



ACC-Deaminase, Phosphate-Solubilizing *Pseudomonas Fluorescens* Enhance Cotton Growth and Yield Under Phosphorus Deficiency Stress

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Abstract: This field study reports the phosphorus (P) deficiency tolerance of cotton (*Gossypium hirsutum* L.) as affected by seed inoculation with phosphate-solubilizing *Pseudomonas fluorescens* containing ACC-deaminase activity under P deficiency stress. The experiment followed a randomized complete block design with split plot arrangement involving two factors and three repeats. Factor A comprised of two P levels, i.e. 0 (control) and 75 kg P ha⁻¹ (recommended). Factor B included three rhizobacterial treatments, i.e. control (no rhizobacterial seed inoculation), seed inoculation with *P. fluorescens* having single activity of ACC-deaminase and seed inoculation with *P. fluorescens* biotype F with ACC-deaminase and P-solubilizing activities both. The soil involved was heavy in texture, non-saline, alkaline-calcareous and low in organic matter and NaHCO₃-extractable P. Recommended P fertilization significantly ($p < 0.05$ to 0.001) increased various parameters of cotton, i.e. plant height (10.5%), sympodial branches (52.2%), stem diameter (37.4%), number of bolls (26.2%), average boll weight (54%), seed cotton yield (36.3%) and P concentration (69.7). The ACC-deaminase rhizobacterial strains of *P. fluorescens*, with or without phosphate-solubilizing activity, also increased various traits of cotton plants. This increase was in a range of 13.4% to 22.6% in case of *Pseudomonas fluorescens* without phosphate-solubilizing activity and 21% to 35.6% (1.4 to 2.0 fold more) in case of *Pseudomonas fluorescens* with phosphate-solubilizing activity. The significant ($p < 0.01$) interaction of recommended dose of phosphorus and *Pseudomonas fluorescens* with phosphate-solubilizing activity resulted in maximum increase in some plant traits of cotton, i.e. plant height (1.3 fold), average boll weight (2.5 fold), seed cotton yield (2.0 fold) and phosphorus concentration (2.4 fold). Both the rhizobacteria increased various plant traits of cotton irrespective of the phosphorus application rate. At both the level of phosphorus, *Pseudomonas fluorescens* with ACC-deaminase and phosphate-solubilizing activities was more effective than its counterpart with only phosphate-solubilizing activity only. *Pseudomonas fluorescens* with dual activities was more effective in increasing plant height and number of sympodial branches at phosphorus deficient level while rest of the plant traits at adequate phosphorus level. The study concluded that *Pseudomonas fluorescens* with dual activities of ACC-deaminase and phosphate solubilization can play significant role as a bio-inoculant to enhance cotton growth and yield under both the phosphorus deficient and adequate conditions.

Keywords: Phosphorus, wheat, rhizobacteria, ACC-deaminase, phosphate-solubilization

1. INTRODUCTION

Phosphorus (P) deficiency restricts plant growth and limits crop productivity (Memon, 1996; Vance *et al.*, 2003). Pakistani soils are frequently poor in P. i.e. $< 10 \text{ mg kg}^{-1}$ Olsen-P (Memon, 1996) which makes P fertilization of crops highly indispensable for acquiring good yields. Despite this fact, phosphatic fertilizers are highly unaffordable input in Pakistan (Zia-ul-hassan *et al.*, 2015). The situation becomes worst due to low P-use-efficiency of plants (20-25% only) (Vishandas *et al.*, 2006). Hence, it is very necessary to explore workable alternates of chemical P fertilization.

Rhizobacteria promotes plant growth under nutrient deficiency stress, by enhanced nutrient solubilization and strengthen root system by controlling endogenous ethylene production through the enzyme 1-aminocyclopropane-1-carboxylic acid (Saleem *et al.*, 2007; Glick, 2012). Soil microbes play vital role in P solubilization like rhizobacteria containing dual

activities of ACC-deaminase and phosphate solubilization (Saleem *et al.*, 2007) by using these microbes to reduce the much expensive phosphatic fertilizers. Some microorganisms are beneficial for plants and commonly known to as rhizobacteria. These rhizobacteria enhance the solubilization of insoluble mineral P complexes and make the precipitated P forms available for plant absorption (Rengel and Marschner, 2005) by promoting healthy root system through phytohormone production (Maynard and Hochmuth, 2007), for example improvement in surface area and number of effective root hair (Biswas *et al.*, 2000). The phosphate solubilizing rhizobacteria also release organic acids to solubilize inorganic P, such as citric and gluconic acids (Rodriguez *et al.*, 2004).

