ACC-Deaminase, Phosphate-Solubilizing *Pseudomonas fluorescens* Increase Phosphorus and Decrease Cadmium Concentration to Enhance Wheat Yield

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Abstract: Phosphorus (P) fertilization of wheat at higher dose may result in grain cadmium (Cd) accumulation. This field study envisaged yield and comparative P and Cd accumulation of wheat under different P doses, i.e. 0, 45 and 90 kg P_2O_5 ha^{-1} (P0, P45 and P90, respectively) and seed inoculation with rhizobacterial strains, i.e. no *Pseudomonas fluorescens* (SM0), with ACC-deaminase *P. fluorescens* (SM1) and with phosphate-solubilizing, ACC-deaminase *P. fluorescens* (SM2). The soil was non-saline, alkaline clay loam, poor in organic matter and P content. Both P nutrition and rhizobacteria positively affected wheat growth, yield and nutrient concentration. Increased 1000-grain weight (TGW), yield and P concentration of wheat was noted at P90 over P0 (24-132%) and P45 (3.7-37%), and in case of SM2 (13-57%) and SM1 (5.4-34%) over SM0, and for SM2 over SM1 (1.4 to 2.4-fold). Grain-Cd concentration decreased at P90 over P0 (34%) and P45 (17%), and at P45 over P0 (21%). It decreased over SM0 at SM2 (22%) and SM1 (8%), and over SM1 at SM2 (2.7-fold). Straw-Cd concentration decreased at P90 over P0 (25%) and P45 (18%), and over P0 at P45 (8%). It decreased over SM0 at SM2 (18%) but increased at SM1 (9%). At all P levels, SM2 was more effective over SM1 or SM0. TGW and straw-P increased for P90 interacting with SM2 over SM0 (8.6 and 29%) and SM1 (6 and 14%), and for SM1 over SM0 (2.5 and 13%). Grain- and straw-Cd decreased due to interaction of P90 with SM2 (30 and 23%) or SM1 (6 and 7%) over SM0, and for SM1 over SM0 (26% and 17%). We conclude that adequate P nutrition and seed inoculation with ACC-deaminase, phosphate-solubilizing *Pseudomonas fluorescens* increase growth and yield of wheat due to its increased P and decreased Cd concentration.

Keywords: ACC-deaminase, cadmium, phosphorus, *Pseudomonas fluorescens*, rhizobacteria, Wheat.

INTRODUCTION

Phosphorus deficient soils restrict crop productivity [1]. According to literature [2], most of the Pakistani soils (~90%) are P-deficient (<10 mg kg^{-1}) with <25% P-use-efficiency by plants [3]. Hence, P fertilization of crops in Pakistan becomes very indispensable for obtaining good returns. Wheat (*Triticum aestivum* L.) consumes ~45% of the total P fertilizers used in Pakistan [4]. Cadmium (Cd) is a nonessential heavy metal which restricts the normal growth and development of plants [5]. In plants, Cd is mostly accumulated in the root. However, a little amount is transported to plant shoots and grains [6]. Cd is usually present in chemical phosphatic fertilizers in traces from where it can accumulate to wheat grain and straw and consequently badly affect human health. Root Cd accumulation decreases grain-Cd accumulation in wheat plants [7]. The microorganisms beneficial for plant growth and development are commonly known as plant growth promoting rhizobacteria [8]. These bacteria possess P-solubilizing activity by releasing numerous organic acids, e.g. gluconic, citric [9] which reduces pH which solubilizes calcium phosphate and hence facilitates P availability to plants [10]. The use of P-solubilizing rhizobacterial inoculants, as biofertilizers for integrated P nutrition of crops, has recently gained momentum in Pakistan [4,11]. By using the P-solubilizing rhizobacteria, unaffordable costs of chemical P fertilizers can also be reduced. On the basis of above literature this filed study was conducted to evaluate the cadmium accumulation of wheat as function of seed inoculation with ACC-deaminase rhizobacteria under varying P application rates.

