Research Article

Combining phosphorus (P) with phosphate solubilizing bacteria (PSB) improved wheat yield and P uptake in alkaline soil

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Abstract

Phosphate solubilizing bacteria can reduce dependence on chemical phosphorus (P) fertilizers by mineralizing and solubilizing indigenous soil P. What's why, we assessed the interactive effect of phosphate solubilizing bacteria (with and without PSB) and phosphorus levels (60, 90 and 120 kg P₂O₅ ha⁻¹) on P uptake and yield of wheat crop under field conditions. Two factorial randomized complete block design (RCBD) with three replications was used. The PSB inoculation significantly enhanced plant height (3%), 1000 grains weight (12%), grain (6%), biological (13%) and straw yield (18.5%) of wheat over control. Inoculation with PSB also significantly improved plant P concentration and uptake (26% each) over un-inoculated control. Similarly, with increasing application rates of P from 60 to 120 kg P₂O₅ ha⁻¹ the tested parameter were significantly improved except straw yield. The interactive effect of PSB and P exhibited significant effect on 1000 grains weight while the rest of parameter didn't respond significantly. However, generally PSB inoculation with P enhanced yield attributes and improved P use efficiency over sole application of P. Thus it may be concluded that PSB should be applied with P to enhance wheat yield and P use efficiency.

Keywords: Alkaline soils; PSB inoculation; Phosphorus uptake; Wheat

Introduction

Wheat (*Triticum aestivum* L.) is a major cereal crop of Pakistan and used as a staple food [1]. It is estimated that by 2020 wheat demand in developing countries is going to increase by 1.6% per annum [2]. Thus improving wheat yield is very crucial for maintaining global food security. According to Food and Agriculture Organization (FAO) projection, food productivity should be increased about by 70% in-between 2005-2050 [3, 4]. Adaptation of modern technologies, using good seeds, fertilizers and irrigation could be possible ways for achieving the target to ensure food security [5].

Phosphorus (P) ranks 2nd in term of its requirement by plant and plays vital role in root development, cell division, flower, seeds and fruit formation [6]. Pakistani soils are naturally poor in P due to high pH and lime contents which leads to the limitation of crop growth. The biologically available P exist in soil are primary and secondary orthophosphate. Crop yield can be improved by optimum application of P as chemical fertilizers [7]. However, only 10-30% of the applied P is used by the plant while the rest lost in soil in many ways [8].

Phosphate solubilizing microorganisms (PSM) can be explored for their potential to enhance P availability and the crop yields [9, 10]. As they can play vital role for in enhancing plant growth under P deficient soils [11]. They are Phosphate economical, eco friendly to expensive chemical P fertilizers. PSM's inoculation enhance P

availability and the crop yields by solubilizing the soil fixed and applied P [12]. Species of the genus *Aspergillus*, *Bacillus*, *Pseudomonas*, *Penicillium* and *Rhizobium* are the potential P solubilizers commonly present in alkaline soil [13]. They may produce the low molecular weight organic acids, lowering of pH in the surroundings and solubilize the insoluble phosphates. Organic acids may also act as chelating agents by discharging their functional groups thus, chelate the cations bonded to P thus, solubilize the insoluble phosphates [14]. In addition to organic acid release, the enzyme phosphatase released by PSM has a significant role in solubilization of insoluble P [15]. Siderophores, chelating compounds and mineral acids produced by PSMs and PSB has also been reported responsible for increasing P solubilization [16]. That's why, the present attempt was made to appraise the effect of PSB in enhancing P availability from phosphatic fertilizers applied at different rates on wheat crop with the following objectives.

Materials and methods

Experimental site

A field experiment was conducted at the agronomic research farm of the department of Agriculture university of Swabi. The aim of this study was to evaluate the associative effect of PSB and Phosphatic fertilizer on yield and P uptake in wheat crop. The soil of experimental site was silt loamy, alkaline and calcareous and deficient in organic matter, nitrogen and phosphorus (Table 1).