Rhizobacterial seed inoculation decreases stress ethylene and hence improve plant growth under various types of biotic and abiotic stresses. The ACC-deaminase rhizobacteria can alter gene expression of plants to enhance their growth. The bacteria also produce

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enzymes that metabolize plant growth regulators thereby increasing the uptake of mineral nutrients and enhancing the plant growth by way of an efficient root system (Saleem *et al.*, 2007). The P-solubilizing rhizobacteria are also reported to augment wheat growth and yield through wheat seed coating. These are, therefore, being utilized to develop suitable biofertilizers in Pakistan to minimize the high cost P fertilizers (Malik *et al.*, 2012). Experiments with P-solubilizing rhizobacteria have shown yield increase in grain crops, e.g. rice and wheat (Afzal and Asghari, 2008; Ozturk *et al.*, 2003). The inoculation with phosphobacterium had significant positive effect on yield parameters in wheat crop. These bacteria also release different types of organic acids which are helpful in lowering the pH in rhizosphere (Afzal and Asghari, 2008).

Keeping the above discussion in mind, it may be hypothesized that these dual purpose rhizobacteria (with both ACC-deaminase and P-solubilizing abilities) can potentially decrease P fertilizer use and enhance cotton growth and yield under P deficiency stress by supplementing plant P nutrition. This field study was conducted to evaluate the P-deficiency tolerance of cotton (*Gossypium hirsutum* L.) under P deficiency stress as affected seed inoculation with rhizobacteria (*Pseudomonas fluorescens*) containing ACC-deaminase or phosphate-solubilizing activities or with dual activities.

2. MATERIALS AND METHODS

This study was conducted in a two-factor randomized complete block split-plot design involving three repeats. Factor A comprised of two P doses, i.e. $P_1 = \text{No P fertilizer (control)}$ and $P_2 = 75 \text{ kg P ha}^{-1}$ (100% recommended P fertilizer). Factor B included three treatments involving different rhizobacterial strains, i.e. control (no rhizobacterial seed inoculation), seed inoculation with *P. fluorescens* having only ACC-deaminase activity and seed inoculation with *P. fluorescens* biotype F containing dual activities, i.e. ACC-deaminase and P-solubilization. The size of each experimental unit was $3.0 \text{ m} \times 5.0 \text{ m} = 15.0 \text{ m}^2$. Pure seed of recently developed cotton variety Hari Dost was obtained from the Cotton Botanist, Agriculture Research Institute (ARI), Tandojam. Sowing was done by maintaining a plant spacing of 1.0 ft. The rows were kept 2.5 ft. apart. The crop was raised by following the recommended production technology. Nitrogen (N), P and potassium (K) were supplied through urea (46% N), diammonium phosphate, DAP (18% N and 46% P_2O_5) and potassium sulphate, SOP (50% K_2O), respectively. The crop received blanket dose of these fertilizers at 150 kg N and 60 kg K ha^{-1} . Half of the N, along with full amount of P (according to treatment

plan) and K, was broadcasted and thoroughly mixed to soil at sowing. The leftover N was applied to the crop at first irrigation. The rhizobacterial strains used in this study were obtained from the University of Agriculture, Faisalabad, Laboratory of Soil Biochemistry and Microbiology. These ACC-deaminase rhizobacteria were selected on the basis of their differential phosphate solubilization activity. General purpose media was used for the preparation of inocula. Selected two strains of rhizobacteria were used for inoculation of broth and incubated at $28 \pm 1^\circ\text{C}$ for 48 h with a shaking speed of 78 rpm. Seed inoculation was performed after maintaining a uniform population of rhizobacteria (107-108 CFU). As suggested by Shaharoon *et al.* (2006), "peat and muck soil were ground to pass through a 2-mm 40-mesh sieve and autoclaved at 121°C for 20 min. A 10-mL inoculum of the selected rhizobacteria was mixed with 50 g of peat and 50 g of muck soil and incubated for 24 h at $28 \pm 1^\circ\text{C}$ before being used for seed coating, with muck to peat soil ratio of 1:1 (w/w)". The processed seeds were left overnight in the laboratory for air-drying. The soil under study was analyzed following Ryan *et al.* (2001) and was found to be heavy (40% clay) in texture, non-saline (EC: 0.98 dS m^{-1}), alkaline (pH: 8.1)-calcareous (CaCO_3 : 16.3%), low in organic matter (0.58%), NaHCO_3 -extractable-P (6.3 mg kg^{-1}) and NH_4OAc -extractable K (105 mg kg^{-1}). At the time of maturity, five randomly selected and tagged plants were taken from each sub-plot record different observations, i.e. plant height, sympodia plant⁻¹, stem diameter, bolls plant⁻¹, boll weight, and seed cotton yield plant⁻¹. Leaf P concentration was determined following standard method (Ryan *et al.*, 2001). The analysis of variance and mean separation, through honestly significant difference test (HSD_{0.05}), was done by using Statistix ver. 8.1.

