Short Communication

Indian Journal of Agricultural Sciences **87** (8): 1104–8, August 2017/Short Communication

Effect of plant growth promoting rhizobacteria on growth, yield attributes and yield of wheat (*Triticum aestivum*)

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Received: 23 November 2015; Accepted: 09 May 2017

Key words: Plant growth-promoting rhizobacteria, PGPR, Root growth, Yield, Yield attributes,

Wheat (*Triticum aestivum* L.) is the important and strategic cereal crop for the majority of world's population. It is one of the most important source of calories and protein in the human diet and share 35.5% of total food grain production next to rice (DAC 2015). Presently, the world population could be fed by the current level of food production (Pretty 2008). But it is still unlikely that current growth in agricultural productivity can go side by side with increasing population (Hazell and Wood 2008). In order to produce enough food, agriculture had relied on the application of large quantity of inorganic N fertilizers to the soil, but their use efficiency still remain low, caused by losses through volatilization, denitrification, leaching and conversion into unavailable forms (Sturz *et al*. 2000). Intensive use of chemical fertilizers destroys the soil ecology, disturbs the environmental balance, degrades soil fertility, contaminates ground water and consequently leads to harmful effects on human health (Joshi *et al.* 2006). Therefore, nutrients supplementation through biofertilizers may be a viable alternative for increasing crop yields and sustain the soil fertility. Biological fertilizers also known as bio-fertilizers, are products carrying livings cells of different types of microorganisms which are able to transform elements (N, P) from unavailable to available forms by biological means, such as, N-fixation and solubilization of rock phosphate (Cakmakci *et al*. 2001, Vessey 2003). Plant growth promoting rhizobacteria (PGPR) include variety of soil bacteria which, when grown in association with host plant, lead to stimulation of growth of their host because of increased mobility, uptake and enrichment of nutrients in plant (Cakmakci *et al*. 2006). Thus, present study aimed to evaluate the effect of combined application of bacterial and cyanobacterial species of PGPR on the enhancement of growth and productivity of wheat.

The field experiment was conducted at the Research farm of ICAR-Indian Agricultural Research Institute (IARI), New Delhi, during the *rabi* season of 2014-15. The climate of Delhi is of sub-tropical and semi-arid type with hot and dry summers and cold winters and falls under the agro-climatic zone 'Trans-Gangetic Plains' and it receives mean annual rainfall of 650 mm. The soil of experimental site belongs to order Inceptisols, Mehruli series having sandy clay loam texture in upper 30 cm soil layer and loam below it. The soil contained 0.52% organic carbon, 170 kg/ha available N, 13.4 kg/ha available P, 260 kg/ha available K and pH of 8.2. The chemical analysis of soil was carried out as per procedures described by Prasad *et al*. (2006). The eleven treatments of the experiment on wheat variety HD-2967 with three replications allocated in a randomized block design, included absolute control, control + *Azotobacter* (IARI Inoculant) + CW1 (*Anabaena* sp.) + PW5 (*Providencia* sp.), recommended dose of NPK, 75% N + full PK, 75% N + full PK + *Azot.,* 75% N + full PK + CW1, 75% N + full PK + PW5, 75% N + full PK + *Azot.* + CW1, 75% N + full PK + *Azot.* + PW5, 75% N + full PK + CW1+ PW5 and 75% N + full PK + *Azot.* + CW1 + PW5. Recommended dose of phosphorus (26 kg P/ha), potassium (33 kg K/ha) and nitrogen (150 kg N/ha) were applied in the crop. Full dose of P and K, and 50% N was applied basally at the time of sowing and remaining 50% N was applied at first irrigation. The crop received a total of six irrigations. Two hand weedings were carried out during the crop growth at 17 days after sowing (DAS) and 35 DAS. The bacterial strains *Providencia* sp. (PW5) and *Azotobacter* (IARI inoculant), and cyanobacterial strain *Anabaena laxa* (CW1) were obtained from the germplasm of the Division of Microbiology, ICAR-IARI, New Delhi. The formulations were amended with 1% CMC (carboxymethyl cellulose) as a sticker, prior to application on the seeds. Data on root growth were also recorded in the present study. The root samples were cleaned by washing them carefully in the running water in a sieve. From the fresh roots, volume and length was measured with the help of a root scanner

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and then dry weight was recorded. The statistical analyses of data were carried out using Statistical Analysis System SAS 9.3.

