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Research Communication

Yield and nutrients uptake of wheat (*Triticum aestivum*) and soil microbial parameters as influenced by plant-growth-promoting rhizobacteria

AMIR JAN DAWLATZAI[1], DINESH KUMAR[2], NAIN SINGH[3], ANJALI ANAND[4] AND RADHA PRASANNA[5]

ICAR-Indian Agricultural Research Institute, New Delhi 110 012

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ABSTRACT

A field experiment was conducted at the ICAR-Indian Agricultural Research Institute, New Delhi, during the winter (*rabi*) season of 2014–15, to study the effect of 3 plant-growth-promoting rhizobacteria (PGPR) species, viz. 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_2O_5 60, K_2O 40) kg/ha on yield and nutrients uptake of wheat (*Triticum aestivum* L. emend. Fiori & Paol.), and on soil-microbial parameters. An increase of 22.6% in grain and 13% in straw yields was recorded in treatment receiving 75% N + *Azotobacter* + CW1 + PW5 over 75% N application. Microbial biomass carbon and dehydrogenase activity showed 12% and 37% increase with the application of 75% N + Azo. + CW1 + PW5 over recommended dose of NPK fertilizers, respectively. Further, protein content in grain and total nitrogen uptake were 34.5% and 16.4% higher in treatment receiving 75% N + *Azotobacter* + CW1 + PW5 than application of 75% N. Our results indicate that the combined application of either 2 or 3 species of bacterial inoculants with 75% N, on an average, saved 37.5 kg N/ha, and hence proved economical.

Key words : PGPR, Protein, Soil microbial parameter, Yield

Nitrogen is one of the essential nutrients for growth and development of wheat. Intensive farming practices that result in high yields require huge amount of chemical fertilizers, which are not only costly but may also create environmental problems, and also their low use efficiency. Further, intensive use of chemical fertilizers destroys the soil ecology, disturb the environmental balance, degrade soil fertility, contaminate groundwater and hence lead to harmful effects on human health (Ayala and Rao, 2002; Joshi *et al.,* 2006). In such a scenario, supplementing nitrogen by biofertilizers can be an appropriate and environment friendly alternative for increasing wheat yields and enhance soil fertility.

Biofertilizers are material which carry living organisms, when they are applied to seed, plant surface, or soil, colonize the rhizosphere or the interior of the plant and enhance growth of the host plant by increasing availability of primary nutrients (Vessey, 2003). The use of plant-growth-promoting rhizobacteria (PGPR) is increasing in agriculture. PGPR are known to engage one or more direct and indirect mechanisms of action to improve plant growth and health; however, the main mode of action of many PGPR is by increasing the availability of nutrients for plants in the rhizosphere (Glick, 1995). Further, PGPR produce phytohormones (Dobbelaere *et al*., 2003) and reduce the disease incidence (Kloepper and Schroth, 1978).

One of the important modes of action of PGPR is to decrease the dependence on the application of chemical nitrogenous fertilizers by fixing atmospheric nitrogen through biological processes (Rodriguez *et al*., 1996; Dobereiner, 1997). Symbiotic nitrogen fixators have generally been used in legume production and currently there is a growing interest in the use of free-nitrogen fixators in agricultural production systems (Casanovas *et al.*, 2000). Asymbiotic nitrogen-fixing bacteria, which live in the rhizosphere, increase yields of cereals and other crops (Reinhold and Hurek, 1989).

Therefore, organisms are important for agriculture to increase the circulation of plant nutrients and decrease the need for chemical fertilizers. Hence, the present study was carried out to investigate the effect of combined applica-

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[1]Ph.D. Scholar, [2]Principal Scientist, [3]Technical Officer, Division of Agronomy; [4]Principal Scientist, Division of Plant Physiology, [5]Principal Scientist, Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi 110 012

tion of bacterial and cyanobacteria strains of PGPR on productivity, soil-microbial properties and nutrients uptake of wheat under irrigated condition.

