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GROWTH AND YIELD OF WHEAT AS AFFECTED BY PHOSPHATE SOLUBILIZING BACTERIA AND PHOSPHATE FERTILIZER

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ABSTRACT

Use of biological alternatives to synthetic fertilizers has radically increased due to the high costs and environmental concerns associated with later ones. Phosphate solubilizing bacteria (PSB) have gained importance as supplements to phosphate (P) fertilizers for eco-friendly crop production. A field experiment was conducted to study the effect of different P fertilizer rates and PSB on the growth and yield of wheat. Treatments comprised of control (without P application), 25, 50 and 75 kg P_2O_5 ha^{-1}, and inoculation of PSB (*Bacillus polymyxa*) @ 25 mL kg^{-1} wheat seed. The experiment was laid-out in randomized complete block design (RCBD) with three replicates. The results showed that application of 75 kg P_2O_5 ha^{-1} resulted in maximum plant height (68.29 cm), number of spikelets per spike (18.67) and grain yield (8.51 kg plot^{-1}) and was statistically superior to all other treatments except 50 kg P_2O_5 ha^{-1}. The highest number of grains per spike (50), yield of main spike (2.49 g), grain yield plant^{-1} (2.93 g) and 100-grain weight (3.77 g) were also recorded in plots fertilized with 75 kg P_2O_5 ha^{-1}. Generally, higher bacterial count was observed at 0-15 cm soil depth compared to the lower soil depth (15-30 cm) after three weeks of sowing and at crop harvest. Furthermore, the yield and yield contributing attributes recorded from PSB inoculated plots were statistically identical to those of 25 kg P_2O_5 ha^{-1} plots. Hence it can be concluded that PSB inoculation can contribute to wheat yield potential to some extent in resource poor farming system.

Keywords: Bacteria, Inoculation, P-fertilizer, P-solubilization, Wheat

INTRODUCTION

After nitrogen, phosphorus (P) is the second most essential nutrient required by plant in large quantities (Shahzado *et al*., 2016). Phosphorus plays a vital role in several key physiological processes viz., photosynthesis, respiration, energy storage, cell division and cell enlargement (Marschner, 2012). Moreover, it is essential for seed formation and root development (Memon and Rashid, 2001). Phosphorus is also a structural component of nucleic acids, phospholipids, phosphorproteins and several co-enzymes (Bertrand *et al*., 2003). About 90% of Pakistani soils suffer from moderate to serve P deficiency (Alam *et al.*, 1994). To correct P deficiency, inorganic P fertilizers are generally applied to soils. Some part of P fertilizers is utilized by the plant and the remainder is converted into insoluble forms due to high pH and $CaCO_3$ activity of Pakistani soils (Memon and Rashid, 2001). A significant reduction in the use of P fertilizer can be achieved if in some way, soil insoluble P is solubilized and made available to crop plants (Islam and Hossain, 2012; Sial *et al*., 2017). Some bacterial species have been found capable of mineralizing and solubilizing soil organic and inorganic P (Islam and Hossain, 2012).

Phosphate solubilizing bacteria (PSB) as biofertilizers have been found effective in solubilizing the fixed soil P and applied phosphates resulting in higher crop yields (Panhwar *et al*., 2013). Seed or soil inoculation with PSB, particularly belonging to the genera *Pseudomonas* and *Bacillus*, have been known to improve plant uptake of nutrients and thereby increase the use efficiency of applied chemical fertilizers (Panhwar *et al*., 2014a & b). Several researchers have suggested that application of PSB improves plant P nutrition and increases the yield of cereals including wheat (Ashrafuzzaman *et al*., 2009; Islam and Hossain, 2012; Sial *et al*., 2015). Among the cereals, wheat is the principal source of diet, particularly in Asia and more specifically in South Asian region (FAO, 2018). In Pakistan, wheat is staple food crop and cultivated on more than 9.0 million ha area annually. It consumes more than 50% of P fertilizers used annually (GOP, 2017). Considerable amount of P fertilizer can be reduced by inoculation of soil with some suitable PSB isolates. The current study was carried-out to compare the effect of *Bacillus polymyxa* and chemical P fertilizer on yield and yield related traits of wheat.