Several growth parameters of wheat, viz. plant height, tillers/m^2, dry matter accumulation and leaf area index were significantly influenced by the application of plant growth promoting rhizobacteria (Table 1 and 2). The growth of wheat progressed slowly in the beginning and then increased at a faster rate during tillering to flowering stages, and afterwards it slowed down. Growth parameters, particularly plant height and dry matter production, followed the quadratic growth model.

In general, the highest values of most of the growth parameters, viz. plant height, tillers/m^2, dry matter accumulation and leaf area index were recorded by the application of 75% N + RPK + *Azo* + CW1 + PW5 at the growth stages studied. The combined application of 75% N with either two or three species of PGPR, viz. 75% N + RPK + *Azo* + CW1, 75% N + RPK + *Azo* + PW5, 75% N + RPK + CW1 + PW5 and 75% N + RPK + *Azo* + CW1 + PW5 treatments favoured significantly higher values for plant height, tillers/m^2, dry matter accumulation and leaf area index than 75% N + RPK, absolute control or use of all the three species of PGPR without nitrogen use in wheat. The combined use of either two or three PGPR species with 75% N was as good as use of recommended NPK (RNPK) in improving the growth parameters of wheat. However, combined use of two or three PGPR species with 75% N and recommended PK gave significantly higher values of growth parameters over application of any single PGPR species with 75% N. The highest dry matter yield was produced by application of 75% N + RPK + *Azo* + CW1 + PW5, which was 37%, 37% and 14.5% higher at 60 DAS, 90 DAS and 120 DAS over 75% N + RPK, respectively. Similarly, the highest leaf area index (LAI) values were recorded with the application of 75% N + RPK + *Azo* + CW1, which were 24% and 23% higher at 60 DAS and 90 DAS over 75% N + RPK, respectively. The highest plant height was recorded with application of 75% N + RPK + Azo + CW1 + PW5, which was 6.3% higher over 75% N + RPK. Overall, application of PGPR was very useful in promoting the growth of wheat crop in the present study. PGPR are known to play several key roles in promoting the plant growth. They can fix atmospheric nitrogen and make available to the associated crop. Further, some specific strains of PGPR are capable of solubilizing and mobilizing the fixed phosphorus and make it available for plant growth. Prasanna *et al*. (2014) reported that *Azotobacter*, *Azospirillum* and *Cyanobacteria* can fix 15-20 kg, 20-30 kg and 25-30 kg N/ha, respectively, under field conditions. Similarly, phosphate

Table 1 Effect of PGPR on plant growth parameters

Treatment	Plant height (cm) at harvest	No. of tillers/m^2 at 60 DAS	No. of tillers/m^2 at 120 DAS	LAI at 60 DAS	LAI at 90 DAS
Absolute control	78.4	367	358	1.82	2.02
Control + *Azo* + CW1 + PW5	83.8	407	398	2.48	2.75
RNPK	95.5	531	523	3.91	4.34
75% N + RPK	90.0	451	442	3.18	3.54
75% N + RPK + *Azo*	89.9	459	450	3.18	3.54
75% N + RPK + CW1	89.4	469	460	3.20	3.56
75% N + RPK + PW5	89.8	462	453	3.15	3.5
75% N + RPK + *Azo* + CW1	95.3	532	523	3.93	4.36
75% N + RPK + *Azo* + PW5	95.5	525	516	3.92	4.34
75% N + RPK + CW1 + PW5	95.4	531	521	3.92	4.35
75% N + RPK + *Azo* + CW1 + PW5	95.7	538	529	3.92	4.34
SEm±	1.72	12.9	13.3	0.22	0.24
LSD (P=0.05)	5.07	38.2	39.2	0.64	0.71

