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SCREENING OF PLANT GROWTH PROMOTING RHIZOBACTERIA FOR SUSTAINABLE WHEAT (*TRITICUM AESTIVUM* L.) CROP PRODUCTION

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Abstract

The study was conducted to isolate and characterize wheat plant growth promoting rhizobacteria (PGPR). The isolation was carried out at tillering stage of wheat crop. The potential of wheat for colonizing different microbes at different combinations of organic and inorganic nutrient sources was determined. Overall, the microbes were more abundant in rhizosphere as compared to non-rhizosphere soil. The highest population of bacteria 9.64 log (cfu g^{-1} soil) and N_2 fixing bacteria 7.67 log (cfu g^{-1} soil) were found in the rhizosphere of wheat where 50 kg N and 33 kg P_2O_5 ha^{-1} integrated with FYM 9 t ha^{-1}. However, the most phosphate solubilizing bacteria 9.08 log (cfu g^{-1} soil) was recorded at sole application of FYM (9 t ha^{-1}). A total of 24 potential isolates varying in colony morphology were screened for further characterization. Most of the PGPR exhibited the capability of nitrogen fixation, P solubilization and IAA production. The P solubilization efficiency ranged from 11.14 to 75.00% with the highest recorded in NIA-PGPR1 (75%). The screened isolates produced indole-3 acetic acid (0.88-8.92 mg L^{-1}) with the highest IAA production of 8.92 mg L^{-1} in NIA-PGPR9. All PGPR strains also had the abilities of biofilm formation. Ten (10) most efficient PGPR isolates were selected for evaluation in enhancing wheat growth under laboratory conditions. All inoculated PGPR isolates positively affected wheat plant growth. The highest plant height (31.5 cm) was observed in plants inoculated with NIA-PGPR1 while maximum root length (8.5 cm) and plant dry biomass (0.172 g) was recorded in NIA-PGPR5. The screened bacterial strains have plant beneficial characters and have potential for development of bio-product in order to enhance wheat plant growth in a sustainable manner.

Key words: Fertilizer, IAA, N_2 fixing bacteria, Phosphate solubilizing bacteria, Plant growth, Rhizosphere.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cultivated crop in the world and is one of the oldest domesticated crops. It is staple food of 8000 years old prominent civilizations of Europe, West Asia and North Africa (Anon., 2014). Wheat occupies around one sixth of total arable land of the world and is the most demanded food grain among all other crops including rice, maize and potato (Anon., 2014). Wheat is staple food in most of South Asian countries and it holds second biggest economy among the population of over 190 million people (Anon., 2015). Wheat is also a prominent staple food crop in Pakistan and grown on a large scale throughout the country. Wheat crop constitutes 60% of the daily diet of people with consumption of 125 kg per capita per year in Pakistan. Hence, it possesses the essential position in agricultural sector. Pakistan is the 10th major wheat producing country in acreage and yield per hectare. In Asia, Pakistan is the 3rd largest producer of wheat grain with the production about 25.5 m tons which fulfill the country's requirement (Anon., 2015).

The continuous increase in population demands for growth in wheat production per unit area and the increase of the land use has resulted in serious depletion of plant nutrients (N, P & K) deficiency in the soils. The fastest and most popular way for maximizing crop production is the application of mineral fertilizers to provide the major nutrients for the growing plants (Imranuddin *et al*., 2017). The application of fertilizers is important for increasing the wheat production (Joy *et al*., 2017). These fertilizers perform a vital role in improving the crop yield particularly on alkaline and calcareous soils of Pakistan (Ahmad, 2000; 2016). However, application of these nutrients in lesser quantities results in low crop yield while their over application increase the environmental pollution. Furthermore, the continuous use of fertilizers will not help to retain crop productivity mainly due to poor soil health hence the application of organic manures can be a useful strategy in this situation (Alam & Shah, 2003). Moreover, the integrated use of these organic sources with chemical fertilizers has been proven an effective way to get an increased crop production. The balanced and integrated use of organic and inorganic fertilizers may improve the soil organic matter content and soil physical properties such as texture, soil pH and soil EC resulting in an improved soil carbon sequestration and crop yields (Alam *et al*., 2003).

The ever increasing prices of mineral fertilizers and non-availability of organic nutrient sources diverted the focus of scientists towards exploring alternative sources of nutrient supply for growing plants in a sustainable manner (Pretty & Bharucha, 2014). Plant growth promoting rhizobacteria (PGPR) are proved to enhance plant growth through the production of IAA, gibberellic acid, indol-3 butyric acid, siderophore, fixing atmospheric nitrogen and solubilizing inorganic phosphate (Naher *et al*., 2013; Panhwar *et al*., 2014). The inoculation of plant beneficial bacteria promoted growth and productivity of wheat (Anand & Nikhilesh, 2015). Furthermore, microbes in the soil rhizosphere are responsible for enhancing the plant health and soil fertility (Khan, 2006). The soil microbes affect plant growth directly or indirectly through various mechanisms (Mantlelin & Touraine, 2004). Indirect growth promotion happens when PGPR results in avoiding growth restricting conditions for crop growth (Glick *et al*., 1999). While direct plant growth promotion by PGPR comprises either supplying nutrient to the plant or assisting the plant for nutrient uptake.