3. RESULTS

Both the sources of variance, i.e. P doses and rhizobacterial strains significantly affected plant height, sympodial branches, number of bolls stem diameter, boll weight, seed cotton yield and leaf P concentration. While their interaction significantly affected plant height, boll weight, seed cotton yield and P concentration only (**Table 1**). These results suggest that rhizobacterial strains significantly affected all the parameters even at higher P doses which again advocate the scope of utilizing these rhizobacteria as bio-fertilizers for field application.

Plant height: The application of 75 kg P ha^{-1} increased plant height up to 10.5% over control (Table 2). Rhizobacterial strains also increased plant height of cotton over their control. This increase was up to 15.5% in case of *Pseudomonas fluorescens* which possessed only ACC-deaminase activity and up to 24.3% in case

of *Pseudomonas fluorescence* having dual activities of ACC-deaminase and P solubilization. The rhizobacteria possessing ACC-deaminase and P-solubilizing activities both increased plant height by 1.6 fold as compared to its counterpart having only ACC-deaminase activity. The interaction between phosphorus doses and rhizobacteria (P × R) also significantly affected plant height of cotton. Maximum increase in plant height of cotton (1.3 fold) was noted as a result of interaction between *Pseudomonas fluorescence* having dual activities of ACC-deaminase and P solubilization and recommended phosphorus dose (75 kg ha⁻¹). The ACC-deaminase, P-solubilizing *Pseudomonas fluorescence* enhanced plant height of cotton up to 29.7% and 10.9% under control and recommended P treatments, respectively. This increase was up to 2.3 and 1.2 fold more against the increase in plant height of cotton (up to 12.9% and 9.5%, respectively) noted in case of *Pseudomonas fluorescence* having only ACC-deaminase activity (Table 1 and 2).

Table 1. P-values from analysis of variance of various parameters of cotton as affected by different phosphorus levels and rhizobacterial strains

Parameter	Phosphorus dose (P)	Rhizobacterial strains (R)	P × R
Plant height (cm)	0.0115	0.0000	0.0046
Sympodial branches (plant ⁻¹)	0.0128	0.0012	0.0662
Stem diameter (mm)	0.0003	0.0024	0.9723
Number of bolls (plant ⁻¹)	0.0228	0.0002	0.0740
Average boll weight (g)	0.0079	0.0004	0.0047
Seed cotton yield (g plant ⁻¹)	0.0013	0.0000	0.0017
Phosphorus concentration (%)	0.0011	0.0005	0.0032

Sympodia plant⁻¹: Recommended P application (75 kg ha⁻¹) increased sympodial branches up to 52.2% over control (Table 2). Rhizobacterial strains also increased sympodial branches of cotton over their control. This increase was up to 18.9% in case of *Pseudomonas fluorescence* which possessed only ACC-deaminase activity and up to 35.6% in case of *Pseudomonas fluorescence* having dual activities of ACC-deaminase and P solubilization. The rhizobacteria possessing ACC-deaminase and P-solubilizing activities both increased sympodia by 1.9 fold as compared to its counterpart having only ACC-deaminase activity. The interaction between phosphorus doses and rhizobacteria (P × R) was non-significant in affecting sympodial branches of cotton. ACC-deaminase, P-solubilizing *Pseudomonas fluorescence* enhanced sympodial branches of cotton up to 77.0% and 14.3% under control and recommended P treatments, respectively. This increase was up to 2.0 and 1.5 fold more against the increase in sympodial branches of cotton (up to 37.7% and 9.2%, respectively)

noted in case of *Pseudomonas fluorescence* having only ACC-deaminase activity (Table 2).