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena* sp.; PW5- *Providencia* sp.; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K); LAI- Leaf area index; DAS-Days after sowing

Table 2 Effect of PGPR on dry matter accumulation in wheat

Treatment	Dry matter accumulation (g/m^2)			
	30 DAS	60 DAS	90 DAS	120 DAS
Absolute control	18	57	341	669
Control + *Azo* + CW1 + PW5	18	100	407	774
RNPK	19	197	672	1058
75% N + RPK	19	145	495	933
75% N + RPK + *Azo*	19	151	517	931
75% N + RPK + CW1	19	151	513	929
75% N + RPK + PW5	20	147	510	931
75% N + RPK + *Azo* + CW1	20	199	679	1070
75% N + RPK + *Azo* + PW5	20	198	677	1067
75% N + RPK + CW1 + PW5	19	198	673	1061
75% N + RPK + *Azo* + CW1 + PW5	19	199	678	1069
SEm±	3	14	22	26
LSD (P=0.05)	NS	42	64	77

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena* sp.; PW5- *Providencia* sp.; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K); NS- Non-significant; DAS-Days after sowing solubilizing bacteria (PSB), a kind of PGPR, can mobilize 20-30 kg P_2O_5/ha to plant roots (Prasanna *et al*. 2014). Thus, the increased availability of nitrogen and phosphorus may help the wheat plants to accomplish better growth.

The higher plant growth may also be correlated with the increased root growth. Increased root length may have increased the root access to more water and nutrients, particularly micro nutrients which may have resulted in balanced nutrition of the crop. As a general role, accessibility of plants to water and essential nutrients would influence the height of wheat crop through nodes and internodes. These results are in close agreements with the findings of Nain *et al*. (2010) and Esmailpour *et al.* (2012).

Yield attributes of wheat were significantly affected by application of different combinations of bacterial and cyanobacterial strains (Table 3). There was a significant increase in effective tillers/m^2, spike length, spike weight and test weight by application of 75% N + RPK + *Azo* + CW1, 75% N + RPK + *Azo* + PW5, 75% N + RPK + CW1 + PW5, and 75% N + RPK + *Azo* + CW1 + PW5 treatments over 75% N + RPK. In general, combinations of either two or three species of PGPR with 75% N + RPK produced statistically similar values of different yield attributes studied, all being at par with recommended dose of NPK (RNPK). In general, there was a significant reduction in yield attributes if only one species (*Azo*, CW1 or PW5) or no species (75% N + RPK) of PGPR was combined with 75% N + RPK as compared to all the former treatments. Further, effective tillers/m^2, spike length, spike weight recorded 15.8, 7.1, 28 and 10.2% higher values respectively, by application of 75% N + *Azotobacter*+ CW1 + PW5 as compared to the treatment receiving 75% N. Similar results were also reported by Abd EI-Lattief (2013).

The results of the present study showed that 75% N + RPK + *Azo* + CW1, 75% N + RPK + *Azo* + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + *Azo* + CW1 + PW5 have recorded significantly higher root length, volume and dry weight over the application of 75% N + RPK + *Azo*, 75% N + RPK + CW1, 75% N + RPK+ PW5, RNPK, 75% N + RPK (Table 4). The highest root length of 4.08 cm/cm^3 of soil, volume of 6.78 mm^3/cm^3 of soil and dry weight 0.93 mg/cm^3 of soil were obtained from application of 75% N + RPK + Azo + CW1 + PW5.The application of PGPR was quite effective in enhancing the wheat root growth in terms of its length, volume and weight. This effect of improved root growth was more pronounced when two or three species of PGPR were combined with mineral N fertilizer (75% N). Improved root systems, including root

