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Growth and productivity of wheat affected by phosphorus-solubilizing fungi and phosphorus levels

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ABSTRACT

Phosphorus (P) availability limits crop growth in most of cultivable soils in north-west India. The beneficial rhizosphere microorganisms such as phosphate-solubilising fungi (PSF) were found to increase P availability in soil and improve crop yields. In view of this, field experiments were conducted during 2009–2011 to evaluate the effect of seed inoculation with PSF (*Penicillium bilaii*) at different rates of fertilizer P on P content in leaves and grain yield of irrigated wheat in India. The soil was low in Olsen P at the Bathinda site and medium at the Ludhiana site. In no-P treatment, PSF significantly increased grain yield by 12.6% over non-inoculated control. The effect of PSF on grain yield was generally more pronounced in a soil with low Olsen-P compared to medium Olsen-P level. Inoculation of PSF along with 50% P fertilizer increased wheat yield equivalent to 100% P with no PSF. Spike density was significantly higher in PSF + 50% P than all the other treatments. There is need to study a long-term effect of *Penicillium bilaii* on P-fertilizer saving in wheat on soils varying in P availability, pH and P fixation capacity for different wheat-based cropping systems.

Keywords: plant nutrition; solubilization; cereal production; macronutrient; biofertilizer; *Triticum aestivum* L.

Phosphorus (P) is second largest limiting element for plant growth. The phosphate rock which is used in the manufacture of P fertilizers is non-renewable resource. We have to address to future shortages for sustainable crop production in India. At global level, India ranks as second largest wheat producing nation and contributing approximately 11.9% to the world wheat production from about 12% of global area (USDA 2010). One of the major constraints in boosting up the wheat production is the unbalanced use of plant nutrients, particularly P and K. Average P uptake of wheat is about 3.8 kg P/t of grains (Timsina and Connor 2001). P recovery by wheat from fertilizers is quite low i.e. 15–20% of the applied P is recovered by wheat while the remaining P is fixed as insoluble P fractions in soils (Rodríguez and Fraga 1999). Only 0.1% of the total P exists in a soluble form available for plant uptake because of its fixation into an unavailable form. Increasing prices of P-fertilizers in developing countries like India and high fixation of P in soil have led to the search for sustainable way of P nutrition of crops. In this regards phosphate-solubilizing microorganisms (PSM) have been seen as the best eco-friendly means for P nutrition of crops.

A number of bacteria (*Bacillus* spp., *Pseudomonas* spp., *Burkholderia* spp.) and fungi (*Aspergillus* spp., *Penicillium* spp.) are known to increase availability of P to plants and benefit plant growth either by mineralization of organic phosphate or by solubilisation of insoluble inorganic phosphates in soils through the production of organic acids (Rodríguez and Fraga 1999, Saber et al. 2009, Saxena et al. 2014). The group of these bacteria and fungi are called as phosphate-solubilizing microorganisms and are considered to have potential use as biofertilizer to improve the plant growth and grain yields of different crops (Rodríguez and Fraga 1999, Sahu and Jana 2000, Adesemoye and Kloepper 2009). Inoculation with P solubilising

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fungi (*A. niger* and *Penicillium* spp.) increased dry biomass of chickpea plants by 22–33% compared with non-inoculated control (Kapri and Tewari 2010). Previous studies showed that *A. niger* and *Vibrio proteolyticus* were the most active P solubilizing species of fungi and bacteria, respectively (Vassilev et al. 2007). Ekin (2010) recorded the highest seed yield of sunflower with 50% less of the recommended fertilizer P (43.7 kg P/ha) when used in conjunction with P solubilising bacteria. While arbuscular mycorrhizae fungi are known to increase fertilizer P use efficiency by increasing its mobility in soil and reducing fixation, these fungi have only an advantage at low P availability in soil (Ghorbanian et al. 2012). Most of the studies on the PSM are carried out on pulse crops and too on P solubilising bacteria but meagre information is available on P solubilising (PSB) fungi for cereal crops like wheat. The present study was therefore, undertaken to evaluate the effect of P solubilising fungi on growth and productivity of wheat crop.