The field experiment was conducted at the Research Farm of ICAR-Indian Agricultural Research Institute (IARI), New Delhi (28°40' N, 77°12' E, 228.6 m above the mean sea-level), during the winter (*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 belongs to Order Inceptisols, Mehrauli 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 *p*H 8.2. The chemical analysis of soil was carried out as per the procedures described by Prasad *et al*. (2006).

The experiment was laid down in a randomized block design with 3 replications. The treatments were: 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.* + CW, 75% N + full PK + *Azot.* + PW5, 75% N + full PK + CW1+ PW5 and 75% N + full PK + *Azot.* + CW1 + PW5. Wheat variety 'HD 2967' was sown in rows, 22.5 cm apart, using a seed rate of 100 kg/ha. Recommended doses of P (60 kg/ha P_2O_5), K (40 kg/ha K_2O) and N (150 kg N/ha) were applied. Full doses of P and K, and 50% N was applied basal. The remaining 50% N was top-dressed at first irrigation. The crop received total 6 irrigations. Two hand-weedings were carried out during the crop-growth period at 17 days after sowing (DAS) and 35 DAS. The bacterial strain *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. Their plant-growth-promoting activities had been earlier evaluated under gnotobiotic, net house and field conditions for wheat and rice crops (Nain *et al*., 2010). The formulations were amended with 1% CMC (carboxymethyl cellulose, from Himedia, India) as a sticker, prior to application on the seeds. Microbial biomass carbon, dehydrogenase activity and fluoresein diacetate (FDA) hydrolysis in soil samples was estimated as per Nunan *et al*. (1998), Casida *et al*. (1964) and Green *et al*. (2006), respectively. The statistical analyses of data was carried out using Statistical Analysis System (SAS) 9.3.

The results indicated that both grain and straw yields were significantly influenced by the application of plant-growth-promoting rhizobacteria (PGPR) (Table 1). Combined application of either 2 or 3 species of PGPR with 75% N + recommended dose of phosphorus (P) and potassium(K) (RPK) resulted in 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 1 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

Table 1. Effect of plant growth-promoting rhizobacteria on grain and straw yields of wheat and soil microbial parameters

Treatment	Grain yield (t/ ha)	Straw yield (t/ha)	MBC (µg/g of soil)	Dehydrogenase activity (µg TPF/g soil/day)	FDA hydrolysis (µg F./g soil/h)
Absolute control	2.91	6.10	654	21.7	0.012
Control + Azo.[a] + CW1[b] + PW5[c]	3.21	6.69	700	30.7	0.015
RNPK[d]	4.96	8.23	747	38.3	0.012
75% N + RPK[e]	4.19	7.30	767	40.2	0.018
75% N + RPK + Azo.	4.21	7.63	757	43.0	0.018
75% N + RPK + CW1	4.15	7.43	752	41.0	0.015
75% N + RPK + PW5	4.14	7.27	750	40.3	0.015
75% N + RPK + Azo. + CW1	5.00	8.20	820	52.4	0.019
75% N + RPK + Azo. + PW5	4.94	8.27	826	51.7	0.020
75% N + RPK + CW1 + PW5	4.94	8.23	823	50.8	0.015
75% N + RPK + Azo. + CW1 + PW5	5.14	8.23	837	52.4	0.015
SEm±	0.07	0.18	15	2.3	0.001
CD (P=0.05)	0.21	0.54	45	6.8	0.003