MATERIALS AND METHODS

To compare the effect of chemical P fertilizer and bacterial inoculant (*Bacillus polymyxa*) on yield and yield related traits of wheat (*Triticum aestivum* L.), a field experiment was conducted at experimental farm of Nuclear Institute of Agriculture (NIA), Tando Jam. Five treatments viz., control (T_1), 25 kg P_2O_5 ha^{-1} (T_2), 50 kg P_2O_5 ha^{-1} (T_3), 75 kg P_2O_5 ha^{-1} (T_4) and PSB inoculum containing 14.2×10^3 cfu mL^{-1} applied @ 25 mL kg^{-1} wheat seed (T_5) were applied in a randomized complete block design (RCBD) with three replications.

Wheat variety Kiran-95 evolved at NIA, Tando Jam was the test crop. The bacterial species *Bacillus polymyxa*, previously isolated from the rhizospheric soil of wheat crop was re-cultured on commercial Pikovskaya's agar medium for further confirmation (Halo zones formation on agar medium). After confirmation, single colony was inoculated into 25 mL Pikovskaya's broth by the help of a wireloop and incubated at 30 ºC for three days. After incubation for three days, the broth was used for wheat seed inoculation @ 25 mL kg^{-1} seed used for sowing. The sowing of crop was done in the mid-November with the help of single row hand drill. The soil samples were collected at the depth of 0-15 and 15-30 cm for the bacterial counting in one gram of soil after three weeks of sowing and at crop harvest. At crop maturity, five plants were randomly selected and harvested for data recording. The date regarding plant height, number of spikelets spike^{-1}, number of grains spike^{-1}, yield of main spike, 100-grain weight, grain yield plant^{-1} were recorded and subjected to analysis of variance (ANOVA) technique using computer based statistical software. The least significant difference (LSD) test was applied to compare treatment means superiority at 5% probability level (Steel *et al*., 1997).

RESULTS AND DISCUSSION

The plant height was significantly affected ($P < 0.05$) by the various treatments (Table 1). The maximum plant height (68.29 cm) was observed at 75 kg P_2O_5 ha^{-1} and it was statistically at par with that observed at 50 kg P_2O_5 ha^{-1}, while control plots showed minimum plant height (52.95 cm) (Table 2). A non-significant difference in plant height was found between T_2 (25 kg P_2O_5 ha^{-1}) and T_5 (PSB inoculation containing 14.2×10^3 cfu mL^{-1} applied at 25mL kg^{-1} wheat seed). Increase in plant height at higher rates of P might be due to sufficient availability of P nutrient which promoted vegetative growth of plants (Abbas *et al*., 2016). Sarker *et al*. (2014) also observed an increase in plant height by the application of P fertilizer alone and in combination with PSB.

Table 1. Analysis of variance (ANOVA) for various traits of wheat crop

Source of variation	Df	Plant height	Spikelets spike^{-1}	Grains spike^{-1}	Yield of main spike	Grain yield plant^{-1}	100- grain weight	Grain yield plot^{-1}
Replicate	2	16.118	0.6	4.467	0.047	0.012	0.305	0.288
Treatment	4	108.187**	22.233**	288.433**	0.890**	1.081**	1.357**	7.572**
Error	12	6.286	0.933	2.883	0.011	0.025	0.116	0.440
$LSD_{0.05}$	---	4.72	1.82	3.20	0.20	0.30	0.64	1.25

** = $P < 0.01$; $LSD_{0.05}$ = Least significant difference at 5% probability level

Table 2. Effect of P fertilizer and P-solubilizing bacteria on yield and yield attributes of wheat