MATERIAL AND METHODS

Experimental sites. The experiment was established at the Punjab Agricultural University (PAU) experimental farm in Ludhiana, in the central part of Punjab, India (30°54'N, 75°48'E, 247 m a.s.l.) in the first year and at two locations, one in Ludhiana and another at the PAU Regional Station Bathinda in the south-western part of Indian Punjab. The loamy sand soil at both the locations was low in both available N (189.4 kg/ha) and Walkley and Black organic carbon (0.16–0.30%) in 0–15 cm soil layer. The 0.5 mol/L $NaHCO_3$-extratable Olsen-P was medium (9.7 mg/kg) in Ludhiana but deficient (5.5 mg/kg) in Bathinda and both the soils were sufficient (81–83 mg/kg) in 1 mol/L NH_4OAC-extractable K.

Experimental design, treatments, and crop management. The experiment was conducted in randomized complete block design with four replications. Six treatments included in the study were the combinations of three doses of fertilizer P [0, 50 and 100% of the recommended (26 kg P/ha)] without and with seed phosphate-solubilising fungi (PSF) inoculation (*Penicillium bilaii*; Strain 201 + Strain 208/culture was in wettable powder formulation containing 21.6% pure culture of *Penicillium bilaii* fungus and 78.4% other ingredients) of Novozymes South Asia Pvt. Ltd. Bangalore, India. The PSF formulation was dissolved in 100 mL of distilled water and wheat seed was treated with this solution at 6.0 mL/kg seed to make a concentration of 10^{-4} of *Penicillium bilaii*. The required amount of the seed (112.5 kg/ha) was placed in the plastic bag and one third of the PSF solution was sprayed on the seeds followed by uniform mixing with seeds the process was repeated three times. The treated seeds were placed in shade for slow drying and the sowing was done on the same day. Wheat (cv. PBW 550) was sown with the row spacing of 22.5 cm. Irrigations (75 mm depth each) were applied at four critical stages; crown root initiation, late tillering, boot and milk stages. Uniform application of 120 kg N as urea and 25 kg K/ha as muriate of potash was made on all the plots. Nitrogen was applied in three equal splits; at sowing, crown root initiation and late tillering stages. The whole of P (as per treatment) and K fertilizers were applied at the time of sowing by broadcasting followed by soil incorporation.

Chemical analysis of soil and plant samples. Soil samples were collected from the surface (0–15 cm) layer from all the plots after the harvest of wheat in 2009–2010 and 2010–2011 at the Ludhiana site only. Ten second fully opened leaves from the top of the plant were randomly collected at 30, 60 and 90 days after sowing. Leaf, straw and grain samples were analyzed for total P content by using di-acid digestion (Yoshida et al. 1976). The observations on spike density, grains/spike, 1000-grain weight, straw and grain yield were recorded at the time of harvest. Spike density was recorded from 1-m row length from two rows within each plot. Ten tillers were randomly selected from within each plot for determining the number of grains/spike. Grain and straw yields at maturity were determined from an area of 10 m^2. The soil samples were analyzed for 0.5 mol/L $NaHCO_3$-extratable Olsen-P (Olsen et al. 1954).

Statistical analysis. The various data collected in the experiment were subjected to analysis of variance using the IRRISTAT version 4.1 package programme (IRRI, Los Banos, Philippines). A combined analysis of variance over the locations and year for characters were also performed at $P = 0.05$.

RESULTS AND DISCUSSION

Yield attributes. Spike density (averaged for three experiments) was significantly affected by

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Table 1. Effect of phosphorus-solubilizing fungi (PSF) and fertilizer P on spike density, grains/ear and grain weight of wheat (data are means of 3 experiments)

P rate (% of RDF)	Spike density/m^2			Number of grains/spike			1000-grain weight (g)		
	no PSF	PSF	mean	no PSF	PSF	mean	no PSF	PSF	mean
0	332^c	356^b	353^b	50.9^c	52.9^{ab}	51.9^c	37.0^b	38.2^a	37.6^b
50	360^b	386^a	369^a	52.8^{ab}	52.0^b	52.4^b	38.1^b	39.5^a	38.8^{ab}
100	383^{ab}	390^a	379^a	53.2^{ab}	53.9^a	53.6^a	39.1^{ab}	39.1^{ab}	39.1^a
Mean	358^b	377^a		52.3	52.9		38.1^b	38.9^a	
$LSD_{0.05}$	PSF – 14.5; P – 17.9; PSF × P – 25.3			PSF – ns; P – 0.98; PSF × P – 1.39			PSF – 0.75; P – 0.91; PSF × P – 1.30		

Means sharing the same letter in a column do not differ significantly at P = 0.05; RDF – recommended dose of fertilizer; ns – non-significant