Table 3 Effect of PGPR on yield attributes of wheat

Treatment	Effective tillers/ m^2	Spike length (cm)	Spike weight (g)	Test weight (g)
Absolute control	343	10.0	2.0	30.3
Control + *Azo* + CW1 + PW5	368	10.5	2.2	34.0
RNPK	481	12.0	3.0	42.0
75% N + RPK	417	11.2	2.5	37.2
75% N + RPK + *Azo*	417	11.1	2.6	37.0
75% N + RPK + CW1	420	11.1	2.5	37.7
75% N + RPK + PW5	419	11.1	2.5	37.7
75% N + RPK + *Azo* + CW1	478	12.0	3.0	42.3
75% N + RPK + *Azo* + PW5	474	12.1	3.0	42.3
75% N + RPK + CW1 + PW5	476	12.0	3.0	42.0
75% N + RPK + *Azo* + CW1 + PW5	483	12.0	3.2	41.0
SEm±	8.02	0.093	0.08	0.96
LSD (P=0.05)	23.7	0.27	0.24	2.84

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena* sp.; PW5- *Providencia* sp.; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K)

Table 4 Effect of PGPR on root growth, grain and straw yields of wheat

Treatment	Root length (cm/cm^3 of soil)	Root volume (mm^3/ cm^3 of soil)	Root dry weigh (mg/cm^3 of soil)	Grain yield (t/ha)	Straw yield (t/ha)
Absolute control	3.02	3.93	0.36	2.91	6.10
Control + *Azo* + CW1 + PW5	3.09	4.10	0.39	3.21	6.69
RNPK	3.57	5.20	0.61	4.96	8.23
75% N + RPK	3.48	5.43	0.60	4.19	7.30
75% N + RPK + *Azo*	3.60	5.57	0.62	4.21	7.63
75% N + RPK + CW1	3.59	5.63	0.63	4.15	7.43
75% N + RPK + PW5	3.62	5.10	0.62	4.14	7.27
75% N + RPK + *Azo* + CW1	4.01	6.54	0.88	5.00	8.20
75% N + RPK + *Azo* + PW5	4.03	6.61	0.87	4.94	8.27
75% N + RPK + CW1 + PW5	4.05	6.51	0.89	4.94	8.23
75% N + RPK + *Azo* + CW1 + PW5	4.08	6.78	0.93	5.14	8.23
SEm±	0.12	0.29	0.06	0.07	0.18
LSD (P=0.05)	0.36	0.85	0.17	0.21	0.54

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena* sp.; PW5- *Providencia* sp.; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K) hair, are the most common phenotypic phenomena noticed after PGPR inoculation in most crops. The increased root length may have increased the root access to more water and nutrients, particularly of micro nutrients. Khalid *et al.* (2004) showed that inoculation of wheat seedlings with PGPR under gnotobiotic (axenic) conditions increased root elongation (up to 17.3%), root dry weight (up to 13.5%), shoot elongation (up to 17.3%) and shoot dry weight (up to 36.3%) over control.

The highest values of crop growth rate and crop relative growth rate were recorded by application of 75% N + RPK + Azo + CW1 + PW5 at the growth stages studied (Table 5).The combined application of 75% N with either two or three species of PGPR, viz. 75% N + RPK + *Azo* + CW1, 75% N + RPK + *Azo* + PW5, 75% N + RPK + CW1 + PW5 and 75% N + RPK + *Azo* + CW1 + PW5 treatments produced significantly higher values for crop growth rate and crop relative growth rate than 75% N + RPK, absolute control or use of all the three species of PGPR without use of nitrogen. The combined application of either two or three PGPR species with 75% N was as good as application of recommended NPK, but significantly higher than application of any single species with 75% N, with respect to crop growth rate and crop relative growth rate of wheat. The higher crop growth rate by 47.6, 40 and 36.8%, and relative growth rate by 11.5, 11.5 and 11.5% of wheat was recorded with the application of 75% N + RPK + Azo + CW1 + PW5 over 75% N + RPK at 60 DAS, 90 DAS and 120 DAS, respectively.

