



Environmental Microbiology

Plant growth promoting rhizobacteria reduce aphid population and enhance the productivity of bread wheat



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ABSTRACT

Plant growth promoting rhizobacteria increase plant growth and give protection against insect pests and pathogens. Due to the negative impact of chemical pesticides on environment, alternatives to these chemicals are needed. In this scenario, the biological methods of pest control offer an eco-friendly and an attractive option. In this study, the effect of two plant growth promoting rhizobacterial strains (*Bacillus* sp. strain 6 and *Pseudomonas* sp. strain 6K) on aphid population and wheat productivity was evaluated in an aphid susceptible (Pasban-90) and resistant (Inqlab-91) wheat cultivar. The seeds were inoculated with each PGPR strain, separately or the combination of both. The lowest aphid population (2.1 tiller⁻¹), and highest plant height (85.8 cm), number of spikelets per spike (18), grains per spike (44), productive tillers (320 m⁻²), straw yield (8.6 Mg ha⁻¹), and grain yield (4.8 Mg ha⁻¹) were achieved when seeds were inoculated with *Bacillus* sp. strain 6 + *Pseudomonas* sp. strain 6K. The grain yield of both varieties was enhanced by 35.5–38.9% with seed inoculation with both bacterial strains. Thus, the combine use of both PGPR strains viz. *Bacillus* sp. strain 6 + *Pseudomonas* sp. strain 6K offers an attractive option to reduce aphid population tied with better wheat productivity.

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Introduction

Bread wheat (*Triticum aestivum* L.) is the most essential cereal crop and staple food for the majority of the mankind.¹ It is grown on an area of >200 million hectares all over the world and meets 21% of global food requirement.² It is a staple food

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of Pakistani people, and contributes 2.2% in GDP and 10.3% toward value added in agriculture. In 2015–16, it was cultivated on an area of 9.26 million hectares with the production of 25.48 million tons.³

Biotic and abiotic stresses are serious environmental constraints which reduce the yield of cereals across the globe.^{4,5} During recent years, the Russian wheat aphids (black aphids) have emerged as a serious threat to wheat production in Pakistan. Its attack is more severe at reproductive and grain filling stages, thus causing a severe decline in crop yield and the quality of produce. Its worst infestation may cause yield decline of 21–92% in aphid susceptible cultivars of wheat.⁶ Indeed, the aphid sucks the cell sap from leaves, which affects the photosynthetic efficiency and reproductive development of plants. It also excretes sugary material which favors the growth of fungi on the leaf surface,⁷ thus affecting the wheat performance negatively.

Although, various pesticides are being employed to control aphid.⁸ However, the use of pesticides in wheat may cause the health problems if the pesticide residues retain in the wheat grain. Moreover, pesticides are greater risk to the long term sustainability of our ecosystem due to their negative impacts on human beings and other soil biological community.⁹

The genetic resistance of wheat to aphid has been reported. For example, Elek et al.¹⁰ tested the diploid, tetraploid and hexaploid wheat lines for their resistance against aphid. They found that the synthesis of hydroxamic acids [especially 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA)], was responsible for inducing resistance against aphid in these wheat lines. Another possible way to reduce the aphid population in wheat is the use of plant-growth-promoting rhizobacteria (PGPR).¹¹ These PGPRs induce resistance against insect pests through synthesis of phytohormones, increase in phosphorus and nitrogen uptake and increase in iron and mineral solubility through chelation growth.¹² Several PGPR strains especially, *Bacillus* and *Pseudomonas* strains are also being used as inoculant biofertilizers,^{13,14} which have direct and indirect effects on insect pest resistance.^{13,15,16} The PGPR (especially *Bacillus* and *Pseudomonas* strains) suppress the soil-borne pathogens by siderophores production and antimicrobial metabolites.¹⁶ Indirect effects include the induced systemic resistance thus enhancing the resistance against various pathogens and pests by the synthesis of physical and chemical barriers in the host.^{17–20} The rhizobacteria mediated induced systemic resistance resembles the systemic acquired resistance which is induced in pathogens including the nematodes, insects, bacterial, fungal and viral pathogens.^{21,22} This induced systemic resistance is promoted by PGPR through signaling pathway whereas systemic acquired resistance is the phenomenon used to explain salicylic acid dependent induced resistance activated by local disease.²³ Several studies have reported that the spotted cucumber beetle (*Diabrotica undecimpunctata*),²⁴ green peach aphid (*Myzus persicae* Sulzer),²⁵ and nymph population of whitefly (*Aleyrodidae*),²⁶ blue green aphid (*Acyrtosiphon kondoi* Shinji) on *Medicago* and white clover plants,²⁷ and population size and population growth of cotton aphids (*Aphis gossypii* Glover) on cucumber,²⁸ was significantly decreased by the application of different PGPR strains.