Table 1. Characteristics of experimental site

Property	Units	Values
Textural Class	-	Silt loam
Organic matter	%	0.92
Lime Content	%	14.33
pH	-	7.9
EC	d Sm ⁻¹	0.72
Total nitrogen	%	0.04
Phosphorous	mg kg ⁻¹	3.89

Treatment combination

This attempt was made to explore the interactive role of P rates and PSB inoculation on P uptake and growth of wheat at the agronomic research farm, University of Swabi. This project was executed using factorial RCB design in triplicates. There were two factors including two types of inoculation (with PSB and without PSB) and three levels of P (60, 90 and 120 kg P₂O₅ ha⁻¹) making a total of 6 treatment each replicated three times.

Experimental procedure

The inoculums of PSB inoculum composed of *Mycobacterium*, *Pseudomonas*, *Rhizobia*, *Burkholderia*, *Pantoea* and *Bacillus* were obtained from Department of Microbiology, Hazara University, Mansehra. The seed of wheat variety Serin was inoculated with PSB as per proposed treatment via using stander inoculation procedure. The plots were treated with 60, 90 and 120 Kg P₂O₅ ha⁻¹ according to the treatment using SSP fertilizer as a source. Inoculated and un-inoculated wheat seed were sown at the rate of 120 Kg ha⁻¹ in 30 cm apart rows while keeping a plant to plant 15 cm in a plot of size 3 x 5 m². Uniform dosage of N and K₂O was applied @ 120 and 60 Kg ha⁻¹ through urea and sulphate of Potash (SOP) fertilizers. Normal recommended cultural practices were adopted during the growth period. Data were recorded throughout the growing season under field conditions.

Agronomic parameter

Plant height (cm) was measured as mean of five randomly selecting plants from each experimental unit at the peak of physiological maturity from base to the tip of spike. The plants from central two rows were harvested, sun dried threshed and weighed in each treatment plot and the yield was converted into kg ha⁻¹. Thousand grains were randomly counted from the harvest of each treatment plot and their weight was measured by sensitive electronic balance. The plants from

central two rows were harvested and sun dried in each treatment plot and was weighed as such for P TDM yield. The yield was then converted into kg ha⁻¹. Straw yield of each treatment plot was measured by subtracting grain yield from biological yield (TMD) of each respective plot and was converted into kg ha⁻¹.

Plant analysis

The wheat plant samples obtained from each experimental plot at harvest was processed for P concentration and uptake. A 0.5g oven dried plant sample was taken into 150 ml flask and treated with 10 ml concentrated nitric acid for 24 hrs. Then, 4 ml HClO₄ was added followed by gentle heating till the emergence of white fumes for obtaining 3 ml of the colorless aliquot. The aliquot was then transferred to 100ml volumetric flask and P was determined by spectrophotometer by using stander procedure of Richards [17]. The total P uptake by wheat was taken as a product of total P and total dry matter yield.

Statistical analysis

The replicated data collected on each parameter was subjected to analysis of variance (ANOVA) of two factor complete randomized block design according to the procedure of Steel and Torrie [18]. In case of significant results the data were further subjected to least significant difference (LSD) test at 5% level of probability for obtaining variations among treatments for various parameters.

Results and discussion

Plant height

Result for the effect of P, PSB and their interaction present in (Table 2). Upon analysis of various result demonstrated that P rates and PSB inoculation significantly affected plant height while their interactive effect was non-significant. It was exhibited by the result that PSB inoculation improved wheat plant height over un inoculated treatment. Application of P at the rate of 120 kg P₂O₅ ha⁻¹ produced taller plants (93 cm),

which was considerably taller than that of 90 kg ha⁻¹ where the lowest plant height was recorded at 60 kg ha⁻¹. These result demonstrated that PSB inoculation along with P fertilization can further enhance the

plant height of wheat and thus can pertain to increased production. Our results are in agreement to [19] who reported that P applied at the rate of 90 kg P₂O₅ ha⁻¹ had significantly increased plant height (93.63cm).