[a]Azo., *Azotobacter* (IARI inoculant); [b]CW1, *Anabaena* sp.; [c]PW5, *Providencia* sp.; [d]RNPK, recommended dose of nitrogen (N), phosphorus (P) and potassium (K); [e]RPK, recommended dose of phosphorus (P) and potassium(K)

gave 22.7, 19.3, 17.9, 17.9 and 18.4% higher grain yield, respectively, over 75% N + RPK. Thus, the highest grain yield was recorded by 75% N + RPK + Azo. + CW1 + PW5. It is hence clear that a significant grain yield response to PGPR inoculation was achieved in the present study. The combined use of either 2 or 3 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. In addition, application of 75% N + RPK resulted in significantly lower grain and straw yields compared with RNPK, however the former was at par with 75% N + RPK + Azo., 75% N + RPK + CW1 and 75% N + RPK+ PW5. There was a positive correlation between total NPK uptake and grain yield (Fig. 1). The R^2 values between grain yield and total N, P and K uptake were 0.88, 0.80 and 0.68 respectively. Hence it can be inferred that combined application of either 2 or 3 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. 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, Turan *et al*. (2010) showed that combined PGPR inoculation with the strain of OSU-142 + M-13 + *Azospirillum* sp. 245 have significantly enhanced grain yield of wheat over full doses of nitrogen application.

Our study showed that soil-microbial biomass carbon and dehydrogenase-enzyme activity measured at flowering stage of wheat were significantly influenced by application of plant-growth-promoting rhizobacteria (PGPR) (Table 1). Application of 75% N + recommended dose of phosphorus (P) and potassium (K) (RPK) + Azo. + CW1, 75% N + RPK + Azo. + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo. + CW1 + PW5 resulted in significantly higher microbial-biomass carbon and dehydrogenase-enzyme activity over both RNPK and 75% N + RPK treatments. However, there was no significant difference observed for the soil microbial-biomass carbon and dehydrogenase-enzyme activity among 75% N + RPK + Azo., 75% N + RPK + CW1, 75% N + RPK+ PW5, RNPK and 75% N + RPK treatments. The highest microbial biomass carbon and dehydrogenase-enzyme activity were obtained by application of 75% N + RPK + Azo., + CW1 + PW5 which were 12 and 37% higher than application of RNPK respectively. Further, application of 75% N + RPK + Azo + CW1 and 75% N + RPK + Azo. + PW5 showed significantly highest values for FDA hydrolysis over all the other treatments. The highest values for FDA hydrolysis were recorded with application of 75% N + RPK + Azo. + PW5, being 67% higher than application of RNPK. The application of PGPR was absolutely effective in increasing the FDA hydrolysis. FDA hydrolysis was higher when *Azotobacter* was inoculated alone or either

