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Research article

Interactive effects of phosphorus and *Pseudomonas putida* on chickpea (*Cicer arietinum* L.) growth, nutrient uptake, antioxidant enzymes and organic acids exudation

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ABSTRACT

Phosphorus (P) availability in alkaline soils of arid and semi-arid regions is a major constraint for decreased crop productivity. Use of plant growth promoting rhizobacteria (PGPR) may enhance plant growth through the increased plant antioxidation activity. Additionally, PGPR may increase nutrient uptake by plants as a result of induced root exudation and rhizosphere acidification. The current study was aimed to investigate combined effects of P and *Pesudomonas putida* (PGPR) on chickpea growth with reference to antioxidative enzymatic activity and root exudation mediated plant nutrient uptake, particularly P. Half of the seeds were soaked in PGPR solution, whereas others in sterile water and latter sown in soils. Plants were harvested 8 weeks after onset of experiment and analyzed for leaf nutrient contents, antioxidant enzymes activities and organic acids concentrations. Without PGPR, P application (+P) increased various plant growth attributes, plant uptake of P and Ca, soil pH, citric acid and oxalic acid concentrations, whereas decreased the leaf POD enzymatic activity as compared to the P-deficiency. PGPR supply both under −P and +P improved the plant growth, plant uptake of N, P, and K, antioxidative activity of SOD and POD enzymes and concentrations of organic acids, whereas reduced the rhizosphere soil pH. Growth enhancement by PGPR supply was related to higher plant antioxidation activity as well as nutrient uptake of chickpea including P as a result of root exudation mediated rhizosphere acidification.

1. Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop in developing countries (Yadav et al., 2010) and a major source of dietary protein. In Pakistan, it is cultivated on the soils of rain-fed dry areas where crop yield has been declined over the last few decades due to decreased availability of P in soil-plant system, as it forms insoluble complexes with calcium, aluminum and iron (Gyaneshwar et al., 2002; Hao et al., 2002). P-deficiency is one of the major limiting factors for decreased agricultural production (Schachtman et al., 1998; Lynch and Brown, 2008), as it is an essential macronutrient, and required for numerous functions like energy transport, nucleic acid synthesis, membrane synthesis and stability, enzyme activation/inactivation, redox reactions, signal transduction, photosynthesis, glycolysis, respiration and nitrogen fixation (Abel et al., 2002; Vance et al., 2003; Khan et al., 2009). Additionally, intensive cultivation, low inputs of fertilizers due to high cost, and P-fixation in Pakistani soils have resulted in poor soil fertility (Afzal et al., 2010).

Use of biofertilizers along with chemical fertilizers may serve as an effective approach for enhancing the crop nutrient requirements, thereby leading to the sustainable crop production. Biofertilizers consist of beneficial microbes, which form colonies in

Abbreviations: DW, Dry weight; FW, Fresh weight; PGPR, Plant growth promoting rhizobacteria; POD, Peroxidase; SOD, Superoxide dismutase.

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soils and promote plant growth by increasing nutrient availability when applied as a seed dressing or on plant surfaces (Vessey, 2003). These microorganisms can enhance the availability of deficient or immobile nutrients in soils after solubilizing their mineral forms. For example, PGPR can promote plant growth by P-solubilization, biological nitrogen fixation, availability of trace elements such as Fe and Zn and the production of plant growth regulators (Ponmurugan and Gopi, 2006; Yadav et al., 2010; Panhwar et al., 2012). Other potential mechanisms for this interaction include the role of P-solubilizing bacteria (PSBs) in lowering the rhizosphere soil pH (Khan et al., 2009) or due to the production of low molecular weight organic acids (Deubel et al., 2000). Numerous researchers have reported an increase in plant growth and P uptake by different crop species (Gaind and Gaur, 1991; Wahid and Mehana, 2000; Peix et al., 2001), while reports are also available where inoculation did not enhance P uptake by plants (Laheurte and Berthelin, 1988). This is possible because many PSBs also produce growth regulators, which may enhance plant growth without increasing P uptake (Leinhos and Vacek, 1994). In this regard, various microbial species like *Allorhizobium*, *Agrobacterium*, *Arthrobacter*, *Azorhizobium*, *Bacillus*, *Bradyrhizobium*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Hyphomicrobium*, *Mesorhizobium*, *Micrococcus*, *Pseudomonas*, *Rhizobium*, *Serratia* and *Sinorhizobium* have been proven to possess plant growth promoting (PGP) traits (Vessey, 2003). Likewise, use of PGPR has improved the growth and yield of various crops such as bean (Nassar et al., 2003; Figueiredo et al., 2008), pea (Tokala et al., 2002), rice (Gopalakrishnan et al., 2013), tomato (El-Tarabily, 2008) and wheat (Sadeghi et al., 2012). Therefore, use of PGPR including PSBs has been suggested as a sustainable solution for improving crop production (Vessey, 2003).