Stem diameter: The recommended dose of P (75 kg ha⁻¹) increased stem diameter up to 37.4% over control (Table 2). Rhizobacterial strains also increased stem diameter of cotton over their control. This increase was up to 17.6% in case of *Pseudomonas fluorescence* which possessed only ACC-deaminase activity and up to 24.5% in case of *Pseudomonas fluorescence* having dual activities of ACC-deaminase and P solubilization. The rhizobacteria possessing ACC-deaminase and P-solubilizing activities both increased stem diameter by 1.4 fold as compared to its counterpart having only ACC-deaminase activity. The interaction between phosphorus doses and rhizobacteria (P × R) was non-significant in case of stem diameter of cotton. The ACC-deaminase, P-solubilizing *Pseudomonas fluorescence* enhanced stem diameter of cotton up to 28.6% and 21.7% under control and recommended P treatments, respectively. This increase was up to 1.3 and 1.4 fold more against the increase in stem diameter of cotton (up to 21.4% and 15.0%, respectively) noted in case of *Pseudomonas fluorescence* having only ACC-deaminase activity (Table 2).

Table 2. Plant height, sympodia and stem diameter of cotton as affected by ACC-deaminase rhizobacteria, without† or with‡ phosphate-solubilizing activity, at deficient and adequate phosphorus levels

Rhizobacteria	Phosphorus dose (kg ha ⁻¹)		Rhizobacteria mean
	00	75	
Plant height (cm)			
Control	95.3d	112.7bc	104.0
<i>P. fluorescence</i> (ACC)†	107.7c	123.3ab	115.5
<i>P. fluorescence</i> (ACC + PS)‡	123.7ab	125.0a	124.3
Phosphorus dose mean	108.9	120.3	
Sympodia/plant			
Control	20.3	39.7	30.0B
<i>P. fluorescence</i> (ACC)†	28.0	43.3	35.7A
<i>P. fluorescence</i> (ACC + PS)‡	36.0	45.3	40.7A
Phosphorus dose mean	28.1B	42.8A	
Stem diameter (mm)			
Control	14.0	20.0	17.0B
<i>P. fluorescence</i> (ACC)†	17.0	23.0	20.0A
<i>P. fluorescence</i> (ACC + PS)‡	18.0	24.3	21.2A
Phosphorus dose mean	16.3B	22.4A	
Honestly significant difference at alpha 0.05: Plant height: P-dose 5.33; rhizobacteria 5.26; P × R 10.23; Sympodia: P-dose 7.23; rhizobacteria 5.15; Stem diameter: P-dose 0.479; rhizobacteria 2.31			

Bolls plant⁻¹: The P application at 75 kg ha⁻¹ increased number of bolls up to 26.2% over control (Table 3). Rhizobacterial strains also increased number of bolls of

cotton over their control. This increase was up to 12.2% in case of *Pseudomonas fluorescence* which possessed only ACC-deaminase activity and up to 21% in case of *Pseudomonas fluorescence* having dual activities of ACC-deaminase and P solubilization. The rhizobacteria possessing ACC-deaminase and P-solubilizing activities both increased number of bolls by 1.7 fold as compared to its counterpart having only ACC-deaminase activity. The interaction between phosphorus doses and rhizobacteria (P × R) was non-significant in case of number of bolls of cotton. The ACC-deaminase, P-solubilizing *Pseudomonas fluorescence* enhanced number of bolls of cotton up to 33.9% and 11.8% under control and recommended P treatments, respectively. This increase was up to 1.7 and 1.8 fold more against the increase in number of bolls of cotton (up to 20.2% and 6.5%, respectively) noted in case of *Pseudomonas fluorescence* having only ACC-deaminase activity (Table 3).

Table 3. Number of bolls, boll weight, seed cotton yield and P concentration of cotton as affected by ACC-deaminase rhizobacteria, without† or with‡ phosphate-solubilizing activity at deficient and adequate phosphorus levels

