3(PO_4)_2$ 5.00 g, $(NH_4)_2SO_4$ 0.500 g, KCl 0.20 g, $MgSO_4$ 0.10 g, $MnSO_4$ 0.0001 g, $FeSO_4$ 0.0001 g, agar 15.00 g. Tri-calcium phosphate $Ca_3(PO_4)_2$ was added to the media to measure its phosphate solubilizing activity. The halo transparent zone formed around the colony was presumptive confirmation of phosphate solubilization and was measured after 3 days of incubation at 30°C.

Table 1: Characterization of selected bacterial isolates for specific plant growth promoting traits

Site of collection of soil sample	Bacterial Isolates	Colony morphology	Gram staining (G^+/G^-)	Phosphate solubilization (zone of clearance) (cm)	HCN production
RGSC	Ac1	Punctiform, convex, white, entire	+	1.7	-
RGSC	Ac2	Punctiform, convex, white, entire	+	1.2	++
RGSC	Ac3	Punctiform, convex, white, entire	+	1.1	+
RGSC	Ac4	Irregular, pulvinate, white, curled	-	1.2	-
Chandauli	Ac5	Irregular, punctiform, white, curled	+	1.4	-
Chandauli	Ac6	Punctiform, convex, white, entire	+	1.7	++
Chandauli	Ac7	Punctiform, convex, white, entire	+	1.5	-
AF.BHU	Ac8	Circular, convex, white, smooth	+	-	-
AF.BHU	Ac9	Punctiform, convex, white, entire	-	-	-
AF.BHU	Ac10	Circular, convex, white, smooth	+	-	-
AF.BHU	Ac11	Circular, slightly yellowish, curled	+	1.7	+
AF.BHU	Ac12	Irregular, punctiform, white, curled	-	0.9	++
Riva, M.P	Ac13	Irregular, convex, white, curled	+	0.8	-
Riva, M.P	Ac14	Punctiform, convex, white, entire	-	-	-
Riva, M.P	Ac15	Irregular, punctiform, white, curled	+	1.5	-
Riva, M.P	Ac16	Punctiform, convex, white, entire	+	1.6	++
Riva, M.P	Ac17	Circular, convex, white, curled	-	-	-
Riva, M.P	Ac18	Irregular, punctiform, white, curled	+	1.1	+
Riva, M.P	Ac19	Circular, convex, white, curled	+	1.3	+
AF.BHU	Ac20	Irregular, pulvinate, white, curled	+	-	-
AF.BHU	Ac21	Spindle, pulvinate, white, curled	+	1.3	++
AF.BHU	Ac22	Irregular, pulvinate, white, curled	+	-	-
AF.BHU	Ac23	Irregular, pulvinate, white, curled	+	1.4	+
AF.BHU	Ac24	Irregular, convex, white, curled	+	-	-
AF.BHU	Ac25	Irregular, pulvinate, white, curled	+	1.2	+
AF.BHU	Ac26	Irregular, convex, white, curled	+	1.9	+++

HCN production: The HCN production by bacterial isolates was determined by color change of filter paper following Alstrom and Burns[17]. One hundred microliter of bacterial suspension was inoculated on nutrient agar medium containing 4.4 g L^{-1} glycine. Filter paper soaked in a reagent solution (2.5 g picric acid 0.125 g and sodium carbonate, 1000 mL of distilled water) was placed in the upper lid of petri dishes. To prevent volatilization, the plates were sealed with parafilm and incubated at 28°C for 4 days. The plate without inoculation of bacterium served as control. A change in colour of the filter paper from yellow to light brown, brown or reddish brown was recorded as weak (+), moderate (++) or strong (+++) reactions, respectively.

IAA production: The IAA production was detected by the modified method as described by Bric *et al.*[18]. Quantitative analysis of IAA was performed using the method of Loper and Schroth[19], YEMA-broth amended with 5 mM tryptophan inoculated with bacterial culture incubated at 28°C for 48 h. One milliliter sample of each culture was centrifuged (10,000×g for 10 min) and the supernatant was collected for further analysis, which involved the addition of 2 mL of salkowski reagent, followed by incubation for 1 h at room temperature under dark conditions. Development of pink colour indicated IAA production. Optical density was taken at 530 nm in UV-vis spectrophotometer. Concentration of IAA produced by cultures was measured with the help of standard graph of IAA obtained in µg mL^{-1}.