This is also possible that P-deficiency may result in the formation of reactive oxygen species (ROS) like $O_2^{\bullet -}$ (Grossman and Takahashi, 2001; Juszczuk et al., 2001; Bargaz et al., 2013), which could possibly induce oxidative stress along with nutrient-specific alterations in plant metabolic system (Kandlbinder et al., 2004). However, plants evolve an efficient antioxidative defense system, comprising of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) (Del-Rio et al., 2003; Masood et al., 2012) and non-antioxidant enzymes like glutathione, ascorbate and tocopherol to scavenge these ROS (Del-Rio et al., 2003). It was thus hypothesized that inoculation of seeds with *Pseudomonas putida* enhances nutrient availability, particularly P, while increasing the root exudation in response to decrease in rihizosphere soil pH. Secondly, PGPR inoculation may enhance antioxidant enzymatic activity, which helps in the adaptation of plants under P-deficiency. Overall, objective of our study was to evaluate the effect of biological enhancer on chickpea growth and to explain it with measured parameters.

2. Materials and methods

2.1. Seed inoculation and plant growth conditions

A pot experiment was conducted in greenhouse of Quaid-i-Azam University, Islamabad, Pakistan located between latitude of 33.74° N and a longitude of 73.13° E to assess the effects of *P. putida* and P on the growth of chickpea (*Cicer arietinum* L. cultivar Dasht). Half of the seeds were inoculated with *P. putida* (Khewra 2P), isolated from Khewra salt range of Pakistan (Yasmin and Bano, 2011), while others not. *P. putida* inoculum was prepared in 250 ml of broth and kept overnight in shaker-incubator (ECELLA E23, Califorina, USA) with a relative speed of 120 rpm and temperature of 28 ± 2 °C. Next day, broth culture was centrifuged at a relative centrifugation force of 1640 *g* for 20 min and pellet was spun and solubilized using sterile water after decanting supernatant. Now, half of the seeds were soaked in bacterial culture for 3–4 h, while others in sterile water before sowing into respective pots. Prior to seed inoculation, qualitative analysis of *P. putida* for P-solubilization was performed on Pikovskaya's agar plates containing insoluble tricalcium phosphate (Gaur, 1990) (Fig. S1). All the seeds were sterilized with 1 mM $CaSO_4 \cdot 2H_2O$ solution before inoculation treatments.

Surface soil (0–15 cm depth) was collected from a non-cultivated field, autoclaved and filled into the pots (25 × 15 cm^2) at the rate of 2.5 kg pot^{-1}. The soil had an EC; 3.2 dS m^{-1}, pH; 7.5, total N; 470 μg g^{-1}, available P; 5.9 μg g^{-1} and extractable K; 230 μg g^{-1}. Texture of the soil was silt loam according to international textural triangle (Moodie et al., 1959), which contained 8% sand, 65% silt and 27% clay. Chickpea seeds were sown in pots with following treatments; +P (recommended NPK fertilizers) and -P (only N and K fertilizers) both with and without inoculation. The amounts of NPK fertilizers were calculated according to 40 kg N, 90 kg P_2O_5 and 60 kg K_2O per hectare viz. urea, di-ammonium phosphate and sulphate of potash. Overall, experiment was consisted of 4 treatments with three replications of each and repeated twice. Six seeds were initially sown in each pot and latter thinned to 3 uniform seedlings. Plants were allowed to grow for 8 weeks maintaining soil moisture contents at 60% field capacity by regular irrigations. Growth parameters like root length, shoot length, root and shoot weights were also recorded at plant harvesting. Thereafter, plant samples were either stored at −80 °C for subsequent analysis of antioxidant enzymes or oven-dried at 65 °C for the determination of total ion concentrations.

2.2. Soil and root exudates collection

Plants were carefully excavated and soil adhering/adjacent to the roots was collected by gentle shaking. Root exudates were collected using rhizobox system according to the procedure described by udDin et al. (2015).

2.3. Enumeration of leaf chlorophyll contents

Chlorophyll contents in intact leaves during the experiments were recorded with the help of Spad meter (Spad 502, Minolta Camera Co. Ltd. Japan). All the leaves except older were selected for chlorophyll measurements.

2.4. Leaf nutrient analysis

For the determination of nutrient content in chickpea leaves, 1 g dry ground material was digested with acid mixture; HNO_3:$HClO_4$ (4:1 v/v) on hot plate until the brown fumes turned into white. On cooling, leaf digests were diluted with distilled water and filtered. Afterwards, collected filtrates were used for the estimation of minerals (Ca, Mg and K) by atomic absorption spectrometer (Spectra AA240 FS, Varian, New Jersey, USA). P contents from the same leaf digests were determined with the help of spectrophotometer at a wavelength of 700 nm according to ascorbic acid method.

To determine N content in chickpea leaves, 100 mg ground leaf samples were digested with 5 ml of H_2SO_4 and few drops of H_2O_2 until the clear solution was not obtained. On cooling, digests were filtered and used for the estimation of leaf N by ammonia *Kjeldhal* distillation apparatus.