Table 2. Response of wheat plant height (cm) to P application rates and PSB inoculation

Phosphorus (kg P ₂ O ₅ ha ⁻¹)	Inoculation		Mean
	With PSB	Without PSB	
60	90.020	87.232	88.622 c
90	91.890	90.020	90.955.b
120	93.963	92.020	92.992 a
Mean	91.958 a	89.754 b	-----

Values with unlike letters in each row and column are significantly different ($\alpha = 0.05$) from each other LSD ($\alpha = 0.05$) for PSB= 0.84, P= 1.03 and PSB x P = NS

1000 Grain weight

Result about the effect of P and PSB and their interaction are mentioned in (Table 3). Upon analysis of various result indicated that P, PSB inoculation and their interaction significantly affected 1000 grains weight (g) of wheat. PSB inoculation improved wheat 1000 grain weight over un inoculated treatment. A 120 kg P₂O₅ ha⁻¹ produced 1000 grain (47.91 g) which was appreciably heavier than that of 90 kg ha⁻¹ whereas, the lighter grains was observed for 60 kg ha⁻¹. The interaction effect of PSB and P indicated that P applied @ 120 kg P₂O₅ ha⁻¹ along with PSB produced heavier 1000 grains which was statistically more than the rest of treatment

combinations while the lighters grains were noted for 60 kg P₂O₅ ha⁻¹ without PSB produced. P applied at the rate of 60 kg ha⁻¹ with PSB produced statistically higher than 1000 grain weight to that of 90 kg ha⁻¹ P without PSB and similar to 120 kg ha⁻¹ P without PSB. These result demonstrated that PSB inoculation can reduce or may minimize dependence on chemical P fertilizer up to 100% under exist soil and climatic conditions and may increase P use efficiency when applied with phosphatic fertilizers. These findings are in confirmation to those of [19] who reported a significant increase in 1000 grains weight (46.80g) over control at that 90 kg P₂O₅ ha⁻¹.

Table 3. Wheat 1000 grains weight as influenced by P application rates and PSB inoculation

Phosphorus (kg P ₂ O ₅ ha ⁻¹)	Inoculation		Mean
	With PSB	Without PSB	
60	45.563 c	42.240 e	43.902 c
90	49.197 b	43.430 de	46.313 b
120	51.213 a	44.610 cd	47.912 a
Mean	48.658 a	43.427 b	-----

Values with unlike letters in each row and column are significantly different ($\alpha = 0.05$) from each other LSD ($\alpha = 0.05$) for PSB= 0.84, P= 1.03 and PSB x P = NS

Grain yield (kg ha⁻¹)

Result regarding the response of wheat grain yield P, PSB and their interaction is presented in (Table 4). Upon analysis of various result indicated that P and PSB inoculation had

significant effect on grain yield while the effect of their interaction was non-significant. It was exhibited by the result that PSB inoculation improved wheat yield over un inoculated control treatment. Application

of phosphorous at the rate of 120 kg P₂O₅ ha⁻¹ produced higher grain yield (2615 kg ha⁻¹) which was appreciably higher than that of obtained (2541 kg ha⁻¹) at 90 kg ha⁻¹ where the lowest yield (2347 kg ha⁻¹) was observed for 60 kg ha⁻¹. The interaction P and PSB was non-significant in this case, however it indicated that P applied at the rate of 120 kg ha⁻¹ along with PSB produced relatively higher yield than the rest of treatment

combinations. These result also demonstrated that PSB inoculation can reduce or may minimize dependence on chemical P fertilizer up to some extent under exist soil and climatic conditions. According to Khan *et al.* [10] Wheat grain yield was appreciably improved (by 22%) from 2920 kg ha⁻¹ to 3560 kg ha⁻¹ with application of 90 kg P₂O₅ ha⁻¹ over control.