Catalase activity: Catalase activity of bacterial isolates was assayed spectrophotometrically by monitoring the decrease in absorbance of H_2O_2 at 240 nm. Catalase was measured according to the method of Beers and Sizer[20]. The enzyme was extracted in 50 mM phosphate buffer (pH 7). The assay solution contained 50 mM phosphate buffer and 10 mM H_2O_2. The reaction was started by addition of enzyme aliquot to the reaction mixture and the change in absorbance was followed after 2 min of the starting reaction. Unit activity was taken as the amount of enzyme, which decomposes 1 M of H_2O_2 in 1 min.

Temperature tolerance: Bacterial growth at different temperatures was determined in YEM-broth inoculated with 50 µL of isolated cultures (10^9 CFU mL^{-1}) and incubated at temperature 20, 30, 40 and 45°C. Optical Density (OD) was measured after 48 h at 420 nm.

pH tolerance: Growth of bacteria at different pH was determined in YEM-broth maintained at pH 5, 7, 9 and 11, inoculated with 50 µL of isolated cultures (10^9 CFU mL^{-1}). Optical Density (OD) was measured at 420 nm.

Seed treatment with PGPR: Out of the 26 bacterial isolates, the isolates with promising plant growth promoting traits were tested on wheat in the soil system. Seeds of wheat (*Triticum aestivum* L.) variety HUW1512 (BHU22) were surface sterilized with 0.2% $HgCl_2$ for 2 min, then rinsed in SDW for 10 min. Seeds were soaked for 7-8 h in 25 mL of YEMA having prescreened bacterial suspensions in their log phase containing 10^9 CFU mL^{-1} and kept at 28±2°C in rotator shaker (90 rpm). Control seeds were soaked in sterile medium. The seeds were then dried overnight aseptically in laminar air flow and used for green house experiments. The seeds were sown in earthen pots containing sterile soil and placed in a temperature controlled growth chamber. Six plants per pot were maintained throughout experimental period. The soil was maintained at 60% water holding capacity. The effect of PGPR treatment on seed germination and seedling vigour of wheat was examined. Germination and vigour analysis were carried out by paper towel method[21]. After 7 days of germination, the percentage germination was calculated as following Eq. 1:

$$\text{Germination (\%)} = \frac{\text{No. of germinated seeds}}{\text{Total No. of seeds}} \times 100 \quad (1)$$

To assess plant vigour, root and shoot length of each individual seedling was measured after 5 days. The vigour index[22] was calculated using the following Eq. 2:

$$\text{Vigour index} = (\text{Mean root length} + \text{mean soot length}) \times \text{germination (\%)} \quad (2)$$

The treatments were arranged in a completely randomized design with three replications. At the end of the experimental period, the plants were uprooted and the shoot and root length and fresh weight were measured. Biomass was dried to constant weight in oven at 80°C for recording the dry weight.

RESULTS

Phosphate solubilization by bacterial isolates: Out of the 26 isolates, 17 isolates formed a clear transparent halo zone around the colony indicating the phosphorus solubilized from tri-calcium phosphate added to the Pikovskaya's agar medium. The halo zone ranged from 0.8-1.9 cm in the different isolates (Fig. 1).

HCN production: The production of HCN in excess may play a critical role in the control of fungal pathogen as described by Flaishman *et al.*[23]. Among all the 26 isolates, 12 isolates were recorded positive for HCN production as shown in Table 1 and Fig. 2.

Indole acetic acid production: Indole acetic acid production by all 26 bacterial isolates was estimated, of which, 17 isolates produced IAA in tryptone yeast medium. The range of IAA production varied from 9.66-215.55 µg mL^{-1} after 72 h of incubation as depicted in Fig. 3a and b. Maximum IAA production was observed by isolate Ac2.