Among the plant hormones most probably produced by the PGPR comprise ethylene, auxin, gibberellins and cytokinin (Afzal *et al*., 2015). Hence, the study was conducted to explore potential PGPR isolated from wheat crop grown under integrated use of FYM and inorganic fertilizers, and evaluation of their plant growth promotion in laboratory conditions.

Materials and Methods

Soil and plant sampling: Rhizospheric soil and plant samples were collected randomly from an ongoing study of wheat grown under integrated use of organic and inorganic fertilizers at the experimental agricultural farm (5°26' 0" N / 68°32' 0" E at latitude of 14 m above sea level in Sindh Province, Pakistan) of Nuclear Institute of Agriculture (NIA), Tandojam, Sindh. Rhizospheric soil samples were taken from the wheat after 45 days of sowing and kept in ice box at 4^0C temperature before analyses. The treatments of the ongoing study were as control, five levels of mineral fertilizers (N & P_2O_5) 150-100, 113-75, 100-67, 75-50 and 50-33 kg ha^{-1} with three doses of FYM (0, 3 & 9 ton ha^{-1}). Wheat variety NIA-Sarang served as test genotype and experiment was arranged in split plot combination of treatments with three replications.

Soil analysis: The soil samples were taken for soil physico-chemical properties, organic matter and nutrient content of soil. Soil texture was determined using Bouyoucos hydrometer method (Gee & Bauder, 1986), organic matter contents were determined following the procedure of Walkely & Black (1934), soil organic carbon was assessed using the formula (SOC% = SOM/1.724 × 100) (Walkely & Black, 1934), electrical conductivity was measured through Digital Electrical Conductivity Meter using 1:5 soil water ratio (Benton, 2001), soil pH was measured in soil to water (1:5) ratio (Benton, 2001) by means of standard pH meter -at 30°C, the total N was analyzed using by Kjeldahl digestion method (Bremner & Mulvaney, 1982) and AB-DTPA extractable phosphorus and potash were analyzed followed by Soultanpur & Schwab (1977).

Characterization of PGPR for the growth promotion of wheat seedlings

Enumeration and isolation of PGPR: Bacterial abundance was assessed from rhizospheric and non-rhizospheric soil of wheat plant whereas, nutrient agar media containing: 0.5% Peptone, 0.3% yeast extract, 1.5% agar and 0.5% sodium chloride pH 6.8 at 25°C (Lapage *et al*., 1970) was used for enumerating total bacterial colony forming units (cfu), while Nfb media was used for nitrogen fixing bacteria as described by Parasad *et al*., (2001). However, Pikovskaya media was applied for enumeration of phosphate solubilizing bacteria (Pikovskaya, 1948). Ten gram of soil sample adhered root and non-rhizosphric soil were placed in 250 mL Erlenmeyer flask containing 90 mL sterilized water. The contents were shaken for 15 to 20 min on mechanical shaker to have a homogenous mixture. A sequence of dilutions was organized from 10^{-1} to 10^{-8} and 0.1 ml aliquots were spread on to the selective media plates and incubated at 28±2°C in an incubator for 72 h of incubation (Lapage *et al*., 1970). The number of colony forming units was counted on colony counter and the population was enumerated according to their dilution and the amount plated. After bacterial enumeration, isolation was carried out using above mentioned media plates. The purity of isolates was determined by observing their morphological characteristics while screening was done on the basis of cell structure and colony morphology.

Determination of N_2 fixing activity: The N_2 fixing activity of bacterial isolates was determined by inserting one loop of bacterial pre-culture of (72 h) in the Nfb medium (Prasad *et al*., 2001). The plates were observed for pellicle formation after 48-72 hours. The isolates capable of pellicle formation were recorded positive for N_2 fixing activity.

Determination of phosphate solubilization: The bacterial isolates' phosphate solubilization ability was analyzed by spotting 10 µl of 48h cultures on to pikovskaya agar plates. The agar plates were incubated (30°C) for seven days and perceived for development of halo zone. The P solubilizing efficiency was determined according to the below formula (Nguyen *et al*., 1992).

$$\text{P Solubilization efficiency} = \frac{\text{Solubilization diameter (halozone)}}{\text{Growth diameter of cology}} \times 100$$

Determination of indole-3 acetic acid (IAA) production: The bacterial isolates were cultured in nutrient broth containing 2 mg L^{-1} tryptophan and incubated at 28 ± 2°C for 48-72 hours. The cultures were centrifuged at 7000 rpm for 7 min and 1 mL of the supernatant was assorted with 2 mL of Salkowsky's reagent (Gorden & Weber, 1950). The concentrations were measured by using standards (1-20 ppm), absorbance curve development on spectrophotometer at 535 nm.