Table 4. Wheat grain yield in response to P application rates and PSB inoculation

Phosphorus (Kg P ₂ O ₅ ha ⁻¹)	Inoculation		Mean
	With PSB	Without PSB	
60	2434.3	2260.7	2347.5 c
90	2600.0	2482.7	2541.3 b
120	2676.7	2554.7	2615.7 a
Mean	2570.3 a	2432.7 b	-----

Values with unlike letters in each row and column are significantly different ($\alpha = 0.05$) from each other LSD ($\alpha = 0.05$) for PSB= 45.36, P= 78.57 and PSB x P = NS

Biological yield

Result regarding the effect of PSB, P and their interaction on biological yield is present in (Table 5). Upon analysis of various result indicated that P and PSB inoculation significantly affected biological yield of wheat while their interaction was observed non-significant. It was exhibited by the result that PSB inoculation improved wheat biological yield over un-inoculated treatment. Phosphorous applied at the rate of 120 kg P₂O₅ ha⁻¹ resulted maximum biological yield of 7129 kg ha⁻¹ which was

statistically similar to that of 90 kg P₂O₅ ha⁻¹ where the lowest was observed for 60 kg P₂O₅ ha⁻¹ which was similar to that obtained from plots treated with 90 while lower than that of 120 kg P₂O₅ ha⁻¹. These result demonstrated that PSB inoculation may improve BY of wheat can reduce or may minimize dependence on chemical P fertilizer up to a great extent under exist soil and climatic conditions. The increase in BY may be attributed to the fact that PSB increase nutrient and water use efficiency and P utilization.

Table 5. Biological yield of wheat as affected by P application rates and PSB inoculation

Phosphorus (kg P ₂ O ₅ ha ⁻¹)	Inoculation		Mean
	With PSB	Without PSB	
60	6294.0	5898.0	6096.0 b
90	6694.3	6154.0	6424.2 ab
120	7896.0	6362.0	7129.0 a
Mean	6961.4 a	6138.0 b	-----

Values with unlike letters in each row and column are statistically different ($\alpha = 0.05$) from each other LSD ($\alpha = 0.05$) for PSB= 651.03, P= 797 and PSB x P = NS

Straw yield

Upon analysis of various result indicated that PSB inoculation had significant effect while

P rates and P* PSB showed non-significant effect on straw yield kg ha⁻¹ of wheat (Table 6). The PSB inoculation significantly

enhanced wheat straw yield over un inoculated control treatment. Higher application of P (120 kg ha⁻¹) resulted straw yield to 4513 kg ha⁻¹ whereas the lowest (3748 kg ha⁻¹) was observed for 60 kg ha⁻¹. The interaction effect of PSB and P indicated that P applied at the rate of 120 kg ha⁻¹ along with PSB produced higher straw yield than

the rest of treatment combinations. The increase in straw yield over control in PSB inoculated plots may be due to taller plants. These result demonstrated that PSB inoculation along with P fertilization may improve straw yield of wheat comparatively better than sole application of P.

Table 6. Straw yield of wheat as affected P application rates and PSB inoculation

Phosphorus (Kg P ₂ O ₅ ha ⁻¹)	Inoculation		Mean
	With PSB	Without PSB	
60	3859	3637	3748.5
90	4094	3671	3882.8
120	5219	3807	4513.3
Mean	4391.1 a	3705.3 b	-----

Values with unlike letters in each row and column are significantly different ($\alpha = 0.05$) from each other LSD ($\alpha = 0.05$) for P= NS and PSB x P = NS

Wheat phosphorus concentration (%)

Analysis of variance exhibited that inoculation and P application rates significantly affected P concentration of wheat while non-significant effect was observed in case of their interaction (Table 7). Inoculation significantly improved wheat P concatenation by 25.3% over un-inoculated control. Similarly, P concentration was gradually increased with increasing level of P. However, the performance of 120 kg P₂O₅ ha⁻¹ was at par to 90 but significantly better

than 60 kg P₂O₅ ha⁻¹. PSB improve P concentration and uptake by plant by enhancing P solubalization through soil acidification and organic and mineral acids (nitric and sulphuric acids) production [20-22]. Jilani *et al.* [23] and Yazdani *et al.* [24] reported that PSB inoculation may reduce P application as external sources by 50%. Zaida *et al.* [25] reported significant improvement in P accessibility for plants through PSB inoculation.

