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BIOPRIMING OF WHEAT SEEDS WITH RHIZOBACTERIA CONTAINING ACC-DEAMINASE AND PHOSPHORATE SOLUBILIZING ACTIVITIES INCREASES WHEAT GROWTH AND YIELD UNDER PHOSPHORUS DEFICIENCY

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ABSTRACT

The performance of two *Pseudomonas fluorescens* strains (biotype F and G) that differ in 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity and phosphorus (P) solubilization was evaluated for their effect on wheat growth and yield under P deficiency stress. The field experiment was conducted in a P-deficient soil by employing two-factor factorial randomized complete block design. Factor A comprised of rhizobacterial biopriming of wheat seeds (seed inoculation with two rhizobacterial strains, viz. B_1: *Pseudomonas fluorescens* biotype G and B_2: *Pseudomonas fluorescens* biotype F and a control treatment, i.e. B_0: with no bacterial inoculation of seed). Factor B included two P fertilizer levels (P_0: 0% and P_1: 100% of the recommended dose). The rhizobacterial biopriming significantly increased most of the growth traits and yield of wheat, either alone or in interaction with P-nutrition. Rhizobacterial seed inoculation was effective at both P-levels and *P. fluorescens* biotype F and G were more effective at adequate and deficient P-levels, respectively. The study concluded that rhizobacterial biopriming is a promising tool in enhancing wheat yield under P deficiency stress.

Keywords: ACC-deaminase, phosphorus, pseudomonads, *Triticum aestivum* L., wheat.

INTRODUCTION

Phosphorus (P) is considered as an essential plant nutrient. Its deficiency adversely affects sustainable crop production (Vance *et al.*, 2003). P is an important non-renewable global resource and world P reserves are rapidly being mined (Lambers *et al*., 2006). In Pakistan, the majority of soils are poor in their P status and contain less than 10 mg kg^{-1} Olsen's P (Memon, 2005). For this reason, P nutrition becomes highly indispensable for crop production. Moreover,

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the P fertilizer efficiency is not more than 25% in Pakistan (Ahmad and Rashid, 2003). Wheat is highly susceptible to P deficiency stress and hence almost 50% of total phosphatic fertilizers are applied to wheat in Pakistan (Vishandas *et al*., 2006). The importance and role of plant growth promoting rhizobacteria (PGPR) for improved plant growth and better crop yields is now well recognized (Arshad *et al*., 2008; Nadeem *et al*., 2010; Shahzad *et al*., 2010; Ramesh *et al*., 2014; Saxena *et al*., 2014). These PGPR promote plant growth by synthesizing and secreting indole-3-acetic acid (Saleem *et al*., 2007) or by facilitating nutrient uptake and increasing plant tolerance to various stresses (Zahir *et al*., 2009; Shahzad *et al*., 2010). Plants produce ethylene as an important growth hormone to mediate a wide array of responses related to the growth and development of plants (Arshad and Frankenberger, 2002). Plant ethylene synthesis is positively related with their 1-Aminocyclopropane-1-Carboxylic Acid (ACC) concentration (Saleem *et al*., 2007; Arshad *et al*., 2008).

The ACC-deaminase regulates ethylene production which inhibits plant growth by metabolizing root-exudates-ACC into α-ketobutyric acid and ammonia (Saleem *et al*., 2007; Arshad *et al*., 2008; Shahzad *et al*., 2010). Shaharoona *et al*. (2008) used two ACC-deaminase rhizobacteria, i.e. *Pseudomonas fluorescens* and *P. fluorescens* biotype F to observe their impact on growth traits, crop yield, and nutrient-use-efficiency of wheat at different NPK levels (0%, 25%, 50%, 75% and 100% of recommended doses) and concluded that these *Pseudomonas* species have potential to improve plant growth and with lower fertilizer input, when integrated with balanced doses of chemical fertilizers normally less than recommended levels (Shaharoona *et al*., 2008). There is a dire need to device suitable strategies to cope both with the increasing shortage and elevating prices of P fertilizers and for decreasing the use of chemical fertilizers for environmental promotion. Phosphate solubilizing rhizobacterial inoculants increase P uptake under P deficiency stress and hence increase crop yield (Rodriguez and Fraga, 1999; Ramehs *et al*., 2014; Saxena *et al*., 2014). The use of ACC-deaminase rhizobacteria, with P-solubilizing activity, as seed inoculants may offer promising results in terms of sustained yields and low phosphatic fertilizer requirements of wheat grown under phosphorus deficient soils. This field study evaluates the response of wheat to biopriming with ACC-deaminase rhizobacteria under phosphorus deficiency stress.