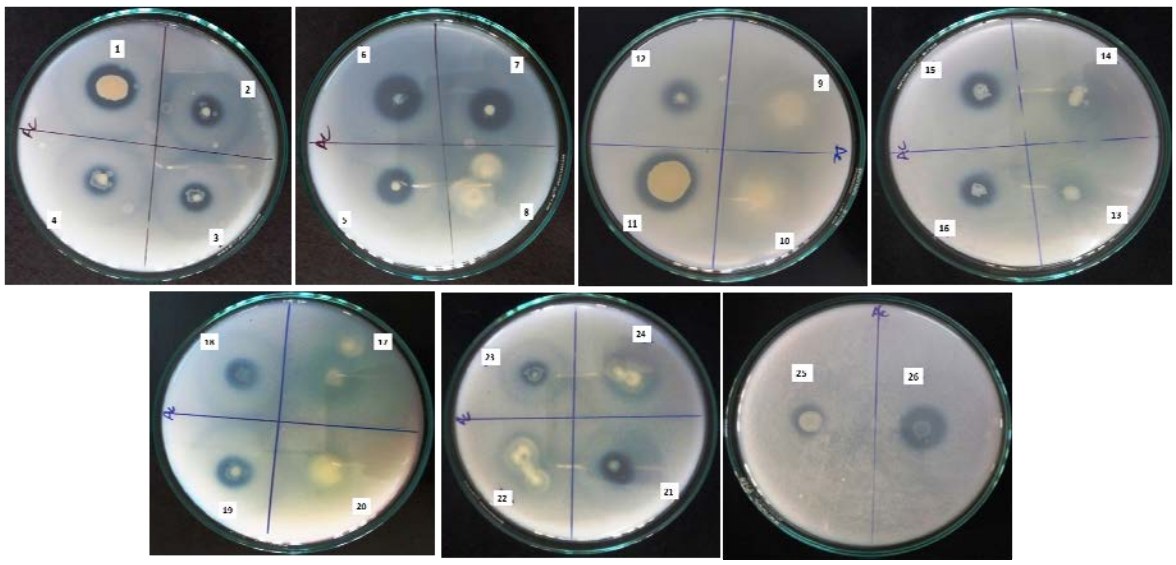

Fig. 1: Phosphate solubilization by bacterial isolates

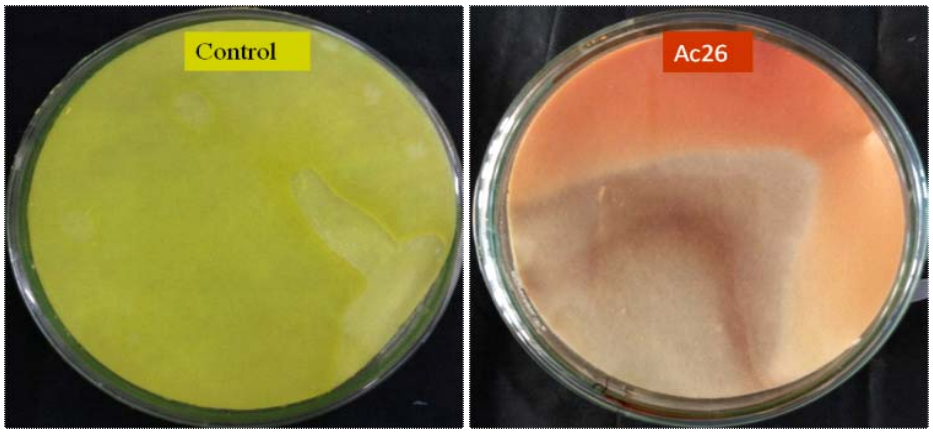

Fig. 2: HCN production by bacterial isolates