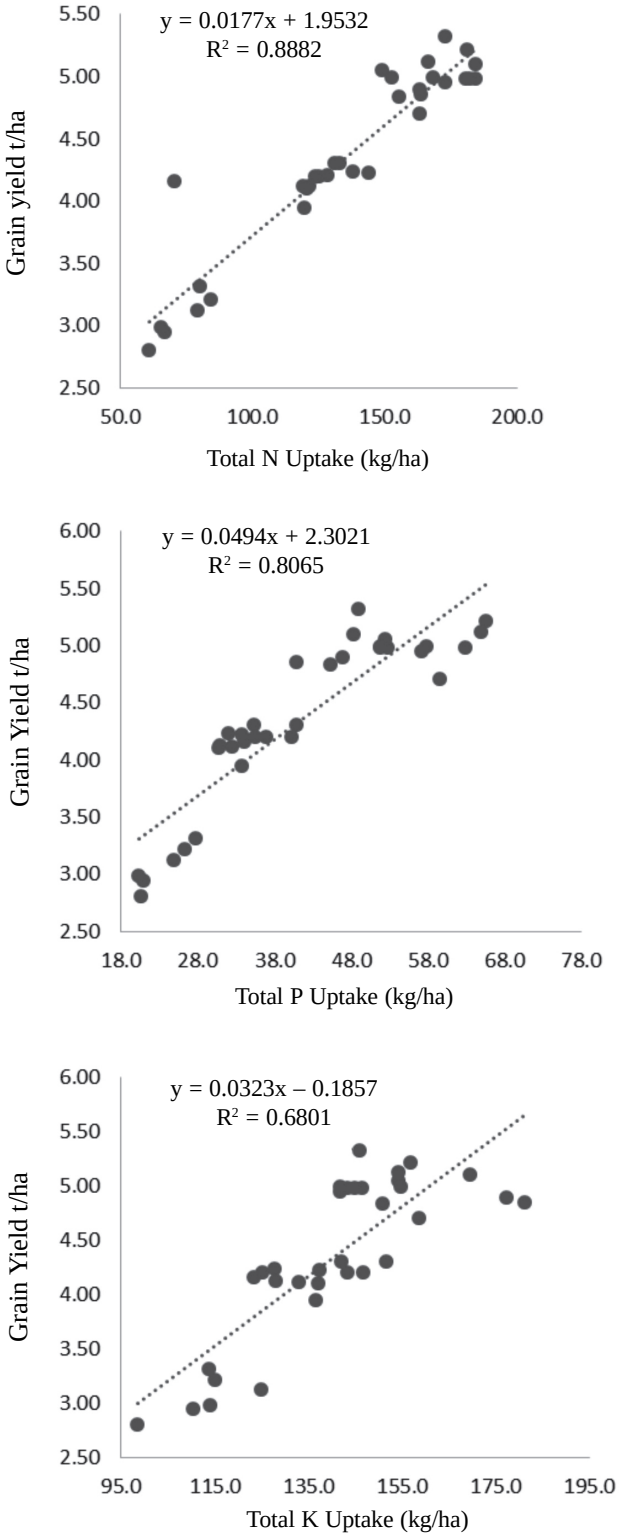

Fig. 1. Correlation between nutrient uptake and grain yield of wheat

with CW1 or CW5 and application of 75% N fertilizer. Similarly, Nain *et al*. (2010) observed the highest values for FDA hydrolysis with application of 2 bacterial strains (PW1 + PW7) and 1 *Cyanobacteria* strain (CW2). Further, Rana *et al*. (2012) reported a significant enhancement in FDA hydrolase with application of (AW5 + AW7) treatment compared to all the treatments at the mid- and harvest stages of the wheat crop.

Uptake of N in grain and straw, and protein content in grain were significantly influenced by application of PGPR (Table 2). Application of 75% N + RPK + Azo. + CW1, 75% N + RPK + Azo. + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo., + CW1 + PW5 treatments significantly increased N uptake in grain and straw, and protein content in grain over 75% N + RPK, whereas the former treatments were at par with RNPK treatment. Application of 75% N + RPK + Azo., 75% N + RPK + CW1, 75% N + RPK+ PW5 did not significantly increase N uptake in grain and straw, and protein content in grain over RNPK; however, they were at par with application of 75% N + RPK. The highest protein content in grain was obtained from combined application of either 2 or 3 species of PGPR.

Nitrogen is the most important nutrient for plant growth and productivity. In addition to enhancement of plant growth and yield, PGPR are directly involved in increased concentration and uptake of nitrogen and synthesis of phytohormones. This may be correlated with their ability to fulfil the N requirement of the wheat crop and the application of single species may have failed to meet nitrogen requirement. Hasanpor *et al*. (2012) reported highest amount for protein percentage (12.4%) with combined inoculation of mycorrhizae and *Azotobacter* in wheat. Turan *et al.* (2010) showed that highest N concentration in leaf, grain, and straw were obtained from mixed inoculation with the OSU-142 + M-13 + *Azospirillum* sp.245 +40 kg N/ha, which increased N concentration of leaf, grain and straw of wheat crop by 52.6%, 83.4%, and 83.0%, respectively, over the control treatment. The increased N concentration in grain eventually results in increased protein content.