Treatments	Plant Height cm	Spikelets spike^{-1}	Grains spike^{-1}	Yield of main spike (g)	Grain yield plant^{-1} (g)	100-grain weight (g)	Grain yield plot per kg plot^{-1}
Control (T_1)	52.95 c	12.0 c	24.67 d	1.0 d	1.31 d	1.92 c	4.41 c
25 kg P_2O_5 ha^{-1} (T_2)	63.33 b	14.33 b	43.33 bc	1.88 c	2.12 c	2.93 b	6.7 b
50 kg P_2O_5 ha^{-1} (T_3)	66.87 ab	17.67 a	46.33 b	2.08 b	2.53 b	3.26 b	8.01 a
75 kg P_2O_5 ha^{-1} (T_4)	68.29 a	18.67 a	50.0 a	2.49 a	2.93 a	3.77 a	8.51 a
PSB (25 mL kg^{-1} seed) (T_5)	63.55 b	14.33 b	43.0 c	1.88 c	2.14 c	2.98 b	6.71 b
$LSD_{0.05}$	4.72	1.82	3.20	0.20	0.30	0.64	1.25

The data values are means of three replications and same letters within each column are not significantly different at 5% probability level. PSB = Phosphate solubilizing bacteria

Table 3. Bacterial count at different depths after sowing and harvesting of the crop

Treatments	Bacterial count after three weeks of sowing (cfu g^{-1} soil)		Bacterial count at crop harvest (cfu g^{-1} soil)	
	0-15 cm	**15-30 cm**	**0-15 cm**	**15-30 cm**
Control (T_1)	13×10^2 c	10.6×10^2 d	13.67×10^2 c	99×10^2 c
25 kg P_2O_5 ha^{-1} (T_2)	16.2×10^3 b	13.2×10^3 c	16.43×10^3 b	12.3×10^3 b
50 kg P_2O_5 ha^{-1} (T_3)	17.2×10^4 a	15.23×10^4 b	18.33×10^4 a	13.6×10^4 a
75 kg P_2O_5 ha^{-1} (T_4)	17.5×10^4 a	16.2×10^4 a	18.67×10^4 a	13.6×10^4 a
PSB (25 mL kg^{-1} seed) (T_5)	16×10^3 b	13.3×10^3 c	16.23×10^3 b	12.3×10^3 b
LSD	4.67	6.25	3.32	10.40

The data values are means of three replications and same letters within each column are not significantly different at 5% probability level. PSB = Phosphate solubilizing bacteria

The spikelets count $spike^{-1}$ of wheat also varied significantly ($P < 0.05$) among the treatments. The highest number of spikelets $spike^{-1}$ (17.67 and 18.67) were recorded at 50 and 75 kg P_2O_5 ha^{-1}, respectively whereas wheat plants in control plots showed lowest count of spikelets $spike^{-1}$ (Table 2). A non-significant difference in spikelets count $spike^{-1}$ was noted between T_2 and T_5. An increasing trend in number of spikelets per spike with successive increments in P indicates the usefulness of phosphorus in seed formation and grain filling (Abbas *et al*., 2017; Irfan *et al*., 2018). Moreover, the inoculation of beneficial microbes improved the plant growth and plant yield related traits (Panhwar *et al.,* 2014a).

The data given in table 2 showed that highest number of grains $spike^{-1}$ (50) was observed at 75 kg P_2O_5 ha^{-1}, followed by 46.33 grains $spike^{-1}$ recorded at 50 kg P_2O_5 ha^{-1}. The control plots showed the lowest number of grains $spike^{-1}$ 24.67. Similar results were revealed by Sarker *et al*., (2014).

The yield of main spike was significantly ($P < 0.05$) affected by the application of various doses of P fertilizer and PSB inoculum treatment. The highest yield of main spike (2.49 g) was recorded in plots fertilized at 75 kg P_2O_5 ha^{-1} while the lowest yield of main spike (1.0 g) was found in control treatments (Table 2). Statistically similar yield of main spike was noted in T_2 and T_5, which showed that PSB inoculant had the potential to contribute to crop yield by solubilizing the soil indigenous P and making it available for plant uptake. Similar findings were reported by Sarker *et al*. (2014) and Islam and Hossain (2012).