Biofilm production: The inoculated cultures of various PGPR were incubated at 30°C on a Kottermann 4020 shaker at a speed of 80 cycle's min^{-1}. The cultures were observed for the biofilm production after 72 h of incubation. Detection of biofilm production (Watnick & Kolter, 1999) was done using the tissue culture plate method (TCPM). The development of film indicated biofilm production by bacterial isolate.

Evaluation of PGPR efficacy in improving growth of wheat seedlings

Experimental design: The experiment was conducted using sterilized sand in plastic pots at microbiology laboratory, new lab building NIA, Tandojam. The sand was autoclaved at 120°C for 60 min before filling into sterilized plastic pots and wheat seeds (Kiran-95) were surface sterilized by the method of Amin *et al*., (2004) were sown in each pot. Ten efficient NIA-PGPR (1-10) isolates were selected for inoculation of wheat pants.

Inoculum preparation and seed inoculation: The best ten potential PGPR were selected for inoculating wheat plant after their biochemical characterization. Pure culture of each bacterial strain was cultured in nutrient broth (NB) for the period of 48 hours. However, the bacterial cells were collected after centrifugation (13500 rev min^{-1}) for ten minutes and splashed with sterilized phosphate buffer (0.85%) saline, while the optical density (OD_{600}) of cells were observed and set respectively. The population of the applied bacteria was set by drop plate method and live bacterial cells (10^9 cfu mL^{-1}) were applied to the wheat plants. The non-inoculated wheat plants were given the equal quantity of dead cells (autoclaved for 30 min at 121°C). Hoagland solution (1/4 strength) was applied (Hoagland & Arnon, 1950) to the wheat plants as and when required. The treatments were randomized using completely randomized design (CRD) and replicated thrice.

Growth data collection: The plant height, root length and numbers of leaves per plants were observed at harvesting (After 21 days of sowing). However, after harvest plant samples were washed to remove all soil particles and dried in an oven at 70°C for 3 days for plant dry biomass.

Data Analysis: All experimental data was statistically analyzed by using analysis of variance (SAS Institute, 2004, Version 9.1) and treatment means were compared using Tukey's test (p<0.05).

Results

Experimental soil properties: The bacteria were isolated from wheat crop which was grown on non-saline soil having EC less than 1 dS m^{-1} with slightly alkaline in nature (pH >7.0). Generally the organic matter and organic carbon were low where no organic source was applied. The nitrogen was deficient in all sampled treatments, while AB-DTPA extractable phosphorus and potash was sufficient in all sampled treatments (Table 1).

The soil temperature was determined from the various soil depths during the sampling time. The soil temperature varied among the various depths (Gao *et al*., 2007). Low temperature was noted at the soil surface 15.37 (°C), while there was a showing gradual increase in the temperature with increasing depths and maximum temperature (17.24°C) was recorded at the soil depth 45 cm (Table 2).

Isolation and enumeration of PGPR: There were significant differences in the bacterial population in various treatments. Comparatively, bacterial population was higher than the nitrogen (N_2) fixing and phosphate solubilizing bacterial populations. The highest total non-rhizospheric bacterial population (8.65 log cfu g^{-1} soil) was found in N & P_2O_5 (50-33 kg ha^{-1}) + FYM 9 t ha^{-1} followed by (8.04 cfu g^{-1} soil) with only FYM 9 t ha^{-1} applied plot. However, the lowest non-rhizospheric population (6.52 log cfu g^{-1} soil) was found in N & P_2O_5 (100-67 kg ha^{-1}) without FYM application (Table 3).

The N_2 fixing bacterial population was comparatively lower than the total bacterial population. The highest N_2 fixing bacterial population (7.0 log cfu g^{-1} soil) in non-rhizosphere was recorded at FYM 9 t ha^{-1} followed by (6.91 log cfu g^{-1}soil) at FYM 3 t ha^{-1} in without N & P_2O_5 fertilizer application, respectively. Among the treatments, the lowest population (4.95 log cfu g^{-1} soil) was observed in N & P_2O_5 (75-50 kg ha^{-1}) with no FYM application (Table 3).

Similarly, the phosphate solubilizing bacterial (PSB) population varied among different treatments. The significantly highest non-rhizosphere PSB population (7.26 log cfu g^{-1} soil) was noted in treatments having no N & P_2O_5 application followed by (6.90 log cfu g^{-1} soil) in N & P_2O_5 (50-33 kg ha^{-1}) with 9 t ha^{-1}FYM. However, the lowest PSB population (5.73 log cfu g^{-1} soil) was noted in N & P_2O_5 (150-100 kg ha^{-1}) without FYM application (Table 3).

Table 1. Soil physico-chemical properties of the filed experimental area at the time of sampling.