However, to the best of our knowledge, no study has been conducted to check the influence of PGPR strains on the aphid population and productivity of bread wheat. Thus, this study was conducted to evaluate the influence of two PGPR strains (*Bacillus* sp. strain 6 and *Pseudomonas* sp. strain 6K) on bread wheat growth and aphid population.

Materials and methods

Preparation of inoculum and seed inoculation procedure

The *Bacillus* strain 6 and *Pseudomonas* strain 6K – used as positive control – were obtained from the Soil Microbiology Laboratory, Institute of Soil and Environmental Science, University of Agriculture, Faisalabad. The *Bacillus* strain was gram positive, rod shaped, aerobic, endospore forming, fast growing with whitish colonies. The *Pseudomonas* strain was gram negative, fast growing, rod shaped, with off-white colonies and motile cells.

The Inoculum was prepared by growing the two selected PGPR strains in a nutrient broth. It was incubated at 28 °C with 100 rev min⁻¹ shaking. Four days old inoculum (10⁷–10⁸ CFU mL⁻¹) was inserted into germ free peat at 100 mL kg⁻¹ peat and incubated at 28 °C for 24 h proceeding to wheat seed inoculation. The peat was made germ free by baking peat in an oven for 30 min at 180 °F.

Wheat seeds (Pasban-90 and Inqlab-91) were inoculated by incorporating with peat and 10% sugar solution (100 mL kg⁻¹) on peat; whereas control treatment seeds were treated with peat and sugar solution without PGPR. Seeds were dried up under shadow for 6–8 h.

Experimental site and treatments

This experiment was carried out at Student's Farm, University of Agriculture Faisalabad, Pakistan in 2013. The experiment comprised of two wheat cultivars (Pasban-90 and Inqlab-91) and two PGPR strains (*Bacillus* sp. strain 6 and *Pseudomonas* sp. strain 6K). The both bacterial strains were inoculated on seeds alone or in combination. Non-inoculated seeds were also sown as a control treatment. The wheat seeds of both varieties were collected from Wheat Research Institute, Ayub Agricultural Research Institute, Faisalabad. The experiment was carried out in randomized complete block design with factorial arrangement having three replications. The net plot size was 1.8 m × 5 m. The seeds of both varieties were sown with single row hand seed drill by maintaining row to row distance of 22.5 cm on November 25, 2013, using seed rate of 125 kg ha⁻¹.

On the basis of soil analysis, the fertilizer was applied at the rate of 120–90–60 kg N, P, K ha⁻¹, using urea, diammonium phosphate (DAP) and muriate of potash (MOP) as sources, respectively. Half of the nitrogen and the full dose of potassium and phosphorous was applied at the time of sowing. The remaining half dose of nitrogen was applied at tillering stage through broadcasting. Data on aphid population (aphid per tiller) were recorded from twenty tillers in each plot manually and then averaged. Moreover the data regarding plant height (cm), productive tillers (m²), spike length (cm), spikelets per

Table 1 – Effect of plant growth promoting rhizobacteria on plant height, productive tillers and spike length of two wheat varieties.

Treatment	Plant height (cm)		Productive tillers (m ⁻²)		Spike length (cm)	
	Pasban-90	Inqlab-91	Pasban-90	Inqlab-91	Pasban-90	Inqlab-91
T ₀	78.7 f	82.0 de	232 d	263 c	8.9 f	9.04 ef
T ₁	84.1 bc	84.0 bcd	277 bc	273 bc	9.8 de	9.8 de
T ₂	81.8 e	82.3cde	289 bc	288 bc	10.5 cd	11.9 b
T ₃	85.2 b	88.9 a	300 b	339 a	11.1 bc	13.7 a
LSD ($p \leq 0.05$)	1.99		30		0.85	

Figures of interaction sharing the same case letter do not differ significantly at $p \leq 0.05$; T₀ = no seed inoculation; T₁ = seed inoculation with *Bacillus* sp. strain 6; T₂ = seed inoculation with *Pseudomonas* sp. strain 6K; T₃ = seed inoculation with *Bacillus* sp. strain 6 + *Pseudomonas* sp. strain 6K.