The total grain yield per plant comprised of the whole grain collected from all the tillers of a wheat plant at harvest and varied considerably under the influence of various treatments (Table 1). The grain yield per plant significantly increased with the increase of P rates. The highest grain yield per plant 2.93 g was recorded in 75 kg P_2O_5 ha^{-1} treatment. Treatments viz., T_2 (25 kg P_2O_5 ha^{-1}) and T_5 (PSB inoculum) produced statistically similar grain yield $plant^{-1}$. Control plots observed the lowest grain yield per plant (Table 2). The application of P alone and in combination with PSB improved the wheat crop yield and yield parameters positively (Panhwar *et al.,* 2013; Sarker *et al*., 2014).

Hundred grain weight varied between the highest 3.77g at 75 kg P_2O_5 ha^{-1} and the lowest 1.92 g values at control (Table 2). The PSB inoculated plots observed 100-grain weight statistically at par with those of 25 and 50 kg P_2O_5 ha^{-1}. Formation of healthier grains at higher P application rates might be due abundant supply of photosynthates available during grain formation and grain filling stage of the crop (Abbas *et al*., 2016 & 2017; Irfan *et al*., 2018). Similarly, the inoculation of PSB may increase P solubilization and its availability for plant uptake, resulting in healthier grains than uninoculated control (Panhwar *et al.*, 2014b).

The grain yield per plot comprised of grain harvested from a plot of 4 ×4 m^2. Application of 50 and 75 kg P_2O_5 ha^{-1} produced comparable grain yield per plot 8.01 and 8.51 kg $plot^{-1}$. Statistically similar grain yield was recorded from T_2 and T_5 treatments while control treatment showed the lowest grain yield per plot 4.41 kg $plot^{-1}$ (Table 2). Since grain yield is the outcome of various yield components like spikelets $spike^{-1}$, grains $spike^{-1}$, 1000-grain weight etc, thus the higher values of these yield components at 50 and 75 P_2O_5 ha^{-1} finally translated into higher grain yield (Abbas *et al*., 2016 & 2017). Sial *et al*. (2015) have also reported the positive effects of P-solubilizing bacteria and fungi on the grain yield of wheat crop. Microbial application with and without P chemical fertilizer resulted in an increased yield of several crops such as cereals, legumes, fibers, vegetables, oil crops and other crops (Kundu *et al*., 2009).

The bacterial count also varied among the treatments after three week of sowing and at harvest (Table 3). Generally higher bacterial count was observed at 0-15 cm soil depth compared to the lower soil depth 15-30 cm at both recording times. After three weeks of sowing, the bacterial count varied between 13×10^2 (control) and 17.5×10^5 cfu g^{-1} soil 75 kg P_2O_5 ha^{-1} at 0-15 cm soil depth, and it ranged between 10.6×10^2 cfu g^{-1} soil (control) 16.2×10^4 (75 kg P_2O_5 ha^{-1}) at 15-30 cm. The bacterial count ranged from 18.67×10^4 cfu g^{-1} soil (75 kg P_2O_5 ha^{-1}) and 13.67×10^2 (control) at 0-15 cm soil depth and from 13.6×10^4 (75 kg P_2O_5 ha^{-1}) and 99×10^2 cfu g^{-1} soil (control) at 15-30 cm when recorded at the harvest of crop. Treatments viz., T_2 and T_5 recorded statistically similar bacterial counts at both depths as well as recording intervals while control plots showed significantly lowest bacterial counts. The PSB wheat seed inoculation does not showed higher bacterial count compared to higher P treatments (50 and 75 kg P_2O_5 ha^{-1}). This might be due to less inoculum or either due to microbes' endophytic character. The higher bacterial count found at the upper soil surface might be due to soil and plant roots relationship (Panhwar *et al.*, 2014a). Similar results were revealed by Sial *et al*. (2015).

CONCLUSION

Our study concluded that wheat growth and yield was significantly affected by the application of various doses of P fertilizer and PSB inoculum treatments. The optimum dose of 75 kg P_2O_5 ha^{-1} showed the better response for the wheat growth and yield However, PSB inoculation affected the wheat yield and yield contributing attributes and their results were identical to those of 25 kg P_2O_5 ha^{-1}. Hence it can be concluded that PSB inoculation can contribute to wheat yield potential to some extent in resource poor farming system.

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