MATERIALS AND METHODS

The field experiment was conducted on a heavy soil (40% clay), alkaline in reaction (pH: 8.1), non-saline (EC: 1.3 dS m^{-1}), calcareous in nature ($CaCO_3$: 15.7%), low in organic matter (0.58%) and deficient in both $NaHCO_3$-extractable P (5.9 mg kg^{-1}) and NH_4OAc-extractable K (105 mg kg^{-1}). Two pre-isolated strains of ACC-Deaminase rhizobacteria (*Pseudomonas fluorescens* biotype G and *Pseudomonas fluorescens* biotype F) were involved in this experiment (Shaharoona *et al*., 2007). Both these strains had positive phosphate solubilizing activity, however, the ACC-deaminase activity of P. *fluorescens* biotype F

(ACC73) was more than that of P. *fluorescens* biotype G (ACC3), i.e. 393±07 and 207±13 n mol NH_3/g biomass/h, respectively. As suggested by Shahroona *et al*. (2007), "the inocula were developed by raising the rhizobacterial strains (ACC73 and N3) in 250-mL flasks containing the DF minimal salt medium with ACC as substrate (N source). The medium was incubated at 28±1^oC for 48 h in an orbital shaking incubator at 100 rev/min. The optical density of each inoculum was measured and a uniform population of rhizobacteria (10^8-10^9 CFU/mL) was maintained at the time of seed inoculation. Peat was ground to pass through a 2-mm 40-mesh and autoclaved at 121^oC for 20 min. A 100-mL inoculum of the selected rhizobacteria was mixed with 1.0 kg of peat and incubated for 24 h at 28±1^oC before being used for seed coating, with a seed to peat ratio of 1:1 (w/w). Inoculated seeds were placed overnight for air-drying in the laboratory". At proper moisture condition, the inoculated and uninoculated (treated with sterilized peat) wheat seeds (*cv*. Imdad) were drilled in the field @ 50 kg ha^{-1}. The study followed a two-factor factorial randomized complete block design. Factor A comprised of ACC-deaminase rhizobacterial inoculation of wheat seeds (seed inoculation with two rhizobacterial strains, viz. B_1: *Pseudomonas fluorescens* biotype G and B_2: *Pseudomonas fluorescens* biotype F and a control treatment, i.e. B_0: with no bacterial inoculation of seed). Factor B included two P fertilizer levels (P_0: 0% and P_1: 100% of the recommended dose). Each treatment was replicated four times. There were 24 experimental units of 15 m^2 (5 m × 3 m). Recommended doses of P and K, i.e. 85 kg P_2O_5 and 50 kg K_2O ha^{-1} were applied at sowing using Diammonium Phosphate, DAP (46% P_2O_5 and 18% N) and potassium sulphate, SOP (50% K). Moreover, a blanket dose of 170 kg nitrogen ha^{-1} was also applied, ½ at sowing and ½ at tillering, using urea (46% N). At maturity (151 days after sowing), the data for growth parameters of wheat were recorded from ten randomly selected plants. The grain and straw yields were initially determined on square meter basis and then converted into kg ha^{-1}.The statistical analysis was performed by using computer software Statistix® for Windows version 8.1 (Analytical Software ©). Mean separation was done using Tukey's honestly significant difference ($HSD_{0.05}$).