MATERIALS AND METHODS

This field experiment was launched at the Experimental Farm of the Nuclear Institute of Agriculture, Tandojam, Sindh during 2013-14. The experiment was repeated thrice in randomized complete blocks having three P levels, i.e. 0, 50 and 90 kg P_2O_5 ha^{-1} (P0, P45 and P90, respectively) assigned to main plots and three rhizobacterial treatments,

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assigned to sub-plots, viz. no seed inoculation with *Pseudomonas fluorescens* (SM0), seed inoculation with *P. fluorescens* having only ACC-deaminase activity (SM1), and seed inoculation with *P. fluorescens* having dual ACC-deaminase and P-solubilizing activities (SM2). The size of each experimental unit was 24 m × 6 m (24.0 m^2). The pure seeds of wheat (*cv*. Sarsabz) were drilled @ 125 kg ha-1 by maintaining a plant spacing of 10cm. The rows were kept 30cm apart. The crop was raised following recommended practices. Weeds were managed manually. The rhizobacterial strains were selected on the basis of their ACC-deaminase activity or phosphate-solubilizing activity or both. General purpose media was used for the preparation of inocula [12]. Rhizobacterial strains were incubated at 28±1°C for 48h under 78 rpm shaking speed. A population of 10^7-10^8 CFU was ensured before seed inoculation with rhizobacteria. As suggested elsewhere [13], "peat and muck soil were ground to pass through a 2-mm, 40-mesh 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±1°C before being used for seed coating, with muck to peat soil ratio of 1:1 (w/w). Inoculated seeds were placed overnight for air-drying in the laboratory". The requisite doses of P_2O_5 were maintained through soil application of diammonium phosphate (DAP, 46% P_2O_5) which is the most commonly used phosphatic fertilizer in wheat crop. The crop received recommended doses of nitrogen (200 kg ha^{-1}), P (100 kg P_2O_5 ha^{-1}) and potassium (50 kg ha^{-1}). Nitrogen (N) was supplied as urea (46% N), while potassium (K) was given as sulfate of potash, (SOP, 50% K_2O). All the P and K along with one-third dose of N, was applied to the crop by broadcasting to the soil and then thoroughly mixed. The remaining two-third dose of nitrogen was given to the crop in two equal splits, i.e. at first and second irrigations. The soil used in this experiment was analyzed for texture, electrical conductivity, pH, organic matter and Olsen available P by taking a composite sample from the experimental area before wheat sowing, following standard methods [14]. The chemical analyses revealed that the soil of the experimental area was clay loam in texture, alkaline in nature (pH: 7.3), free from salinity hazards (EC: 0.90 dSm^{-1}). Moreover, the soil was poorly fertile and found low in organic matter (0.7%) and available Olsen Phosphorus (5.9ug g^{-1}). The crop was irrigated according to its requirements by following the recent recommendations. All the other recommended agronomic practices were followed throughout the life span of the crop. At maturity, three plants were harvested from each experimental unit to observe 1000-grain weight and grain and straw yield. Phosphorus and cadmium accumulation from the straw and grain of mature plants of all samples, obtained from each experimental unit, was determined following standard methods [15]. The collected data were subjected to analysis of variance, mean separation and correlation using Statistix ver. 8.1. The analysis of variance was done by following a two-factor randomized complete block split-plot design. The treatment means were separated by Tukey's Honestly Significant Difference (HSD) test at alpha 0.05.