Phosphorus uptake in grain and straw was significantly higher in 75% N + RPK + Azo. + CW1, 75% N + RPK + Azo. + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo. + CW1 + PW5 and RNPK treatments over the single application of any of the PGPR species with 75% N and RPK, viz. 75% N RPK + Azo., 75% N + RPK + CW1, 75% N + RPK + PW5, absolute control and inoculation of only Azo. + CW1 + PW5 (Table 2). Potassium uptake in grain and straw was significantly influenced (Table 2) by PGPR. The uptake of K in grain was significantly higher by application of 75% N + RPK + Azo. + CW1, 75% N + RPK + Azo. + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo. + CW1 + PW5 and RNPK over the application of 75% N + RPK + Azo., 75% N + RPK + CW1, 75% N + RPK + PW5 and 75% N + RPK. In addition, the uptake of K in straw was significantly higher by application of 75% N + RPK + Azo., 75% N + RPK + CW1, RNPK, 75% N + RPK + Azo. + CW1, 75% N + RPK + Azo. + PW5, 75% N + RPK + CW1 + PW5 and 75% N + RPK + Azo. + CW1 + PW5 treatments over application of 75% N + RPK and 75% N + RPK + PW5. Application of 75% N + RPK + Azo. + CW1 + PW5 showed the highest values for uptake

Table 2. Effect of plant-growth-promoting rhizobacteria on protein content in grain, and nitrogen, phosphorus and potassium uptake in wheat

Treatment	Protein content in grain (%)	Nitrogen uptake (kg N/ha)			Phosphorus uptake (kg P/ha)			Potassium uptake (kg K/ha)		
		Grain	Straw	Total	Grain	Straw	Total	Grain	Straw	Total
Absolute control	9.9	49.5	15.0	64.5	14.9	1.3	16.2	9.2	98.6	107.8
Control + Azo.[a] + CW1[b] + PW5[c]	10.5	57.8	23.4	81.2	19.0	1.6	20.6	10.1	107.9	118.1
RNPK[d]	12.9	109.8	42.5	152.3	37.9	2.6	40.5	16.2	137.1	153.3
75% N + RPK[e]	10.7	77.0	33.7	110.7	25.1	1.8	26.9	13.4	119.3	132.7
75% N + RPK + Azo.	12.1	87.2	39.5	126.7	26.0	1.9	27.9	13.7	127.3	141.1
75% N + RPK + CW1	12.8	90.9	40.5	131.5	25.0	1.8	26.8	13.7	125.0	138.7
75% N + RPK + PW5	12.0	85.1	37.5	122.6	25.2	1.8	27.0	13.2	118.6	131.8
75% N + RPK + Azo. + CW1	14.3	122.5	50.3	172.8	39.1	2.6	41.7	16.0	133.9	149.9
75% N + RPK + Azo. + PW5	14.4	121.4	50.7	172.1	37.9	2.6	40.5	16.3	139.3	155.5
75% N + RPK + CW1 + PW5	14.4	121.7	50.7	172.4	38.3	2.6	40.9	16.3	138.9	155.2
75% N + RPK + Azo. + CW1 + PW5	14.4	126.5	50.8	177.4	43.7	2.8	46.5	17.5	142.8	160.3
SEm±	0.63	4.7	2.4	6.6	2.5	0.18	2.6	0.55	6.2	6.6
CD (P=0.05)	1.9	14.0	7.3	19.6	7.3	0.53	7.8	1.63	18.4	19.6

[a]Azo, *Azotobacter* (IARI inoculant); [b]CW1, *Anabaena* sp.; [c]PW5, *Providencia* sp.; [d]RNPK, recommended dose of nitrogen (N), phosphorus (P) and potassium (K); [e]RPK, recommended dose of phosphorus (P) and potassium (K)

of K both in grain and straw.

This study clearly showed the positive influence of plant-growth-promoting rhizobacteria (PGPR) on productivity and nitrogen economy in wheat crop. In general, wheat grain yields recorded with application of 75% N + PK+ combination of any 2 or 3 species of PGPR were statistically similar to the recommended dose of NPK fertilizer (RNPK). Thus, it can be inferred that the combined application of either 2 or 3 species of bacterial inoculants with 75% N, on an average, saved 37.5 kg N/ha, and hence it can save money required to buy the N fertilizer.

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