PSF inoculation, P levels, and their interaction (Table 1). The spike density increased significantly with PSF inoculation at 0% P and 50% P treatments (Table 1). On average, application of PSF increased the spike density by about 7% both at 0% and 50% fertilizer P over no PSF application. Spike density with PSF inoculation + 50% P was similar to that produced with the application of 100% P without PSF inoculation. The number of grains/spike (averaged for 3 experiments) was significantly influenced by P fertilizer and PSF × P levels. PSF inoculation significantly increased the number of grains/spike at 0% P level only. A significant increase in number of grains/spike with P application was observed at 50% P treatment over no P treatment. Application of PSF, fertilizer P and their interaction showed a significant effect on 1000-grain weight of wheat. Application of PSF increased the grain weight at 0% and 50% P levels but the differences were significant at 50% P level. On average, application of PSF at 50% P level increased the 1000-grain weight by 3.7% over no PSF treatment.

Grain yield. Grain yield of wheat was significantly influenced by fertilizer P and PSF treatments in all the years at both locations (Table 2) except for PSF in 2009–2010 at the Ludhiana site where it was non-significant. In 2009–2010 in Ludhiana, grain yield was significantly increased with PSF inoculation at 50% P level only. The significant response to applied P was observed up to 50% P level due to the medium level of Olsen P in the soil. In 2010–2011 in Ludhiana, PSF inoculation significantly increased the grain yield by 8.6% over no PSF at 0% P level only. In Bathinda, PSF inoculation significantly increased the grain yield by 39.9% at 0% P and 9.0% at 50% P level over no PSF treatment. Thus, the effect of PSF on grain yield was stronger in Bathinda due to lower amount of Olsen P in the soil compared to that

Table 2. Effect of phosphorus-solubilizing fungi (PSF) and fertilizer P on grain yield (t/ha) of wheat

P rate (% of RDF)	Ludhiana						Bathinda 2010–2011			Mean		
	2009–2010			2010–2011								
	no PSF	PSF	mean	no PSF	PSF	mean	no PSF	PSF	mean	no PSF	PSF	mean
0	6.64^b	6.55^b	6.60^b	5.79^b	6.29^a	6.04^b	4.24^c	5.93^a	5.09^b	5.56^b	6.26^a	5.91^b
50	6.90^{ab}	7.14^a	7.02^a	6.16^a	6.30^a	6.23^{ab}	5.69^b	6.20^{ab}	5.95^a	6.25^a	6.55^a	6.40^a
100	7.03^{ab}	6.96^{ab}	7.00^a	6.39^a	6.41^a	6.40^a	6.33^a	6.36^a	6.35^a	6.59^a	6.58^a	6.59^a
Mean	6.86	6.88		6.11^b	6.33^a		5.42^b	6.16^a		6.13^b	6.46^a	
$LSD_{0.05}$	PSF – ns; P – 0.28; PSF × P – 0.40			PSF – 0.18; P – 0.22; PSF × P – 0.31			PSF – 0.35; P – 0.43; PSF × P – 0.61			PSF – 0.21; P – 0.26; PSF × P – 0.37		

Means sharing the same letter in a column do not differ significantly at P = 0.05; RDF – recommended dose of fertilizer; ns – non-significant

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Table 3. Effect of phosphorus-solubilizing fungi (PSF) and fertilizer on leaf P content (mg/kg) in at three growth stages of wheat (data are means of 3 experiments)

P rate (% of RDF)	30 DAS			60 DAS			90 DAS		
	no PSF	PSF	mean	no PSF	PSF	mean	no PSF	PSF	mean
0	0.140	0.144	0.142^{b}	0.153^{b}	0.164^{ab}	0.159^{c}	0.217	0.219	0.218^{b}
50	0.148	0.152	0.150^{a}	0.174^{ab}	0.181^{ab}	0.178^{b}	0.231	0.233	0.232^{a}
100	0.149	0.154	0.152^{a}	0.192^{a}	0.190^{a}	0.191^{a}	0.234	0.244	0.239^{a}
Mean	0.146^{b}	0.150^{a}		0.173^{b}	0.178^{a}		0.227	0.232	
$LSD_{0.05}$	PSF – 0.02; P – 0.03; PSF × P – ns			PSF – 0.02; P – 0.02; PSF × P – 0.03			PSF – ns; P – 0.12; PSF × P – ns		

Means sharing the same letter in a column do not differ significantly at $P = 0.05$; RDF – recommended dose of fertilizer; ns – non-significant; DAS – days after sowing

in Ludhiana. In earlier studies (Khiari and Parent 2005), PSF inoculation was shown to increase the wheat yield due to their role in P solubilization from the insoluble P fractions. Overall, PSF inoculation increased grain yield by 12.6% over no inoculation when no fertilizer P (0% P) was applied. Seed inoculation with PSF at 50% and 100% P application resulted in a non-significant increase in mean yield. A combination of 50% P fertilizer dose along with PSF produced wheat yield similar to that obtained with 100% of P fertilizer dose. Ekin (2010) reported a saving of 50% P fertilizer in conjunction of PSF in sunflower. Low efficiency with PSF in the present study may be due to unfavourable soil and climatic conditions for their optimum growth and activity.