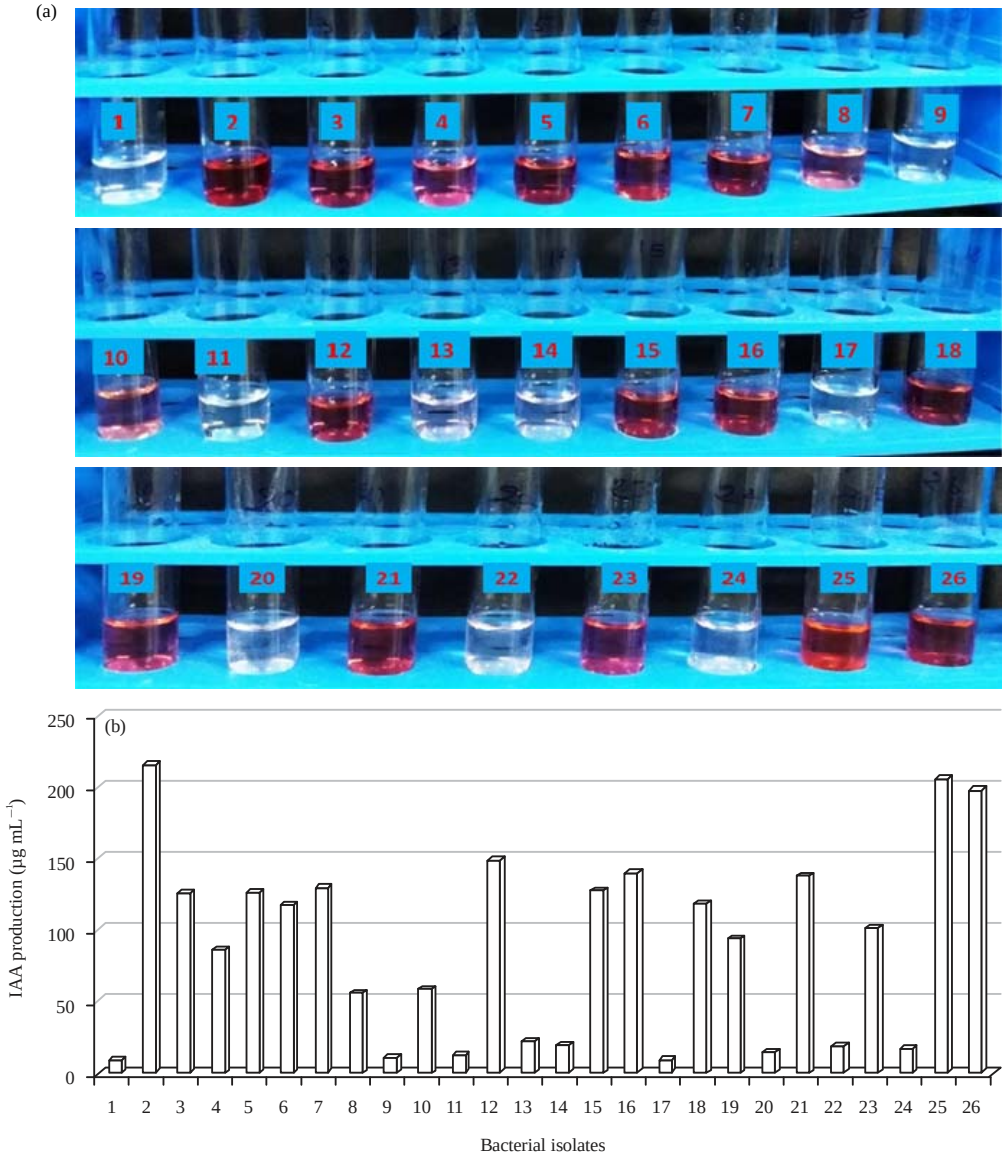

Fig. 3(a-b): (a) Development of pink colour and (b) Bars graph showing IAA production by bacterial isolates