Rhizobacteria	Phosphorus dose (kg ha ⁻¹)		Rhizobacteria mean
	00	75	
Number of bolls/plant			
Control	36.3	51.0	43.7C
<i>P. fluorescence</i> (ACC)†	43.7	54.3	49.0B
<i>P. fluorescence</i> (ACC + PS)‡	48.7	57.0	52.8A
Phosphorus dose mean	42.9B	54.1A	
Boll weight (g)			
Control	2.8c	6.4ab	4.6
<i>P. fluorescence</i> (ACC)†	4.7b	6.5ab	5.6
<i>P. fluorescence</i> (ACC + PS)‡	5.3ab	6.9a	6.1
Phosphorus dose mean	4.3	6.6	
Seed cotton yield (g/plant)			
Control	48.3e	83.0bc	65.7
<i>P. fluorescence</i> (ACC)†	70.3d	90.7ab	80.5
<i>P. fluorescence</i> (ACC + PS)‡	77.7cd	94.0a	85.8
Phosphorus dose mean	65.4	89.2	
P concentration (%)			
Control	48.3e	83.0bc	65.7
<i>P. fluorescence</i> (ACC)†	70.3d	90.7ab	80.5
<i>P. fluorescence</i> (ACC + PS)‡	77.7cd	94.0a	85.8
Phosphorus dose mean	65.4	89.2	
Honestly significant difference at alpha 0.05: Bolls/plant: P-dose 7.43; rhizobacteria 3.37; Average boll weight: P-dose 0.879; rhizobacteria 0.654; P × R 1.49; Seed cotton yield: P-dose 3.74; rhizobacteria 4.92; P × R 9.23; Phosphorus concentration: P-dose 0.021; rhizobacteria: 0.028; P × R: 0.0527			

Boll weight: The P application at 75 kg ha⁻¹ increased average boll weight up to 54% over control (Table 3). Rhizobacterial strains also increased average boll weight of cotton over their control. This increase was up to 21.4% in case of *Pseudomonas fluorescence* which possessed only ACC-deaminase activity and up to 33% in case of *Pseudomonas fluorescence* having dual

activities of ACC-deaminase and P solubilization. The rhizobacteria possessing ACC-deaminase and P-solubilizing activities both increased average boll weight by 1.5 fold as compared to its counterpart having only ACC-deaminase activity. The interaction between phosphorus doses and rhizobacteria (P × R) also significantly affected average boll weight of cotton. Maximum increase in average boll weight of cotton (2.5 fold) was noted as a result of interaction between *Pseudomonas fluorescence* having dual activities of ACC-deaminase and P solubilization and recommended phosphorus dose (75 kg ha⁻¹). The ACC-deaminase, P-solubilizing *Pseudomonas fluorescence* enhanced average boll weight of cotton up to 90.5% and 7.8% under control and recommended P treatments, respectively. This increase was up to 1.3 and 7.8 fold more against the increase in average boll weight of cotton (up to 67.9% and 1.0%, respectively) noted in case of *Pseudomonas fluorescence* having only ACC-deaminase activity (Table 3).

Seed cotton yield: The recommended P nutrition (75 kg ha⁻¹) increased seed cotton yield up to 36.3% over control (Table 3). Rhizobacterial strains also increased seed cotton yield of cotton over their control. This increase was up to 22.6% in case of *Pseudomonas fluorescence* which possessed only ACC-deaminase activity and up to 30.7% in case of *Pseudomonas fluorescence* having dual activities of ACC-deaminase and P solubilization. The rhizobacteria possessing ACC-deaminase and P-solubilizing activities both increased seed cotton yield by 1.4 fold as compared to its counterpart having only ACC-deaminase activity. The interaction between phosphorus doses and rhizobacteria (P × R) also significantly affected seed cotton yield of cotton. Maximum increase in seed cotton yield of cotton (2.0 fold) was noted as a result of interaction between *Pseudomonas fluorescence* having dual activities of ACC-deaminase and P solubilization and recommended phosphorus dose (75 kg ha⁻¹). The ACC-deaminase, P-solubilizing *Pseudomonas fluorescence* enhanced seed cotton yield of cotton up to 60.7% and 13.3% under control and recommended P treatments, respectively. This increase was up to 1.3 and 1.4 fold more against the increase in seed cotton yield (up to 45.5% and 9.2%, respectively) noted in case of *Pseudomonas fluorescence* having only ACC-deaminase activity (Table 3).