Treatments		EC1:2.5	pH	OM	OC	N	P	K
N&P_2O_5 (kg ha^{-1})	FYM (t ha^{-1})	(dS m^{-1})		---------- % ----------			------- (mg kg^{-1}) -----	
0	0	0.359	7.5	0.44	0.255	0.02	35.7	190
150-100	0	0.436	7.3	0.59	0.342	0.05	96.72	195
113-75	0	0.556	7.3	0.44	0.255	0.05	87.04	220
100-67	0	0.561	7.7	0.51	0..296	0.04	60.24	220
75-50	0	0.481	7.4	0.51	0.296	0.04	59.56	230
50-33	0	0.388	7.6	0.55	0.319	0.03	56.28	220
0	3	0.445	7.9	0.68	0.304	0.04	51.02	190
150-100	3	0.422	7.8	0.81	0.470	0.06	118.22	280
113-75	3	0.713	7.9	0.83	0.481	0.05	97.32	270
100-67	3	0.417	7.8	0.73	0.423	0.05	72.34	195
75-50	3	0.481	7.6	0.81	0.470	0.05	63.22	220
50-33	3	0.380	7.5	0.73	0.423	0.04	58.48	210
0	9	0.447	7.7	0.87	0.505	0.06	77.18	240
150-100	9	0.374	7.6	0.89	0.516	0.07	140.32	390
113-75	9	0.519	7.5	0.92	0.534	0.07	137.68	300
100-67	9	0.463	7.5	1.62	0.940	0.06	121.02	310
75-50	9	0.458	7.6	1.03	0.597	0.06	133.28	280
50-33	9	0.438	7.7	1.25	0.725	0.06	117.34	270

FYM = Farmyard manure, OM = Organic matter, OC = Organic carbon, N = Nitrogen, P = Phosphorus and K = Potash

Table 2. Soil temperature (°C) of the experimental area at the time of sampling.

Site	Depth (cm)			
	0	15	30	45
1	15	15.5	16.67	17.2
2	15.5	15.5	16.67	17.2
3	15.6	15.6	16.67	17.3
Mean	15.37	15.54	16.67	17.24

The rhizosphere population among the treatments varied at various FYM rates. The bacteria were more abundant in rhizosphere as compared to non-rhizosphere. Among various bacterial isolates, the highest population of total bacteria was reported on nutrient agar media plates. The highest total bacterial population (9.64 log cfu g^{-1} soil) was found in N & P_2O_5 (50-33 kg ha^{-1}) followed by (9.51 log cfu g^{-1} soil) in N & P_2O_5 (50-33 kg ha^{-1}) + FYM 9 t ha^{-1}, respectively. While, the lowest population (7.30 log cfu g^{-1} soil) was found in N & P_2O_5 (100-67 kg ha^{-1}) without FYM (Table 4).

The N_2 fixing and PSB rhizospheric populations were comparatively lower than the total rhizospheric population. The highest N_2 fixing bacterial population (7.67 log cfu g^{-1} soil) was found in treatments with N & P_2O_5 (50-33 ha^{-1}) + FYM 9 t ha^{-1}. Whereas, lowest population (5.79 log cfu g^{-1} soil) was in N & P_2O_5 (113-75 kg ha^{-1}) without FYM applied treatments (Table 4).

The trend of PSB population was also similar as observed in N_2 fixing bacterial population among the various treatments. However, at the various rates of FYM 0, 3 and 9 t ha^{-1} significantly affected the PSB population. The highest PSB population (9.08 log cfu g^{-1} soil) was observed in without N & P_2O_5 application + FYM 9 t ha^{-1} and the lowest population (5.70 log cfu g^{-1} soil) was recorded in N & P_2O_5 (150-100 kg ha^{-1}) without FYM application (Table 4).

Biochemical characterization: A total of 24 strains (NIA-PGPR) were screened for further studies based on their different colony morphological characteristics (Table 5). Most of the PGPR have ability to show the nitrogen fixation, except NIA-PGPR 15, 16, 17, 18, 20 and 23. Similarly, phosphate solubilizing ability was shown by majority of the strains except NIA-PGPR 3, 4, 6, 7, 9, 11, 12 and 13. In case of the biofilm production almost all PGPR strains were able to produce biofilm excluding NIA-PGPR 19 and 21 (Table 5).

IAA production by PGPR strains: The selected (24) PGPR strains were tested for IAA production and all strains were capable to produce IAA in the broth culture. The highest IAA production (8.92 mg L^{-1}) was recorded in NIA-PGPR 9 and the lowest in NIA-PGPR 15. Most of the strains produced IAA in the range of 3-4 mg L^{-1} (Fig. 1).

Phosphate solubilizing activities by PGPR strains: The selected PGPR strains (24) were tested for phosphate solubilizing ability on Pikovskaya media plates. The P solubilization efficacy of the selected isolates varied according to their hallo zones around their colonies (Fig. 2). The highest P solubilization efficiency was found by NIA-PGPR 1 (75%) followed by the NIA-PGPR 10 (72.73%), with the least solubilization efficiency exhibited by NIA-PGPR 23 (11.14%).

Table 3. Non-rhizospheric (soil) microbial population at various rates of fertilizers and FYM applied treatments in wheat crop.