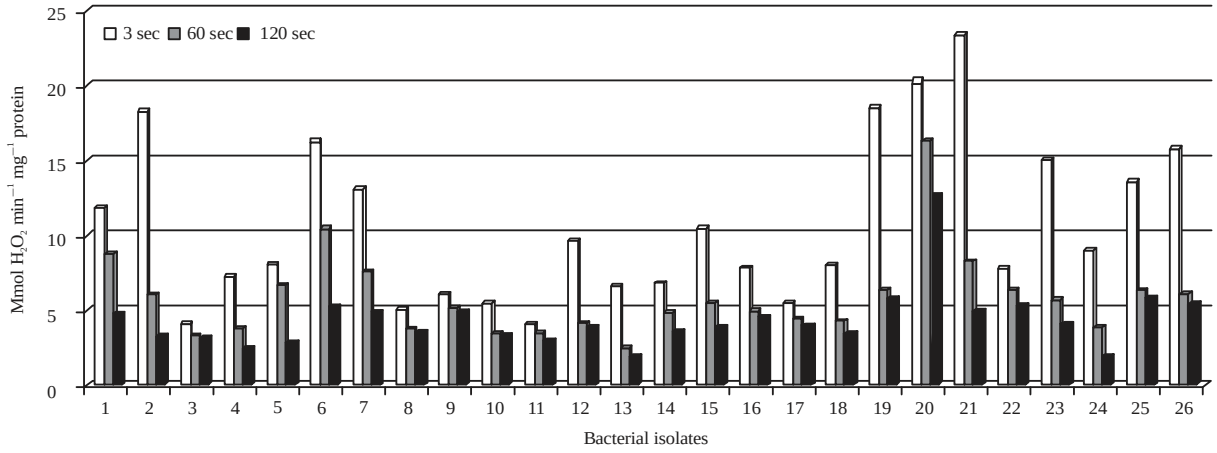

Fig. 4: Catalase activity of bacterial isolates at different time intervals

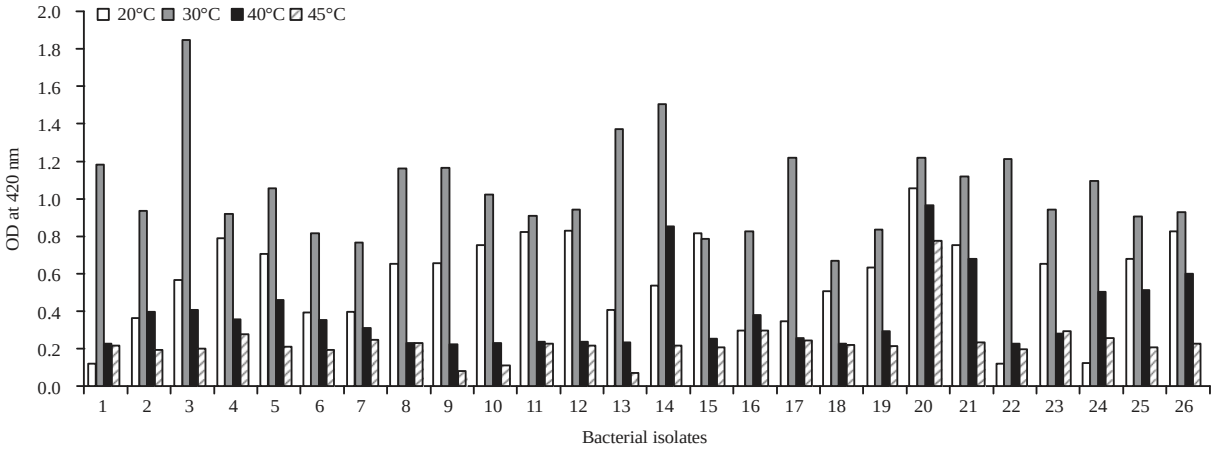

Fig. 5: Growth of bacterial isolates at varying temperature

Catalase test: The breakdown of hydrogen peroxide follow first order kinetics under a variety of conditions and increases linearly with catalase concentration. The decrease in concentration of H_2O_2 was evaluated by measuring absorbance at fix time intervals of 3, 60 and 120 sec at 240 nm. Out of 26 bacterial isolates,10 isolates showed good catalase activity. Maximum catalase activity was observed by Ac21 (Fig. 4).

Effect of temperature on growth of bacterial isolates: Maximum growth of bacterial isolates was observed at 30°C. There was a gradual and uniform decrease in bacterial growth rate with increase in temperature from 30-45°C. Minimum growth of bacterial isolates was observed at 45°C. Temperature below 30°C and above 40°C did not favour growth in most of the isolates (Fig. 5).

Effect of pH on growth of bacterial isolates: Maximum growth of all the bacterial isolates were obtained at pH 7. Minimum growth of most of the bacterial isolates was observed at pH 11 (Fig. 6).

Effect of PGPR on seed germination and vigour index: Considerable increase in percentage seed germination was observed after various bacterial inoculations. The increase in wheat germination ranged from 25-75% by the isolates, with maximum increase observed by the bacterial isolate Ac26. High vigour index in wheat was observed after PGPR inoculation. The bacterial isolate Ac26 showed a maximum value of 2830.69 and was 157% more compared to control (Fig. 7, Table 2).