RESULTS

The two sources of variance behaved independently in enhancing the plant height of wheat.The data further elucidated that the rhizobacterial biopriming did not affect the plant height of wheat and both the strains of rhizobacteria did not improve the plant height as against no inoculation (Table1). Although, rhizobacterial biopriming and the P-levels significantly differed, their 'interaction' was non-significant. Rhizobacterial inoculation enhanced the number of tillers per plant up to 18% as compared to control, however, there was no significant difference between the two strains of rhizobacteria (Table 1). Moreover, the P-levels were significantly different from each other and the application of recommended P (85 kg P_2O_5 ha^{-1}) increased the number of tillers per plant of wheat up to 34% more as compared to control (Table 1). Moreover, the two sources of variance behaved independently in enhancing the spike length of

wheat. Further examination of data reflected that the rhizobacterial inoculation did not affect the spike length of wheat and both the strains of rhizobacteria did not improve the spike length of wheat as against no inoculation (Table 2). Nonetheless, both P-levels were significantly different from each other. The application of recommended P (85 kg P_2O_5 ha^{-1}) increased the spike length of wheat up to 28% as compared to control (Table 2). Likewise, rhizobacterial biopriming and the P-levels significantly differed in affecting number of seeds per spike. However, their interaction was non-significant. The rhizobacterial inoculation enhanced the number of seeds per spike of wheat up to 7% as compared to control. Nonetheless, there was no significant difference between the two strains of rhizobacteria (Table 2). Moreover, the P-levels were significantly different from each other and the application of recommended P (85 kg P_2O_5 ha^{-1}) increased the number of seeds per spike of wheat up to 8.3% more as compared to control (Table 2). The interaction between rhizobacterial inoculation and P-levels increased the number of seed per plant and seed index of wheat up to 67% and 88% as compared to control (no P, no rhizobacterial inoculation). The rhizobacteria *Pseudomonas flourescens* biotype G and biotype F produced maximum number of seeds per plant and seed index under deficient and recommended P regimes, respectively (Table 3). The data illustrated that the interaction between rhizobacterial inoculation and P-levels increased the grain and straw yield of wheat up to 80% and 47% as compared to control (no P, no rhizobacterial inoculation), respectively. The rhizobacteria *Pseudomonas flourescens* biotype G and biotype F produced maximum grain and straw yield under deficient and recommended P conditions, respectively (Table 4). The relationship among various plant growth traits and the grain yield of wheat were determined through Pearson's correlation analysis (Table 5). The analysis results revealed that the grain yield of wheat had highly significant positive relationship with height of plant (r=0.72***), tillers/plant (r=0.83***), length of spike (r=0.85***), seeds/spike (r=0.80***), seeds/plant (r=0.95***) and seed index (r=0.90***).

Table 1. Effect of ACC-deaminase rhizobacterial biopriming on plant height and number of tillers per plant of wheat under deficient & adequate P levels.

Seed inoculation	Plant height (cm)			Number of tillers $plant^{-1}$		
	P_0[†]	P_{85}	Mean	P_0[†]	P_{85}	Mean
No inoculation	72.60	89.10	80.85	1.65	2.42	2.04B
Pseudomonas flourescens biotype G	74.25	92.65	83.45	2.20	2.60	2.40A
Pseudomonas flourescens biotype F	75.95	91.60	83.77	2.00	2.82	2.41A
Mean	74.27B	91.12A		1.95B	2.62A	
Source of variance	strain	P rate	S × P	strain	P rate	S × P
Tukey's HSD, alpha 0.05	NS[≠]	6.77	NS	0.2673	0.1791	NS

[†] P_0 and P_{85} refer to phosphorus rates of 0 and 85 kg ha^{-1}, respectively. [≠] Non-significant
Means followed by similar letters are statistically alike at alpha 0.05.

Table 2. Effect of ACC-deaminase rhizobacterial biopriming on spike length and number of seeds per spike of wheat under deficient and adequate P levels.