Leaf P content. Inoculation with PSF, fertilizer P and their interactions showed a significant effect on mean P concentration in 30 and 60 day-old wheat leaves (Table 3). At 90 days after sowing, however, no significant effect of PSF was observed on P content in leaves. At 30 days of sowing P content in wheat leaves increased with PSF irrespective of P level, but the increase in P content was significant at 0% and 50% P at 60 days of sowing. The mean P content in wheat leaves increased significantly with fertilizer P application over no P at all the three growth stages of wheat. The increase in P content of wheat leaves with PSF over no PSF was greater at early growth stages when P uptake was limited by root growth. At later growth stages extensive root growth helped in greater uptake of P from both soil fertilizer sources thereby reducing the role of PSF on P uptake.

Grain P content. The P content in grain increased with PSF at 0% P level only, which was similar to that in 50% P with no PSF (Table 4). Similarly, total P uptake in grains plus straw was significantly higher in PSF over no PSF at 0% P level only. On average, PSF increased the P uptake

Table 4. Effect of phosphorus-solubilizing fungi (PSF) and fertilizer P content in grains and total P uptake of wheat and Olsen P content in soil at wheat harvest (data are means of 2 experiments in Ludhiana)

P rate (% of RDF)	Grain P (%)			Total P uptake (kg/ha)			Olsen P (mg/kg)		
	no PSF	PSF	mean	no PSF	PSF	mean	no PSF	PSF	mean
0	0.27^{b}	0.29^{ab}	0.28^{b}	19.9^{f}	22.2^{d}	21.1^{c}	9.4^{b}	8.9^{b}	9.2^{b}
50	0.29^{ab}	0.30^{a}	0.30^{a}	21.9^{e}	23.1^{c}	22.5^{b}	9.7^{a}	9.0^{b}	9.4^{b}
100	0.31^{a}	0.31^{a}	0.31^{a}	25.0^{b}	25.7^{a}	25.4^{a}	10.1^{a}	9.6^{a}	9.9^{a}
Mean	0.29^{b}	0.30^{a}		22.3^{b}	23.7^{a}		9.7^{a}	9.2^{b}	
$LSD_{0.05}$	PSF – 0.012; P – 0.015; PSF × P – 0.021			PSF – 0.12; P – 0.15; PSF × P – 0.21			PSF – 0.37; P – 0.45; PSF × P – 0.63		

Means sharing the same letter in a column do not differ significantly at $P = 0.05$; RDF – recommended dose of fertilizer; ns – non-significant

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by 11.6% in 0% P treatment. The increase in P uptake with PSF was possibly due to the increase in available P in the soil through solubilisation of inorganic P fractions in the soil. The increase in P uptake in several crops with the inoculation of PSB was also reported by many researchers (Rodríguez and Fraga 1999, Gulati et al. 2007).

Soil P content. Olsen-P content in soil at wheat harvest was significantly affected by P and PSF treatments. The Olsen-P content was generally lower in PSF compared to no PSF treatments mainly due to the increased P uptake by wheat as reported previously in this study. Olsen-P content increased with increasing level of P, irrespective of PSF. On the contrary, previous reports showed increases in the availability of soluble phosphate by PSF and PSB (Ponmurugan and Gopi 2006, Panhwar et al. 2011). The PSF might have increased the P availability during crop growth and the differences disappeared by the time of wheat harvest.

It is concluded that PSF gave significantly higher (by 12.6%) grain yield than the control alone by increasing solubility of the unavailable P forms in soil. The study showed potential of using PSF (*Penicillium bilaii*) as bio-inoculants along with 50% of recommended P fertilizer dose that produced wheat yield similar to 100% P when no PSF was used. However, more such long-term studies are needed on different soil types varying in P availability, pH and P fixation capacity before PSF can be recommended for adoption by the wheat farmers.

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