Table 7. Wheat P concentration as affected by interaction of P and PSB

Phosphorus (Kg P ₂ O ₅ ha ⁻¹)	P concentration (%)		Mean
	With PSB	Without PSB	
60	0.211	0.161	0.185 b
90	0.235	0.177	0.206 ab
120	0.241	0.185	0.224a
Mean	0.228a	0.182b	----

Values with unlike letters in each row and column are significantly different ($\alpha=0.05$) different from each other. LSD for P = 0.0272, PSB 0.0180, P x PSB = NS

Phosphorus uptake (Kg ha⁻¹)

Results regarding P uptake by wheat are presented in (Table 8). Both PSB inoculation, P application and their interaction significantly affected P uptake in maize. Inoculation with PSB improved P uptake in

wheat by 26% over un-inoculated control. Similarly, P uptake significantly incased with increasing application rate of P. Highest uptake was recorded for plot treated with 120 Kg P₂O₅ ha⁻¹ (22.70 kg ha⁻¹) followed by 90 Kg P₂O₅ ha⁻¹ (20.34 kg ha⁻¹) while the lowest

was observed in plants obtained from plots treated with 60 Kg P₂O₅ ha⁻¹ (17.51 kg ha⁻¹). Interactively, P uptake increased with increasing P both in with and without PSB. However, sole application of 120 kg P₂O₅ ha⁻¹ resulted comparable uptake as 60 kg P₂O₅ ha⁻¹ with PSB treated plots. Furthermore, the performance of 90 kg P₂O₅ ha⁻¹ without PSB was statistically better than 120 kg P₂O₅ ha⁻¹ without PSB.

These findings demonstrated that PSB can increase P uptake both at lower and higher application of P. Additionally, PSB are capable of reducing the use of by 33%. Similar findings have been observed by

several researchers [23, 26]. This enhancement in soil and plant P availability by PSB could be attributed to production of different organic acids [27] by PSB, which acidify surrounding soils [28] as a result solubilize P from Ca₃ (PO₄)₂ in calcareous soils. Similar, converse relationship between pH and soluble phosphate was also reported previously by Illmer and Schinner [20]. These acids can also relocate adsorbed phosphate through ligand exchange reactions. Organic acids may also act as chelating agent for cations like Ca²⁺, Al³⁺ and Fe³⁺ and may increase plant P availability [22, 26, 28].

Table 8. Wheat P Uptake as affected by interaction of P and PSB

Phosphorus (Kg P ₂ O ₅ ha ⁻¹)	P uptake in (kg ha ⁻¹)		Mean
	With PSB	Without PSB	
60	20.8b	14.6e	17.51c
90	23.5a	17.3d	20.34b
120	24.7a	18.6bc	22.70a
Mean	22.9a	17.5b	-----

Values with unlike letters in each row and column are significantly different ($\alpha=0.05$) different from each other. LSD for P = 1.241, PSB = 1.803, P x PSB = 2.111

Conclusion and recommendations

Our findings imply that, PSB inoculation and P fertilization and their interaction significantly improved plant height, 1000 grains weight, grain yield and P uptake in wheat. With increasing rates of P from 60 to 120 kg P₂O₅ ha⁻¹ wheat yield and P uptake were improved, however this increase was more prominent in inoculated treatments than un-inoculated control. Thus, it can be deduce that PSB inoculation may reduce the use of chemical P fertilizer up to 45 kg ha⁻¹ but further studies should be conducted to confirm these results at a variety of soils on other cereals

Authors' contributions

Conceived and designed the experiments: M Adnan, S Fahad & IA Mian, Performed the experiments: A Hussain, Hajira & F Ullah, Analyzed the data: M Saeed, MW

Muhammad, M Roman, R Perveez & F Wahid, Contributed materials/ analysis/ tools: F Subhan, MA Raza, M Zamin, F Ullah, KU Rehman & S Andaleeb, Wrote the paper: M Adnan, A Hussain & S Iqbal.

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