Table 2 – Effect of plant growth promoting rhizobacteria on spikelets per spike, grains per spike, and biological yield of two wheat varieties.

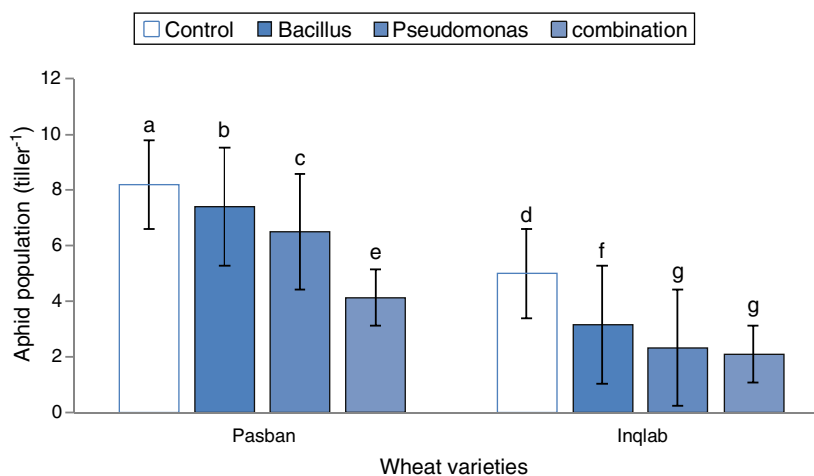
Treatment	Spikelets per spike		Grains per spike		Biological yield (Mg ha ⁻¹)	
	Pasban-90	Inqlab-91	Pasban-90	Inqlab-91	Pasban-90	Inqlab-91
T ₀	15.7 e	16.9 cd	37.2 e	39.2 d	14.1 f	15.1 d
T ₁	16.5 d	17.5 bc	40.3 de	41.8 c	14.5 e	15.4 b
T ₂	16.9 cd	17.8 ab	40.9 cd	44.4 b	14.6 e	15.6 b
T ₃	17.1 c	18.4 a	42.1 c	46.7 a	15.3 c	15.9 a
LSD ($p \leq 0.05$)	0.65		1.45		0.14	

Figures of interaction sharing the same case letter do not differ significantly at $p \leq 0.05$; T₀ = no seed inoculation; T₁ = seed inoculation with *Bacillus* sp. strain 6; T₂ = seed inoculation with *Pseudomonas* sp. strain 6K; T₃ = seed inoculation with *Bacillus* sp. strain 6 + *Pseudomonas* sp. strain 6K.

Table 3 – Effect of plant growth promoting rhizobacteria on straw yield and grain yield of two wheat varieties.

Treatment	Straw yield (Mg ha ⁻¹)		Grain yield (Mg ha ⁻¹)	
	Pasban-90	Inqlab-91	Pasban-90	Inqlab-91
T ₀	6.0 g	7.6 e	3.6 h	4.0 g
T ₁	7.3 f	8.1 c	4.1 f	4.2 e
T ₂	7.9 d	8.6 b	4.4 d	4.5 c
T ₃	8.2 c	9.0 a	4.6 b	5.0 a
LSD ($p \leq 0.05$)	0.12		0.04	

Figures of interaction sharing the same case letter do not differ significantly at $p \leq 0.05$; T₀ = no seed inoculation; T₁ = seed inoculation with *Bacillus* sp. strain 6; T₂ = seed inoculation with *Pseudomonas* sp. strain 6K; T₃ = seed inoculation with *Bacillus* sp. strain 6 + *Pseudomonas* sp. strain 6K.

**Fig. 1 – Aphid population as affected by the use of bacterial strains.**

spike, straw yield (Mgha^{-1}), and grain yield (Mgha^{-1}) were recorded following Farooq et al.²⁹

Statistical analysis

The data collected during the experiment were analyzed using the Fisher's analysis of variance technique (two way anova) and the treatments' means were compared by least significance difference (LSD) test at 5% probability level.³⁰ The data analysis was performed using randomized complete design in factorial arrangement with the help of statistical software 'Statistics 8.1'.