Table 5 Effect of PGPR on crop growth rate and crop relative growth rate of wheat

Treatment	CGR ($g/m^2/d$)			RGR (mg/g dry matter/d)		
	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120 DAS
Absolute control	2.4	8.7	6.0	53.1	55.0	25.0
Control + *Azo* + CW1 + PW5	3.0	10.2	7.5	54.0	55.7	30.9
RNPK	6.0	16.0	12.2	64.2	68.7	38.2
75% N + RPK	4.2	11.7	9.5	59.6	63.8	35.5
75% N + RPK + *Azo*	4.4	12.2	9.9	61.2	65.3	36.3
75% N + RPK + CW1	4.4	12.1	10.2	60.4	64.8	36.0
75% N + RPK + PW5	4.2	12.1	10.1	60.2	64.5	35.8
75% N + RPK + *Azo* + CW1	6.0	16.0	12.0	65.6	70.2	39.0
75% N + RPK + *Azo* + PW5	5.9	16.0	12.1	64.7	69.3	38.5
75% N + RPK + CW1 + PW5	6.0	15.8	12.4	64.3	68.9	38.3
75% N + RPK + *Azo* + CW1 + PW5	6.2	16.4	13.0	66.5	71.2	39.6
SEm±	0.49	0.93	0.47	1.72	0.98	0.62
LSD (P=0.05)	1.45	2.67	1.40	5.12	2.89	1.83

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena* sp.; PW5- *Providencia* sp.; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K); DAS- Days after sowing

It is evident from the data presented in Table 4 that the highest grain yield was recorded when 75% N was combined with RPK and all the three species of PGPR tested (75% N + RPK + *Azo* + CW1 + PW5), while the highest straw yield was produced by application of 75% N + RPK + *Azo* + PW5. Combinations of either two or three species of PGPR with 75% N + RPK produced statistically similar grain and straw yields, all being at par with recommended dose of NPK (RNPK). There was a significant reduction in grain and straw yields if only one species (*Azo*, CW1 or PW5) or no species (75% N + RPK) of PGPR was combined with 75% N + RPK as compared to all the former treatments. Treatments 75% N + RPK + *Azo* + CW1 + PW5, 75% N + RPK + *Azo* + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5 and RNPK gave 22.7, 19.3, 17.9, 17.9 and 18.4% higher grain yield, respectively, over 75% N + RPK.

It is thus clear that a significant grain yield response to PGPR inoculation was achieved in the present study. This response was achieved in both situations of no NPK or NPK application. The grain yield increased more prominently if all the three species of PGPR were combined with 75% N + RPK. Interestingly, the grain yield increase was similar when either two or three species of PGPR were combined with 75% N + RPK. The combined use of either two or three species of PGPR with 75% N + RPK was equally effective in increasing the wheat grain yield as application of recommended dose of fertilizers (RNPK). Thus, these biofertilizers could help in saving the nitrogenous fertilizers in wheat production. Furthermore, there was a positive correlation between different yield attributes and grain yield (Fig 1). The R^2 values between grain yield and different yield attributes such as effective tillers/m^2, spike length, spike weight and test weight were 0.74, 0.90, 0.88 and 0.84, respectively. It suggests that, for example, 74% of the variation in mean grain yield of wheat could be adequately explained by the regression equation computed ($y = 0.0089x + 0.6169$) between grain yield and effective tillers. Hence, it can be inferred that combined application of either one or two species was able to meet the 25 % of nitrogen requirement of wheat crop. However, application of single species was not effective in meeting the nitrogen requirement. Further, this may also be correlated with the increased root parameters as the values recorded were higher for the same treatments. These benefits may also be due to the synthesis of hormones like IAA, which would have promoted the growth of the wheat. Turan *et al*. (2010) have showed that combined PGPR inoculation with the strain of OSU-142 + M-13 + *Azospirillum* sp. 245 significantly enhanced grain yield of wheat over full doses of nitrogen application.