Treatments		Total bacterial population	N_2 fixing bacterial population	Phosphate solubilizing bacterial population
N & P_2O_5 (kg ha^{-1})	FYM (t ha^{-1})	(log cfu g^{-1} soil)		
0	0	7.23^{cd}	6.65^{cd}	6.32^{d}
150-100	0	6.92^{e}	6.36^{e}	5.73^{f}
113-75	0	7.00^{d}	6.11^{f}	5.91^{f}
100-67	0	6.52^{f}	6.34^{e}	5.85^{f}
75-50	0	6.89^{e}	4.95^{h}	6.28^{d}
50-33	0	6.96^{d}	5.04^{g}	6.23^{d}
0	3	7.34^{cd}	6.91^{b}	6.53^{b}
150-100	3	6.83^{e}	6.76^{c}	6.28^{d}
113-75	3	6.80^{e}	6.43^{d}	6.34^{cd}
100-67	3	6.86^{e}	6.32^{e}	6.15^{e}
75-50	3	7.12^{d}	6.67^{cd}	6.04^{e}
50-33	3	7.36^{cd}	6.54^{d}	5.95^{f}
0	9	8.04^{b}	7.00^{a}	7.26^{a}
150-100	9	7.57^{c}	6.74^{c}	6.45^{c}
113-75	9	7.56^{c}	6.76^{c}	6.43^{c}
100-67	9	7.79^{b}	6.80^{b}	6.36^{cd}
75-50	9	7.45^{c}	6.82^{b}	6.59^{b}
50-33	9	8.65^{a}	6.88^{b}	6.90^{b}

The data values are means of five replications and same letters within each column are not significantly different (p<0.05) among the treatments

Table 4. Rhizospheric microbial population at various rates of fertilizers and FYM applied treatments in wheat crop.

Treatments		Total bacterial population	N_2 fixing bacterial population	Phosphate solubilizing bacterial population
N & P_2O_5	FYM			
(Kg ha^{-1})	(t ha^{-1})	---------- (log cfu g^{-1} soil) ----------		
0	0	8.57^{d}	7.23^{c}	6.67^{f}
150-100	0	8.26^{e}	6.70^{e}	5.70^{h}
113-75	0	8.18^{e}	5.79^{g}	5.85^{h}
100-67	0	7.30^{f}	6.85^{e}	6.23^{g}
75-50	0	8.28^{e}	7.00^{d}	6.18^{g}
50-33	0	8.38^{e}	6.11^{f}	6.60^{f}
0	3	8.89^{d}	7.54^{b}	7.40^{e}
150-100	3	8.56^{d}	7.30^{c}	6.69^{f}
113-75	3	8.32^{e}	7.34^{c}	6.26^{g}
100-67	3	8.30^{e}	7.08^{d}	6.43^{f}
75-50	3	8.26^{e}	6.04^{f}	6.76^{f}
50-33	3	8.85^{d}	7.36^{c}	6.90^{f}
0	9	9.21^{c}	7.67^{a}	9.08^{a}
150-100	9	9.08^{b}	7.41^{c}	8.26^{b}
113-75	9	9.38^{b}	7.43^{c}	7.15^{e}
100-67	9	9.02^{c}	7.36^{c}	7.69^{d}
75-50	9	9.51^{b}	7.30^{c}	7.99^{c}
50-33	9	9.64^{a}	7.58^{b}	8.46^{b}

The data values are means of five replications and same letters within each column are not significantly different (p<0.05) among the treatments

Table 5. Biochemical properties of isolated PGPR strains from the wheat crop.

S. No.	Isolates	Colony morphology	Nitrogen fixing ability	Phosphate-solubilizing ability	Biofilm production
1.	NIA-PGPR1	Circular, off white	+ve	+ve	+ve
2.	NIA-PGPR2	Transparent, oval	+ve	+ve	+ve
3.	NIA-PGPR3	Translucent, gummy	+ve	-ve	+ve
4.	NIA-PGPR4	Circular, gummy, off white	+ve	-ve	+ve
5.	NIA-PGPR5	Irregular, off white	+ve	+ve	+ve
6.	NIA-PGPR6	Circular, dull	+ve	-ve	+ve
7.	NIA-PGPR7	Off white, gummy	+ve	-ve	+ve
8.	NIA-PGPR8	Transparent, irregular	+ve	+ve	+ve
9.	NIA-PGPR79	Translucent, rod shape	+ve	-ve	+ve
10.	NIA-PGPR10	Glistening, circular	+ve	+ve	+ve
11.	NIA-PGPR11	Circular, light yellow, sticky	+ve	-ve	+ve
12.	NIA-PGPR12	Irregular, gummy, off white	+ve	-ve	+ve
13.	NIA-PGPR13	Circular, translucent	+ve	-ve	+ve
14.	NIA-PGPR14	Irregular, yellow, rough	+ve	+ve	+ve
15.	NIA-PGPR15	Circular, translucent	-ve	+ve	+ve
16.	NIA-PGPR16	Circular, translucent, off white, shiny	-ve	+ve	-ve
17.	NIA-PGPR17	Circular, light yellow, gummy	-ve	+ve	+ve
18.	NIA-PGPR18	Irregular, yellow, rough	-ve	+ve	+ve
19.	NIA-PGPR19	Circular, yellow gummy	+ve	+ve	-ve
20.	NIA-PGPR20	Dark yellow, circular, rough	-ve	+ve	+ve
21.	NIA-PGPR21	Brown, circular, sticky	+ve	+ve	-ve
22.	NIA-PGPR22	Irregular, yellow	+ve	+ve	+ve
23.	NIA-PGPR23	Oval, light yellow	-ve	+ve	+ve
24.	NIA-PGPR24	Irregular, yellow, rough	+ve	+ve	+ve