Results

Both wheat cultivars differ significantly for plant height, productive tillers, spike length, spikelets per spike, grains per spike, biological yield, straw yield, grain yield and aphid population. Likewise, seed inoculation with both PGPR strains significantly affected the plant height, productive tillers, spike length, spikelets per spike, grains per spike, biological yield, straw yield, grain yield and aphid population. The interaction of wheat cultivars with PGPRs was also significant for plant height, productive tillers, spike length, spikelets per spike, grains per spike, biological yield, straw yield, grain yield and aphid population (Tables 1–3).

The aphid population was significantly reduced by the application of PGPR strains. The minimum population of aphid (2.1 aphids per tiller) was recorded in Inqalab-90 when it was inoculated with mixture of PGPR strains (*Bacillus* + *Pseudomonas* strains) as seed treatment, and that was statistically similar with Inqalab-90 inoculated with *Pseudomonas* strains. The maximum aphid population (8.2 aphids per tiller) was recorded in Pasban-90 without inoculation with any bacterial strains (Fig. 1).

The interaction of wheat cultivars with PGPR strains showed that the highest plant height, productive tillers, spike length, spikelets per spike, grains per spike, biological yield, straw yield and grain yield were recorded in Inqalab-91 with seed inoculation with both of the bacterial strains (*Bacillus* + *Pseudomonas* strains). Seed inoculation with both bacterial strains enhanced the grains per spike by 25.5% than control in Inqalab-91. The grain yield was also enhanced by 38.9% in Inqalab-91 with seed inoculation with both bacterial strains. In Pasban-90, the grain yield was increased by 35.5% when seeds were inoculated with both bacterial strains than control (Tables 1–3).

Discussion

Use of both PGPR strains either alone or in combination was very useful for the control of aphid population. Indeed, the PGPR increase the accumulation of phenolic compounds and phytoalexins, the activities of defense enzymes/genes (encoding glucanase, chitinase, and peroxidase), transcripts and pathogenesis-related proteins, increase the lignification and

modulate the ethylene-modulated signal transduction pathway, and induce the physiological changes within plants,^{31–36} which improve the plants defense against insect pest attack. PGPRs also inhibit the crop pests through release of different volatile and diffusible metabolites (e.g. pyoluteorin and pyrrolnitrin),³⁷ which are toxic to insect pests thus reducing their population as was observed in this study. Likewise, the improvement in aphid suppression and wheat growth might be attributed to the production of siderophores.¹⁶ The indirect effects of PGPR include the induction of systematic resistance thus enhancing the resistance against insect pests by the synthesis of physical and chemical barriers in the host.^{17–20}

Moreover, the PGPRs helps to improve the phosphorus and nitrogen uptake and enhance the activity of indole acetic acid which helps the wheat plant to uptake and translocate the micro and macronutrients (zinc, iron, nitrogen and manganese) in a better way. These nutrients play a significant role in biogeochemical cycling and increases the activities of defense enzymes against pathogen.^{38,39} Several other studies have reported that application of various PGPR strains enhanced the resistance against insect pest in field and vegetable crops.^{24–27}

Better aphid control due to seed inoculation with PGPR resulted in better crop growth which ultimately enhanced the plant growth and the plant height. The better growth due to PGPR application as a result of aphid control finally resulted in better grain partition which enhanced the number of grains per spike. The highest grain yield in both wheat varieties due to application of the PGPR might be attributed to better grains per spike and productive tillers. It is well known that the productive tillers and grains per spike are important yield contributing traits which resulted in better grain yield in this study. In another study, Hilali et al.⁴⁰ speculated that plant height and spikelets per spike was increased in many crops by microbial inoculation. Rodriguez et al.⁴¹ also observed significant increase in wheat yield by the application of P solubilizing and N_2 fixing *Bacillus* sp. The better grain yield in wheat in this study might be attributed to the direct effect of PGPRs on wheat and their indirect effect on aphid suppression. Beside suppressing the pests, the PGPRs also promote the plant growth through production of various plant hormones (e.g. abscisic acid, ethylene, cytokinins, and auxins), indole-3-acetic acid, indole-3-ethanol and 1-aminocyclopropane-1-carboxylate which promote shoot and root growth.⁴² There is dire need to extend this study and understand the whole mechanism how the PGPR affecting defense mechanism and enhancing yield of bread wheat.

In conclusion, combine use of both PGPR strains viz. *Bacillus* sp. strain 6 + *Pseudomonas* sp. strain 6K offers an attractive option to reduce the aphid population and enhance the productivity of bread wheat.

Conflicts of interest

The authors declare no conflicts of interest.

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