RESULTS

The P-values from analysis of variance of various parameters of wheat as affected by different phosphorus (P) doses and rhizobacterial strains are given in Table **1**. These values clearly demonstrate that both the P doses and rhizobacterial strains highly significantly ($p<0.01$) affected grain yield (kg ha^{-1}) and grain P concentration (g kg^{-1}). Further the P dose highly significantly ($p<0.01$) and rhizobacterial strains

Table 1: P-values from Analysis of Variance of Various Parameters of Wheat as Affected by Phosphorus Doses, Rhizobacterial Strains and Their Interaction

Parameter	Phosphorus dose (P)	Rhizobacterial strains (R)	P × R
1000-grain weight (g)	0.0004	0.0000	0.0088
Grain yield (kg ha^{-1})	0.0009	0.0000	0.7990
Straw yield (kg ha^{-1})	0.0005	0.0022	0.1971
Grain P concentration (g kg^{-1})	0.0000	0.0000	0.0934
Straw P concentration (g kg^{-1})	0.0000	0.0000	0.0000
Grain Cd concentration (mg kg^{-1})	0.0004	0.0191	0.0001
Straw Cd concentration (mg kg^{-1})	0.0004	0.0041	0.0157

significantly ($p<0.01$) affected straw yield under the study. Moreover, the interaction between these two main sources of variance was significant ($p<0.01$ and <0.05) for the 1000-grain weight and straw Cd concentration (mg kg^{-1}), and highly significant ($p<0.01$) for straw P concentration (g kg^{-1}) and grain Cd concentration (mg kg^{-1}) and other parameters were non-significant including P and cadmium (Cd) accumulation by wheat grain and straw.

1000-Grain Weight

In general, P nutrition increased 1000-grain weight (TGW). Application of P90 offered maximum 1000-grain weight (TGW), 24% more than P0 and 10% more than P45, whereas, P45 produced 13% more TGW as compared to P0. Similarly, when compared to SM0, TGW increased up to 13% in case of SM2 and 5.4% in case of SM1. The SM2 increased TGW 2.4-fold more as compared to SM1. The interaction of rhizobacteria and P nutrition also increased TGW. Maximum TGW was obtained in case of SM2×P90 which was 8.6% and 5.9% more as compared to TGW obtained at SM0×P90 and SM1×P90, respectively. The TGW in case of SM1×P90 was almost similar (only 2.5% more) to that noted in case of SM0×P0 (Table **2**).

Grain Yield

Adequate P nutrition also enhanced wheat yield (Table **2**). Application of P90 increased grain yield as against P0 (42%) and P45 (14%). Moreover, when compared to SM0, the increase in grain yield noted in case of SM2 and SM1 was 31% and 17% as against SM1. It was also observed that SM2 increased grain yield up to 1.8-fold more as against SM1.

Straw Yield

Straw yield of wheat also responded positively to adequate P nutrition (Table **2**). Application of P90 enhanced straw yield as compared to P0 (27%) and P45 (3.7%). Moreover, as against control, both SM2 and SM1 increased straw yield (15% and 8%, respectively). It was noted that SM2 increased grain yield up to 1.8-fold more as against SM1.

Grain-P Concentration

As compared to P0 and P45 wheat grains accumulated more P (117 and 44%, respectively) when supplied with P90 (Table **3**). Even P45 resulted in increased accumulation of P (50%) by wheat plants as compared to P0.Likewise, both the rhizobacterial

Table 2: 1000-Grain Weight and Yield of Wheat as Affected by ACC-Deaminase *Pseudomonas fluorescence* without† or with‡ Phosphate-Solubilization under Different Phosphorus Doses

Rhizobacteria	Phosphorus dose (kg ha^{-1})			Rhizobacteria mean
	00	45	90	
1000-grain weight (g)				
Control	23.7e	31.8cd	36.1abc	30.5
P. fluorescence (ACC)†	27.8d	32.5bc	37.0ab	32.4
P. fluorescence (ACC + PS)‡	33.0bc	35.7abc	39.2a	36.0
Phosphorus dose mean	28.2	33.3	37.4	
$HSD_{0.05}$: P-dose: 2.3552; Rhizobacteria: 1.5762; P × R: 3.7994				
Grain yield (kg ha^{-1})				
Control	2220	2906	3656	2927C
P. fluorescence (ACC)†	2839	3584	4090	3504B
P. fluorescence (ACC + PS)‡	3215	4161	4605	3994A
Phosphorus dose mean	2758C	3550B	4117A	
$HSD_{0.05}$: P-dose: 433.12; Rhizobacteria: 324.11				
Straw yield (kg ha^{-1})				
Control	3275	4404	4896	4192B
P. fluorescence (ACC)†	3855	4853	5094	4601AB
P. fluorescence (ACC + PS)‡	4461	5316	5061	4946A
Phosphorus dose mean	3864B	4858A	5017A	
$HSD_{0.05}$: P-dose: 340.9; rhizobacteria: 437.7				