Note: +ve = Positive, -ve = Negative

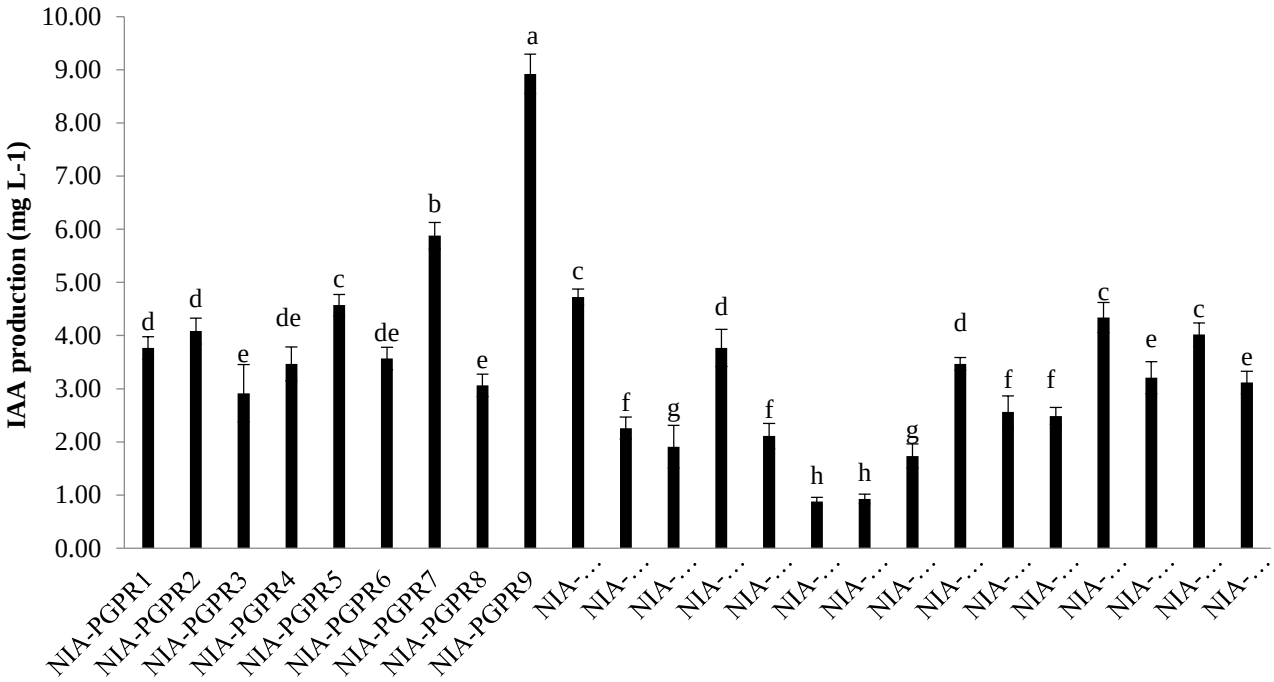

Fig. 1. Production of IAA by PGPR strains isolated from the wheat crop.
The data values are means of five replications and same letters within each column are not significantly different (p<0.05) among the treatments.