DISCUSSION

Plant rhizosphere is a preferred niche of the soil microflora for its nutrient availability. The plants too get benefitted by the microflora particularly the PGPR due to its

Table 2: Influence of selected bacterial PGPR's on growth parameters of wheat crop under controlled conditions

Bacterial isolates	Germination (%)	Mean root length (cm)	Mean shoot length (cm)	Vigour index	Fresh weight (mg)	Dry weight (mg)	Root/shoot ratio
Ac2	66.66	7.55±0.10	16.55±0.15	1606.50	0.134±0.007	0.063±0.001	0.456
Ac3	70.00	8.03±0.16	16.75±0.10	1734.60	0.125±0.006	0.061±0.001	0.479
Ac21	86.66	12.45±0.13	15.66±0.19	2436.01	0.169±0.005	0.096±0.001	0.795
Ac26	93.33	13.08±0.11	17.25±0.10	2830.69	0.181±0.003	0.12±0.003	0.758
Control	53.33	5.50±0.08	15.08±0.13	1097.53	0.058±0.003	0.13±0.025	0.364

Data shows Mean±SD, derived from three independent replicates

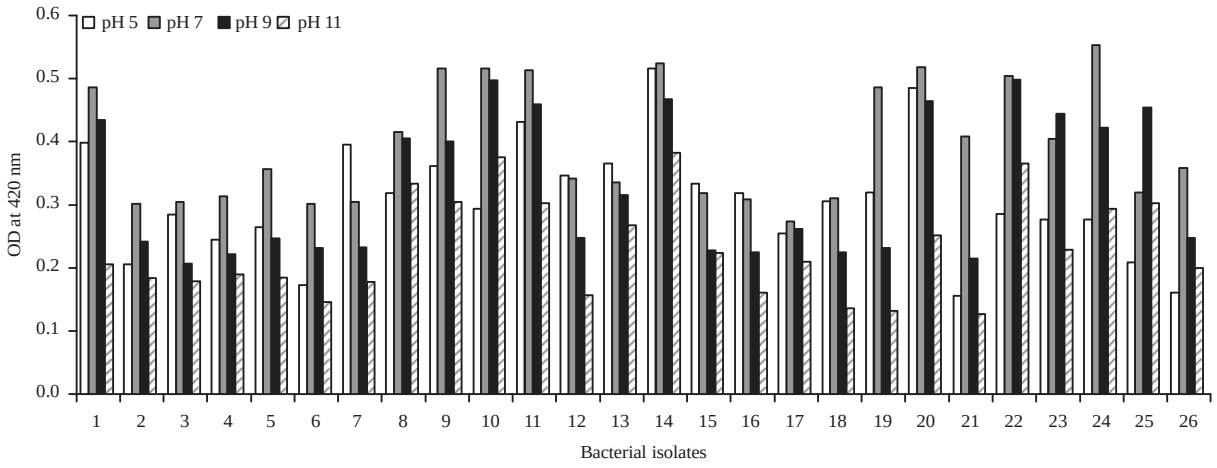

Fig. 6: Growth of bacterial isolates at varying pH

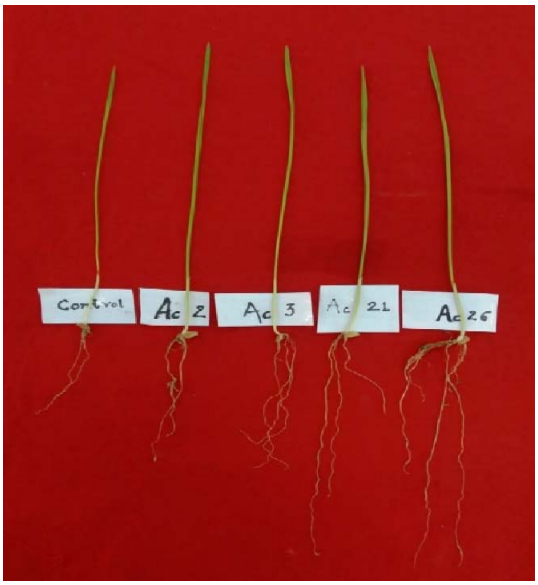

Fig. 7: Effect of PGPR inoculation on seed germination and vigor index of wheat

multifacet growth promoting attributes like production of growth hormones like IAA, phosphate solubilization, enhanced nutrient uptake[24], ammonia production and preventing the growth of plant pathogen by HCN production. In the present investigation 26 bacterial isolates were screened for their growth promoting attributes of which four isolates viz Ac2, Ac3, Ac21 and Ac26 performed extremely well. Most of the bacterial isolates showed more than 1 cm zone of clearance during the phosphate solubilization assay. While most of the studies report only the presence or absence of phosphate solubilization in the test isolates, the present results showed a high level of phosphate solubilization by the microbes, which favored the plant growth directly. As phosphorus is a major nutrient required for plant growth and most of the added phosphorus in form of NPK gets fixed in the soil, inoculating bacterial isolates with good phosphate solubilizing capacity can improve phosphorus mobilization and in turn availability to the plants. Increased phosphorus availability to wheat plants leads to profuse growth of root as evident in the present study and was in accordance to previous studies[25]. Augmentation in the root length was up to 137% after inoculation of strain Ac26. Increased root length not only support plant growth by exploring a greater volume of soil thereby increasing nutrient availability and absorption, but is also of great importance in plants exposed to drought stress as they increase water availability to plants[26,27]. Almost 2 fold increase in root length by Ac26 isolate indicated its potential to promote plant growth under normal soil conditions and can also be tested under drought stressed conditions.

The HCN production was detected in less than 50% of bacterial isolates. The Ac2, Ac3, Ac21 and Ac26 showing a number of plant growth promoting traits were also positive for HCN production. The HCN production by PGPR has been looked at both beneficial as well as harmful trait with respect to their effect on plant growth. The beneficial effects have mostly been discussed in their antifungal role and hence, suppression of plant pathogens[28]. Production of HCN in excess may play a critical role in suppression of fungal disease in wheat seedling. Inoculation of PGPR viz Ac2, Ac3, Ac21 and Ac26 found positive for all tested plant growth promoting traits showed increased percent germination and vigour index of wheat plants. Increase in wheat crop yield up to 49% has been reported in earlier studies[29]. Bacterial strain showing catalase activity must be highly resistant to environmental, mechanical and chemical stress. The isolates Ac2, Ac21 and Ac26 showed high catalase activity.