Table 3: Phosphorus and Cadmium Concentration of Wheat as Affected by ACC-Deaminase *Pseudomonas fluorescence* without † or with ‡ Phosphate-Solubilization under Different Phosphorus Doses

Rhizobacteria	Phosphorus dose (kg ha^{-1})			Rhizobacteria mean
	00	42.5	85	
Grain P concentration (g kg^{-1})				
Control	1.27	2.27	3.23	2.26C
P. fluorescence (ACC)†	1.67	2.90	3.70	2.76B
P. fluorescence (ACC + PS)‡	2.17	3.07	4.40	3.21A
Phosphorus dose mean	1.70C	2.74B	3.78A	
$HSD_{0.05}$: P-dose: 0.22; Rhizobacteria: 0.20				
Rhizobacteria	**Phosphorus dose (kg ha^{-1})**			**Rhizobacteria mean**
	00	**45**	**90**	
Straw P concentration (g kg^{-1})				
Control	0.88f	1.27de	2.30c	1.48
P. fluorescence (ACC)†	1.13e	2.20c	2.60b	1.98
P. fluorescence (ACC + PS)‡	1.40d	2.73ab	2.97a	2.37
Phosphorus dose mean	1.14	2.07	2.62	
$HSD_{0.05}$: P-dose: 0.14; Rhizobacteria: 0.11; P × R: 0.26				
Rhizobacteria	**Phosphorus dose (kg ha^{-1})**			**Rhizobacteria mean**
	00	**42.5**	**85**	
Grain Cd concentration (mg kg^{-1})				
Control	0.143d	0.217ab	0.255a	0.205
P. fluorescence (ACC)†	0.143d	0.176cd	0.188bc	0.169
P. fluorescence (ACC + PS)‡	0.143d	0.172cd	0.178cd	0.164
Phosphorus dose mean	0.143	0.188	0.207	
$HSD_{0.05}$: P-dose: 0.02; P × R: 0.04				
Straw Cd concentration (mg kg^{-1})				
Control	0.248d	0.462ab	0.583a	0.431
P. fluorescence (ACC)†	0.306cd	0.384bc	0.485ab	0.392
P. fluorescence (ACC + PS)‡	0.247d	0.380bc	0.450b	0.359
Phosphorus dose mean	0.267	0.409	0.506	
$HSD_{0.05}$: P-dose: 0.06; rhizobacteria: 0.04; P × R: 0.11				

strains SM2 and SM1 also contributed to increased grain-P concentration (38 and 26%, respectively) against SM0. The grain-P concentration in case of SM2 was 1.4-fold more as compared to SM1.

Straw-P Concentration

Straw-P concentration (Table **3**) was more in case of P90 as compared to P0 and P45 (132 and 37%, respectively). When supplied with P45, P concentration of wheat straw was more (70%) as compared to P0. The rhizobacterial inoculants SM2 and SM1 also increased straw-P concentration of wheat plants (57 and 34%, respectively) against SM0. The straw-P concentration increased (1.7-fold) by SM2 when compared to SM1. The rhizobacteria×P interaction increased straw-P concentration. Maximum straw-P concentration was obtained in case of SM2×P90 which was 29% and 14% more as compared to straw-P concentration obtained at SM0×P90 and SM1×P90, respectively. Straw-P concentration in case of SM1×P90 was 13% more than it was noted in case of SM0×P0.