Reduction in soil fertility, low fertilizer use efficiency and increasing environmental pollution are the primary concern to agriculture in terms of crop productivity. Biofertilizers are appropriate and environmental friendly

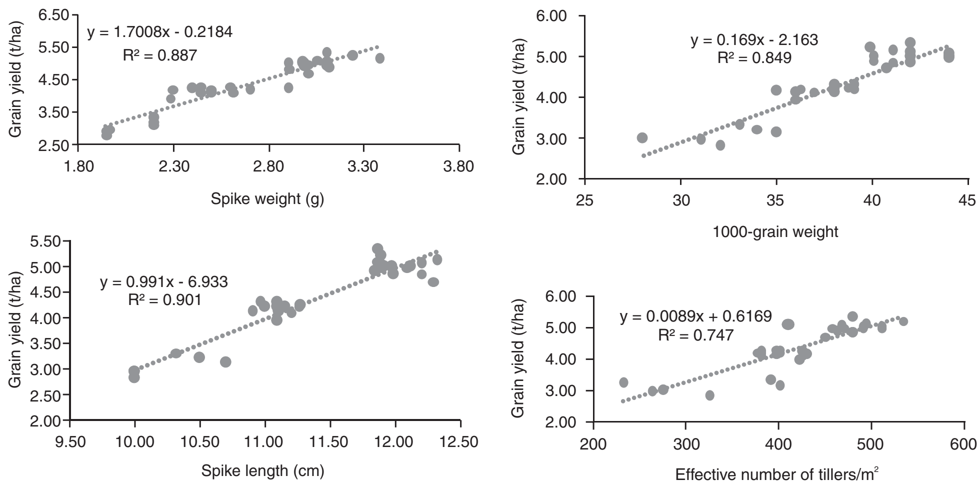

Fig. 1 Correlation between yield attributes and grain yield

tools to supplement the chemical fertilizers. This study clearly showed the potential positive influence of PGPR on productivity and growth of wheat. Different combinations of bacterial strains, viz. *Providencia* sp. PW5, *Azotobacter* (IARI inoculant), and cyanobacterial strain *Anabaena laxa* CW1 significantly increased the growth and yield attributing characters of wheat, which resulted in significant increase in both grain and straw yields. Further, the combined application of these PGPR inoculants resulted in significant increase in root growth.

SUMMARY

A field experiment was carried out at the ICAR-Indian Agricultural Research Institute, New Delhi during *rabi* season of 2014-15 to evaluate the effect of three plant growth promoting rhizobacteria (PGPR) strains, PW5 (*Providencia* sp.), *Azotobacter* (IARI Inoculant) and CW1 (*Anabaena* sp.) along with 75% recommended dose of nitrogen (N) and full dose of phosphorus (P) and potassium (K) fertilizers (N 150, P 26, K 33 kg/ha). An increase of 6% in plant height, 19.6% in number of tillers/m^2 and 14.5% in dry matter/m^2 were obtained by application of 75% N + *Azotobacter*+ CW1 + PW5 over the treatment receiving 75% N alone. Leaf area index was recorded 23% higher by application of 75% N + *Azotobacter* + CW1 as compared to 75% N. Further, an increase of 15.8, 7.1, 28.0 and 10.2% was obtained with respect to effective tillers/m^2, spike length, spike weight and test weight, respectively by the application of 75% N + *Azotobacter*+ CW1 + PW5 as compared to the treatment receiving 75% N alone. Further, the former treatment recorded 14.2, 30.3 and 52.4% higher values for root length, root volume and root dry weight, respectively, over application of recommended dose of NPK. Grain yield was 22.6% higher in treatment receiving 75% N + *Azotobacter* + CW1 + PW5 over 75% N application. Our study clearly showed the positive influence of PGPR on the growth parameters and grain yield of wheat crop.

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