Drought conditions are accompanied by increase in temperature and changes in soil pH, therefore, the bacterial isolates were also tested for their growth behavior at the same extreme soil temperature and pH as observed in soil exposed to drought. All the isolates showed best growth at 30°C. A uniform decrease in bacterial growth of Ac2, Ac3, Ac21 and Ac26 was observed with a decrease of 58, 78, 39 and 35% in growth at 40°C. The Ac21 and Ac26 bacterial isolates showing multiple plant growth promoting activities, showed considerable growth even under altered temperature conditions thereby showing their wide adaptability to high and low temperatures. Fluctuation in pH of growth media also affected bacterial growth. Although an increase or decrease in pH by 2 units affected the growth rate of bacteria, however, it did not inhibit the bacterial multiplication.

Another important trait of plant growth promotion that was detected in most of the bacterial isolates was the production of growth hormone IAA. Isolates Ac2, Ac3, Ac21and Ac26 could produce relatively high concentration of IAA compared to other isolates. Production of high level IAA by isolates has a direct benefit on plant growth as they increase the root length[30]. The IAA production was quantified ranging 9.66-215.55 μg mL^{-1} in the present study. Similar observation of up to 46-50 μg mL^{-1} IAA production has been reported by other researchers[31,32]. Thus the test isolates were extremely promising in promoting root growth during early stage of seed germination and development and thereby resulting in enhanced vigour index. The seed germination percentage almost got augmented by 2 fold on inoculation of bacterial isolate Ac26. Similar reports of two-fold increase in seed germination has been reported by earlier researchers[32,33]. However, the vigour index reported in the present study in much higher compared to earlier reports[32] on wheat.

CONCLUSION

Screening bacteria for a number of growth promoting attributes can be promising approach in accelerating seed germination, plant development and suppression of diseases. In this study, the enhancement in early stages of plant growth parameters by the bacterial inoculants Ac21 and Ac26 was supported by the greater number of PGPR traits possessed by these isolates and hence they could prove effective in improving wheat crop production and maintenance of soil fertility.

SIGNIFICANT STATEMENTS

- Many plant growth promoting bacteria residing in the rhizosphere can serve as an important tool in enhancing seed germination and vigour index of wheat
- High level of phosphate solubilization and IAA production can be critical parameters for screening the growth promoting bacteria
- Seed priming by bacterial isolates Ac21 and Ac26 can enhance seed germination in wheat by 2 fold
- The bacterial strains obtained can be promising in increasing wheat production by an eco friendly approach which is safe for public health and address the food security issues

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