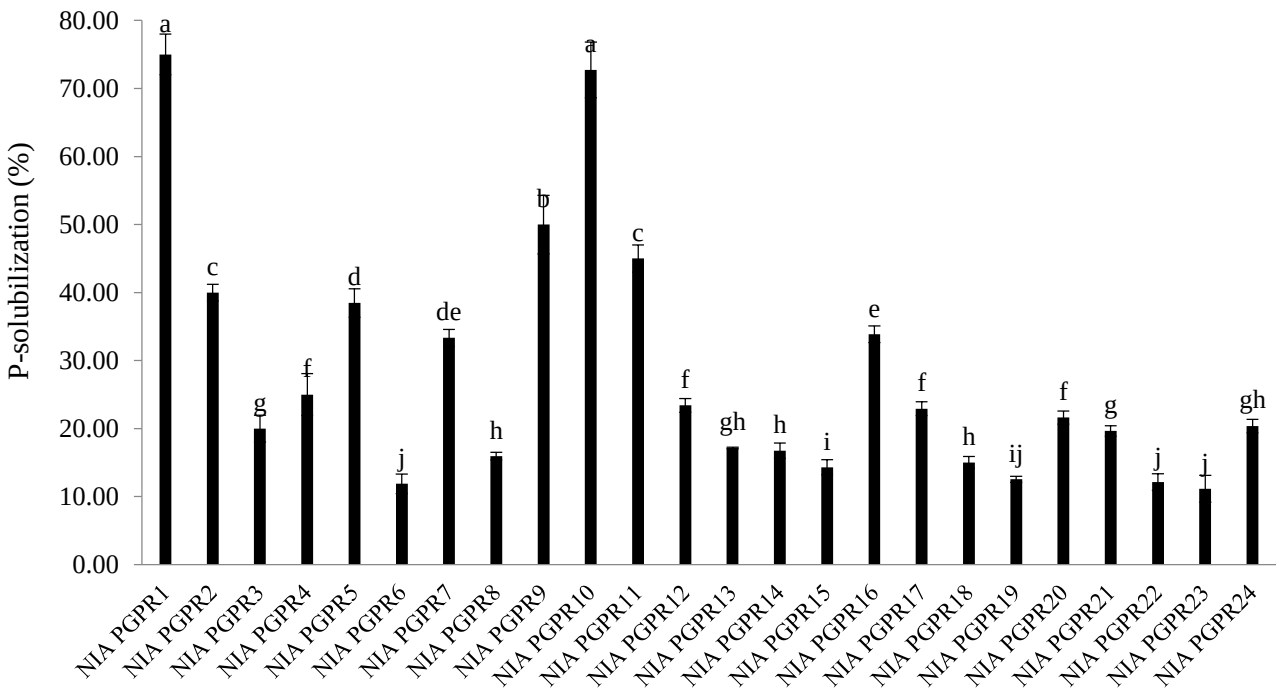

Fig. 2. Phosphate solubilization (%) by PGPR strains isolated from the wheat crop.
The data values are means of five replications and same letters within each column are not significantly different (p<0.05) among the treatments.

Effect of PGPR strains on wheat growth: A total of ten (10) PGPR were selected for evaluation in improving the growth of wheat plants. All PGPR strains were capable to increase the wheat growth as compared to the non-inoculated treatment (Table 6). Significantly (p<0.05) the highest plant height (31.50 cm) was found in treatment inoculated with NIA-PGPR 5, followed by NIA-PGPR 1 (31.25 cm). The significantly (p<0.05) maximum root length was found in treatments inoculated with NIA-PGPR 5 (8.5 cm) followed by NIA-PGPR 7 (8.25 cm). Significantly (p<0.05) the highest numbers of leaves were recorded by NIA-PGPR 1, 3, 4, 5 & 7 (3 plant $^{-1}$), while the highest plant dry biomass was observed by NIA-PGPR 5 (0.172 6) followed by NIA-PGPR 10 (0.170 g).

Table 6. Effect of potential PGPR strains on the growth of wheat plants.

Isolates	Plant height	Root length	Number of leaves	Plant dry biomass
	---------- (cm) ----------		------ (Plant $^{-1}$) -----	---- (g plant^{-1}) ---
Control	25.75e	5.5de	2b	0.095f
NIA-PGPR1	31.25a	6.25c	3a	0.125d
NIA-PGPR2	30b	7.75b	2.5a	0.129c
NIA-PGPR3	30.75b	7.5b	3a	0.153b
NIA-PGPR4	30.25b	8a	3a	0.132c
NIA-PGPR5	31.50a	8.5a	3a	0.172a
NIA-PGPR6	29c	6c	2.5	0.124d
NIA-PGPR7	30.5b	8.25a	3a	0.131c
NIA-PGPR8	29.5c	5.75d	2.5a	0.097c
NIA-PGPR9	28d	6c	2.5a	0.134c
NIA-PGPR10	31a	8a	2.5a	0.170a

The data values are means of five replications and same letters within each column are not significantly different (p<0.05) among the treatments

Discussion

The isolation and characterization of PGPR were carried out from wheat crop growing under various levels of FYM and mineral fertilizers. The soil was slightly alkaline and non-saline in nature. The FYM application did not have significant effect on enhancing the soil organic matter or organic carbon content compared to the control treatments (Brar *et al*., 2015). Earlier research has shown that application of FYM along with inorganic fertilizers enhanced the soil organic matter (Zhang *et al.*, 2006). FYM is one of the major organic sources serving as source of energy to the soil microflora and organic carbon deliberated is an index of the soil health. The microbes were observed in abundance in the treatments where FYM was applied along with lower level of mineral fertilizer. There is a prodigious demand for more research on biological N_2 fixation and P solubilization for the energy conservation. Hence, introduction of potential microbes in soils may support in enhancing the crop production by maintaining proper microbial population and subsequent atmospheric N_2 fixation with providing moresoluble P contents through solubilization of insoluble P from the soil (Chand, 2006). On the other hand, not a single source of plant nutrients can meet the whole nutrient requirements for crops in modern agriculture, they require to be consumed in an integrated manner following a management technology which is suitable, economically feasible, socially satisfactory and ecologically rigorous (Finck, 1998).