Grain-Cd Concentration

The concentration of Cd in wheat grains when supplied with P90 and P45 was lower (34 and 21%,

respectively) as compared to no P application (Table **3**). The grain-Cd concentration was also lower (17%) at P90 as against P45. Both the rhizobacterial strains SM2 and SM1 were also proved effective in decreasing grain-Cd concentration (22 and 8%, respectively) against SM0. The grain-Cd concentration noted in case of SM2 was 2.7-fold lower than it was recorded in case of SM1. The of rhizobacteria×P interaction decreased grain-Cd concentration. Grain-Cd concentration noted in case of SM2×P90 was 30% and 5% lower as compared to grain-Cd concentration recorded at SM0×P90 and SM1×P90, respectively. Grain-Cd concentration in case of SM1×P90 was 26% lower than it was noted in case of SM0×P0.

Straw-Cd Concentration

The straw-Cd concentration at P90 and P45 was lower (25 and 8%, respectively) as compared to no P application (Table **3**). The straw-Cd concentration was also lower (18%) at P90 as against P45. The rhizobacterial strain SM2 decreased straw-Cd concentration (18%) against SM0. However, reverse was true for SM1 which increased (10%) straw-Cd concentration over SM0. Hence, SM2 greatly decreased straw-Cd concentration as compared to SM1. The of rhizobacteria×P interaction decreased straw-Cd concentration. Straw-Cd concentration noted in case of SM2×P90 was 22% and 7% lower as compared to straw-Cd concentration recorded at SM0×P90 and SM1×P90, respectively. Straw-Cd concentration in case of SM1×P90 was 16% lower than it was noted in case of SM0×P0.

DISCUSSION

The present filed study evaluates cadmium accumulation of wheat asa function of seed inoculation with ACC-deaminase rhizobacteria and phosphorus fertilization. Plant growth promoting rhizobacteria (PGPR) influence the growth, yield, and nutrient uptake of crops by increasing nutrient bioavailability and by producing plant hormones, augment other mycorrhiza, minimize fungal and bacterial diseases, help control insect pests [16] and enhance N_2 fixation in legumes [17]. The results of present field study (Tables **1** to **3**) clearly depicted the importance of phosphorus (P) in wheat nutrition and confirmed earlier reports by many workers, emphasizing the need of adequate P nutrition of wheat for improved growth traits and enhanced yield. Accordingly, this study also endorsed that the increasing P levels significantly increased wheat growth and yield across rhizobacterial strains (Table **2**). Almost all the growth traits and yield were significantly affected by adequate P nutrition. Moreover, P and Cd accumulation of wheat plants was also enhanced as a result of P application (Table **3**). Furthermore, the inclusion of two ACC-deaminase rhizobacterial strains of *P. fluorescens*, without or with P solubilizing activity, also significantly enhanced almost all the growth traits and yield of wheat. Moreover, the decreased Cd accumulation by wheat was a function of increased P accumulation from the rhizosphere through pertinent mechanisms (Tables **1** to **3**). Phosphate-solubilizing rhizobacteria enhance P-solubilization through producing acid phosphatase and hence used as bio-inoculants, especially through rhizobacterial or mycorrhizal co-inoculation [18,19]. The results of present studies also endorse earlier findings. The *P. fluorescens* with dual activities of ACC-deaminase and P solubilization more effectively enhanced various plant traits of wheat as against its counterpart with that had only ACC-deaminase activity (Tables **2** and **3**). Similarly, increased grain yield and nutrient uptake by *P. fluorescens* with or without nitrogen fertilization is also reported in literature [20]. Early findings [21] also highlight that *P. fluorescens* increased wheat shoot and root biomass and length, however, the effect was cultivar specific. Our results are in agreement with the findings of previous studies [22] and endorse that the rhizobacterial inoculants are the benign supplement to the chemical P fertilization for enhanced P-use-efficiency and sustainable P nutrition.

CONCLUSION

We concluded from the results of this study that adequate P nutrition and seed inoculation with ACC-deaminase, phosphate-solubilizing *Pseudomonas fluorescens* increase growth and yield of wheat due to its increased P and decreased Cd concentration.

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