Seed inoculation	Spike length (cm)			Number of seeds spike^{-1}		
	P_0[†]	P_{85}	Mean	P_0[†]	P_{85}	Mean
No inoculation	8.75	11.50	10.12	43.00	47.00	44.74B
Pseudomonas flourescens biotype G	10.00	12.25	11.12	46.25	49.75	48.00A
Pseudomonas flourescens biotype F	9.50	12.50	11.00	45.75	49.50	47.96A
Mean	9.42B	12.08A		48.75A	45.00B	
Source of variance	strain	P rate	S×P	strain	P rate	S × P
Tukey's HSD, alpha 0.05	NS[≠]	0.8105	NS	3.015	2.0205	NS*

[†] P_0 and P_{85} refer to phosphorus rates of 0 and 85 kg ha^{-1}, respectively. [≠] Non-significant
Means followed by similar letters are statistically alike at alpha 0.05.

Table 3. Effect of ACC-deaminase rhizobacterial biopriming on number of seeds per plant and 100 grain weight of wheat under deficient and adequate P levels.

Seed inoculation	Number of seeds plant^{-1}			100 grain weight (g)		
	P_0[†]	P_{85}	Mean	P_0[†]	P_{85}	Mean
No inoculation	109.7d	154.5b	132.13	2.27e	3.17bc	2.72
Pseudomonas flourescens biotype G	125.2c	160.0b	142.63	2.85cd	3.65b	3.25
Pseudomonas flourescens biotype F	118.7cd	183.5a	151.12	2.67de	4.27a	3.47
Mean	117.9	166.0		2.60	3.70	
Source of variance	strain	P rate	S × P	strain	P rate	S × P
Tukey's HSD, alpha 0.05	---	---	14.624	---	---	0.4831

[†] P_0 and P_{85} refer to phosphorus rates of 0 and 85 kg ha^{-1}, respectively. [≠] Non-significant
Means followed by similar letters are statistically alike at alpha 0.05.

Table 4. Effect of ACC-deaminase rhizobacterial biopriming on grain and straw yield of wheat under deficient and adequate P levels.

Seed inoculation	Grain yield (kg ha^{-1})			Straw yield (kg ha^{-1})		
	P_0[†]	P_{85}	Mean	P_0[†]	P_{85}	Mean
No inoculation	1942d	3197b	2570	3000e	3976bc	3488
Pseudomonas flourescens biotype G	2682c	3521a	3102	3794c	4144b	3969
Pseudomonas flourescens biotype F	2474c	3743a	3108	3460d	4425a	3942
Mean	2366	3487		3418b	4182a	
Source of variance	strain	P rate	S × P	strain	P rate	S × P
Tukey's HSD, alpha 0.05	---	---	280.58	---	---	237.26

[†] P_0 and P_{85}refer to phosphorus rates of 0 and 85 kg ha^{-1}, respectively. [≠] Non-significant
Means followed by similar letters are statistically alike at alpha 0.05.

Table 5. Relationship between various plant growth traits and wheat grain yield as affected by rhizobacterial biopriming under P-deficiency stress.

Growth parameter	r-value	p-value
Plant height	0.7187	0.0001
Number of tillers per plant	0.8286	0.0000
Spike length	0.8465	0.0000
Number of seeds per spike	0.7993	0.0000
Number of seeds per plant	0.9508	0.0000
Seed index	0.9015	0.0000