Phosphorus concentration (%): The application of 100% recommended dose of P, i.e. 75 kg ha⁻¹ increased phosphorus concentration up to 69.7% over control (Table 3). Rhizobacterial strains also increased phosphorus concentration of cotton over their control. This increase was up to 13.4% in case of *Pseudomonas fluorescence* which possessed only ACC-deaminase

activity and up to 26.8% in case of *Pseudomonas fluorescence* having dual activities of ACC-deaminase and P solubilization. The rhizobacteria possessing ACC-deaminase and P-solubilizing activities both increased phosphorus concentration by 2.0 fold as compared to its counterpart having only ACC-deaminase activity. The interaction between phosphorus doses and rhizobacteria (P × R) also significantly affected phosphorus concentration of cotton. Maximum increase in phosphorus concentration of cotton (2.4 fold) was noted as a result of interaction between *Pseudomonas fluorescence* having dual activities of ACC-deaminase and P solubilization and recommended phosphorus dose (75 kg ha⁻¹). The ACC-deaminase, P-solubilizing *Pseudomonas fluorescence* enhanced phosphorus concentration of cotton up to 77.8% and 4.8% under control and recommended P treatments, respectively. This increase was up to 1.9 and 2.5 fold more against the increase in phosphorus concentration of cotton (up to 40% and 1.9%, respectively) noted in case of *Pseudomonas fluorescence* having only ACC-deaminase activity (Table 3).

4. DISCUSSION

The results of this field study (Table 1 to 3) clearly depicted the importance of phosphorus (P) in cotton nutrition and confirmed earlier reports by many workers, emphasizing the need of adequate P nutrition of cotton for improved growth traits and enhanced yield. Accordingly, this study also endorsed that the increasing P levels significantly increased cotton growth and yield across rhizobacterial strains (Table 2 and 3). Almost all the growth traits and yield were significantly affected by adequate P nutrition. Moreover, P accumulation of cotton plants was also enhanced as a result of P application, which was obvious (Table 3). Furthermore, the inclusion of two ACC-deaminase rhizobacterial strains of *Pseudomonas fluorescens*, without or with P solubilizing activity, also significantly enhanced almost all the growth traits, yield and P accumulation of cotton plants (Table 2 and 3). The *Pseudomonas fluorescens* with dual activities of ACC-deaminase and P solubilization more effectively enhanced various plant traits of cotton as against its counterpart having only ACC-deaminase activity (Table 2 and 3). (Baig *et al.* 2012) demonstrated that Bacillus strains of rhizobacteria with dual abilities increased both the growth and yield of wheat plants but also enhanced their P uptake.

Early studies on the subject highlighted that the beneficial effects of rhizobacteria are strain specific and highly depends up on the plant nutrient status (Belimov *et al.*, 2002). Later on, it was reported that the rhizobacteria containing ACC-deaminase activity promote the growth of plants by decreasing the deleterious effects of stress ethylene under a variety of plant stresses. These rhizobacteria alter plant gene

expression and positively modulate the growth of plants (Glick *et al.*, 2007. Shaharoon *et al.* 2008) elucidated that rhizobacteria enhance root growth, however, such activity is governed by the nutrient status of rhizosphere. These workers observed that the promotion of wheat growth and yield by two ACC-deaminase *P. fluorescens* species declined with the increasing rates of chemical NPK fertilizers. Contrary to these results, (Naveed *et al.* 2008) found that the biofertilizer with rhizobacterial strain *P. fluorescens* biotype G (N3) enhanced the grain yield and nutrient uptake of maize, irrespective of the presence or absence of N fertilizer. These variations can be explained from the findings of Egamberdieva (2010) who observed that the beneficial effects of rhizobacteria were cultivar specific.

In an elegant study, (Malik *et al.* (2012) observed that the rhizobacterial seed coating enhanced important yield components and increased grain wheat yield. Likewise, Saber *et al.* (2012) found that the N-fixing and P-solubilizing bacteria had positive effects on wheat growth and yield. (Qureshi *et al.* 2012) explained that these rhizobacteria are involved in some important processes in soil, e.g. nutrient decomposition, mobilization, mineralization, solubilization, coupled with N-fixation and production of different plant hormones. Nitrogen fixation in legumes by PGPR bacteria has also been documented (Lugtenberg and Kamilova, 2009). Recently, (Sharma *et al.* 2011) explained that these rhizobacteria reduce soil pH by producing organic acids and mineralize organic P through acid phosphatase.

Recently, (Afzal *et al.* 2014) found that *P. fluorescens* with dual abilities of ACC-deaminase and P-solubilization effectively enhance wheat growth and yield irrespective of P nutrition status. (Zia-ul-hassan *et al.* 2015) also reported that the biopriming of wheat seeds with ACC-deaminase, P-solubilizing rhizobacteria is an effective strategy to enhance growth and yield of wheat under P-deficiency stress. Therefore, biofertilizers developed through these rhizobacteria significantly reduce nutrient requirement by sustaining crop yield (Qureshi *et al.*, 2012).

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