The soil temperature was recorded during the sampling to determine the soil temperature and its effect on soil micro biota. The temperature of soil was not much differing from the soil surface hence, it could not affect soil microbial population. Overall, total bacteria were in abundance followed by phosphate solubilizing and N_2 fixing bacteria in rhizospheric soil. The application of organic source of nutrients (FYM) affected the microbial abundance of both the rhizospheric and non-rhizospheric soil as the higher bacterial populations were recorded in FYM applied treatments. Furthermore, bacteria were in more abundance in the rhizosphere compared to non-rhizosphere as the microbial populations are generally different in rhizosphere (Islam *et al*., 2016). For successful and effective plant growth promotion, it is essential for PGPR to colonize in the rhizosphere. The higher levels of mineral fertilizer (N & P_2O_5) had negative effect on colonization of beneficial bacteria and their low population was recorded in the rhizosphere as it had been observed earlier by Chand *et al*., (2010). It had been proven in a long term study of wheat that the bacteria were in much abundance in FYM applied treated plots as compared non-applied rhizospheric soil. The higher microbial population may be due to the application of organic manures which provides adequate biomass as a feed for the microbes and supports in enhancing microbial population in soil (Singh *et al*., 2012).

The microbes with multiple plant beneficial traits were isolated from the wheat crop including N_2 fixing bacteria and phosphate solubilizing bacteria. The screened bacteria had the capability to produce IAA in a substantial quantity as reported by Abbasi *et al*., (2011) that PGPR isolated from wheat rhizosphere could enhance its growth by phytohormones production. Similarly, Zahid *et al.*, (2015) found that all the isolates produced IAA phytohormone as it was considered very common ability among PGPR (Panhwar *et al*., 2012; Naher *et al*., 2009). Atmospheric nitrogen fixation by the beneficial bacteria in the soil rhizosphere and endorhizosphere make N nitrogen available to plants and has positive effects on wheat growth (Fischer *et al.*, 2007). The screened PGPR isolates had the capability to fix atmospheric N_2 and supplement N nutrient for enhancing wheat growth as reported by Naher *et al*., (2013) in rice crop. All the isolates exhibited potential for solubilizing inorganic phosphate and the efficiency was upto 75%. Inorganic phosphate solubilization contributed much in enhancing wheat growth as reported by Zhang *et al*., (2012) that PSB proved in enhancement of root dry weight up to 25.6%.

Most of the isolates screened in the current study had the ability of biofilm formation. The biofilm formation ability of the bacterial strains is considered an important approach for their effective survival in plant rhizosphere. From the earlier research it was observed that *In vitro* biofilm formation has significantly positive correlation with root colonization (Kasim *et al.*, 2016). Hence, such type of PGPR with their biofilm formation abilities should

play well in avoiding competing organisms, nutrient uptake and quick reactions for the changing environmental conditions. The plants associated biofilms abilities are capable to defend themselves from the outer stresses and other microbial competitions in rhizosphere, and to develop positive effects for the plant growth promotion (Seneviratne *et al.*, 2010).

The inoculation of selected PGPR isolates proved their efficacy in growth development of wheat plants. The wheat plants showed significant differences after inoculation of selected PGPR isolates. An enhancement in plant growth was observed in inoculated wheat plant as compared to non-inoculated ones. Similarly, Yadav & Yadav (2011) reported that PGPR bioinoculants have proved the potential to improve the plant biomass and productivity of a widespread range of crops. PGPR inoculants proved to have beneficial traits for the host plants and could be applied in the development of latest, safe and effective seed treatments as a substitute or supplement to the chemical fertilizers (Islam *et al*., 2016). These PGPR results in increased shoot and root lengths and their colonization on the surface of the roots had significant positive effect directly/indirectly on the growth and development of the wheat plants (Gerhardt *et al.*, 2009).

Besides the direct effects on the growth of plant, use of bioinoculants in soil significantly enhances the water holding capacity of soil, develops soil texture, soil structure, supplies essential nutrients and proliferates beneficial microbes which increases the root and shoot biomass and eventually soil organic carbon (Sharma, 2011). Similar results of favorable effects of various PGPR species were obtained by Mehta *et al*., (2015) and Zhahid *et al*., (2015) on different crops *In vitro* and in field experiments in mutable ecological conditions. Thus, it is significant to find and categorize PGPR strains from the local regions which could be suitable for increasing crop growth and yield (Panhwar *et al.*, 2014; Verma *et al.*, 2013).

Conclusion

Soil microbes are more abundant in the soil rhizosphere zone as compared to the non-rhizosphere. Application of organic source of nutrients like FYM to soil not only adds up to soil organic matter/carbon but also increase the population of beneficial bacteria. Biofilm formation helps bacteria to be greater in rhizosphere and to withstand the abrupt environmental changes. To have an effective bio-product, the PGPR strains with multiple plant beneficial traits such as production of IAA, N_2 fixation, P solubilization and biofilm formation should be screened for inoculation purpose. Isolating indigenous PGPR for enhancing crop production has an advantage as locally isolated strains might play better role in having flexibility to local climatic conditions. However, a series of glasshouse and fields experiments with various soil types and climatic conditions are required to proceed from screening of a PGPR to a biofertilizer product. The screened isolates of the current study can provide the basis to develop a bio-product for improved and sustainable wheat crop production in the local climatic conditions.

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