DISCUSSION

The results of the present field study (Table1 to 5) endorsed the beneficial effects of the inoculation of wheat seed by selected ACC-deaminse rhizobacterial strains in improving most of the growth traits and yield of wheat, both under phosphorus deficient and adequate conditions, i.e. 0 and 85 kg P_2O_5 ha^{-1}. These results again signify the importance of the inclusion of these ACC-deaminase rhizobacteria, with P-solubilizing activity, in plant nutrition programs to support chemical fertilization in low input sustainable agriculture, as already established in the literature. Plant growth improvement, yield enhancement and environmental promotion by rhizobacteria is now well recognized (Shaharoona *et al*., 2006a; Shaharoona *et al*., 2006b; Arshad *et al*., 2007; Saleem *et al*., 2007; Shaharoona *et al*., 2007; Zahir *et al*., 2009; Nadeem *et al*., 2010; Shahzad *et al*., 2010). These rhizobacteria fix atmospheric nitrogen (Shahzad *et al*., 2010), solubilize nutrients (Saleem *et al*., 2007), produce siderophores, synthesize various enzymes and phytohormones, such as auxins, cytokinins and gibberellins to modulate plant growth and development at various stages (Zahir *et al*., 2009; Nadeem *et al*., 2010; Shahzad *et al*., 2010). ACC-deaminase microbial inoculants sustain plant growth and development under various stresses by maintaining stress-induced ethylene production in the roots of host plants by increasing root length (Saleem *et al*., 2007). The growth promotion in maize plants, i.e. length and weight of the roots and shoots, grown under different environments, by 21 selected rhizobacterial isolates was due to their ability to hydrolyse ACC and decrease endogenous ethylene synthesis. Consequently, the potential inhibitory effects of higher ethylene concentration were eliminated (Shaharoona *et al*., 2003). Later on, Shaharoona *et al*. (2006c) observed that maize seed inoculation significantly increased various plant growth traits and biomass production of maize even under adequate N supply. Jaleel *et al*. (2007) noted that *P. fluorescens* improved leaf number, height and biomass of *Catharanthus roseus* under drought stress. Shaharoona *et al*. (2007) observed increased root elongation, root weight, tillers/pot, seed index and yield of wheat grain and straw in response to rhizobaterial inoculants under both pot and field conditions.

Shahroona *et al.* (2007) also reported that, "*Pseudomonas fluorescens* (ACC50), which exhibited a relatively high in vitro ACC-deaminase activity, chitinase activity, auxin production, and P solubilization and more intensive root colonization, was the most efficient isolate under the field conditions". Later on, Arshad *et al*. (2008) noted that ACC-deaminase rhizobacterial inoculants significantly decreased peas bad effects of drought stress on growth and yield. Shaharoona *et al*. (2008) studied two ACC-deaminase rhizobacteria, *Pseudomonas fluorescens* and *P. fluorescens* biotype F for their impact on growth, yield, and nutrient-use-efficiency of wheat under different NPK levels (0%, 25%, 50%, 75%, and 100% of recommended doses). They advocated that the integrated use of chemical fertilizers and seed priming with ACC-deaminase phosphate solubilizing rhizobacteria have the potential to improve plant growth and save costly fertilizer input (Shaharoona *et al*., 2008). Shahzad *et al*. (2010) reported improvement in root and shoot growth, nodulation and grain yield of chickpea by rhizobacterial inoculation under various experimental conditions. Nadeem *et al*. (2010) observed that the rhizobacteria inoculated wheat plants under salinity stress had more K^+ to Na^+ ratios and possessed more water and chlorophyll, although had low proline as against control. The benefits of rhizobacterial biopriming of wheat seed in shape of growth promotion and yield enhancement was highly likely due to their positive phosphate solubilization activity and ACC-deaminase activity, as reported by Shaharoona *et al*. (2007). The *Pseudomonas fluorescens* biotype F performed better under adequate P conditions because of its comparatively greater ACC-deaminase activity (393±7 n mol NH3/g biomass/h) as compared to *Pseudomonas fluorescens* biotype G (207 ± 13 n mol NH3/g biomass/h). Many other workers also supported this premise (Shaharoona *et al*., 2003; Shaharoona *et al*., 2006c; Saleem *et al*., 2007). Earlier too, it has been documented that the phosphate solubilizing rhizobacteria increase P-uptake under P deficiency stress and enhance crop yield (Rodriguez and Fraga, 1999). Some recent studies on wheat (Ramesh *et al*., 2014; Saxena *et al*., 2014) and soybean (Ramesh *et al*., 2014) also support these results endorsing the beneficial effects of these P-solubilizing microorganisms.

CONCLUSION

The above results endorsed the benefits of phosphate solubilizing rhizobacterial inoculation of wheat seed under both low and high input sustainable agriculture systems. The *Pseudomonas fluorescens* biotype F is especially suitable under low phosphorus input agriculture system while *Pseudomonas fluorescens* biotype G is better for high phosphorus input agriculture system. Further research is warranted to validate these results.

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