2.5. Antioxidative enzymatic activity determination

Antioxidative activity of superoxide dismutase (SOD) and

peroxidase (POD) enzymes in chickpea leaves was determined with the help of spectrophotometer according to established protocols of Beauchamp and Fridovich (1971) and Van-Assche et al. (1988), respectively.

2.6. Organic acids analysis

Organic acids concentrations in root exudates were determined using high performance liquid chromatography (HPLC) fitted with Flexer FX-10 isocratic pump (PerkinElmer, MA, USA). For this purpose, 20 μl of each sample was injected into the C_{18} column (Brownlee Analytical C-18 3 μm; 150 × 4.6 mm^2) after preparing them in 80% ethanol. Mobile phase was separated in the presence of 18 mM KH_2PO_4 solution, whereas pH of the solution was buffered to 2.1 with H_3PO_4. Retention time was adjusted to 10 min with a flow rate of 1 ml min^{-1} at 28 ± 2 °C. Organic acids were then analyzed with UV detector at a wavelength of 215 nm.

2.7. Statistical analysis

The experiments followed the completely randomized design (CRD) with factorial arrangements and data were checked for normal distribution prior to statistical analysis. ANOVA was obtained for significance and treatment means were compared using LSD and Tukey's tests of sigma stat packages (SPSS, Inc., Chicago, IL, USA) at $p \leq 0.05$ (Elliott and Woodward, 2007). We also obtained Pearson's correlation coefficients for studying the relationship among the measured parameters.

3. Results

3.1. Plant growth and leaf chlorophyll contents under the influence of P-deficiency and P. putida seed inoculation

Without inoculation, normal supply of P (+P) improved the shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and root dry weight: shoot dry weight of chickpea by 23, 31, 17, 33, 48, 43 and 15%, respectively as compared to the P-deficient treatment (Table 1). Factor 'PGPR' interacted with P-deficiency and improved the root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and root/shoot ratio by 25, 15, 30, 38, 39, and 11%, respectively. Consistently, interaction between 'PGPR' inoculation and '+P' improved the shoot length, root length, shoot fresh weight, root fresh weight, root dry weight and root dry weight: shoot dry weight by 26, 19, 14, 32, 30, and 38%, respectively as compared to '+P' alone. In general, *P. putida* seed inoculation in +P treatment resulted in the higher values for any of the measured plant growth parameters as compared to all other treatments. Interaction between 'PGPR' and '+P' also increased the leaf chlorophyll contents by 29%, but individually both factors remained insignificant.

3.2. Leaf nutrient content as influenced by P-deficiency and P. putida seed inoculation

Table 2 shows the data of minerals contents in chickpea leaves as influenced by P-deficiency and *P. putida* inoculation. Without inoculation, factor '+P' increased the P and Ca accumulation in chickpea leaves by 69 and 31%, whereas decreased the Mg uptake of plants by 15%. Use of PGPR under P-deficiency increased the leaf N, P, K and Ca content by 20, 30, 17 and 28%, whereas decreased the leaf Mg by 32% as compared to P-deficiency alone. Similarly, interaction between 'PGPR' and '+P' was significant for increasing the leaf N, P, and K contents by 22, 20, 25%. In comparison to inoculated P-deficient treatment, inoculated +P treatment only influenced the leaf P.

3.3. Soil pH as influenced by P-deficiency and P. putida seed inoculation

Without inoculation, '+P' resulted in the maximum value of soil pH as compared to the P-deficient treatment (Fig. 1). This increase in soil pH was 0.18 units over −P treatment. PGPR inoculation strongly interacted and decreased the soil pH by 0.18 and 0.17 units in both treatments as compared to their non-inoculated respective. Overall, soil pH was higher in '+P' treatment both with and without PGPR inoculation.

3.4. Antioxidative enzymatic activity of chickpea leaves as influenced by P-deficiency and P. putida seed inoculation

In the absence of *P. putida*, both P-deficient and normal supply of P did not exhibit any significant difference for SOD activity (Fig. 2a). On contrary, P-deficiency resulted in the higher POD activity when compared with the '+P' treatment (Fig. 2b). Interestingly, PGPR application in both P-deficient and normal P enhanced the SOD enzymatic activity to 1.56 and 1.57 μmol g^{-1} FW min^{-1} as compared to their un-inoculated respective, however, remained insignificant when compared with each other. Similar to SOD, PGPR supply in any treatment combinations enhanced the POD enzymatic activity as compared to their un-inoculated respective. In general, interaction between '+P' and 'PGPR' induced the antioxidant enzymatic activity of POD enzyme to 0.88 μmol g^{-1} FW min^{-1} as compared to the individual factors; 'PGPR' and '+P'.

Table 1
Combined effects of P and *P. putida* inoculation on various plant growth parameters. Values exhibit means ± standard error of three replicates, whereas case letters indicate significant differences among the treatments at $p \leq 0.05$ level.

Treatments	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g pot^{-1})	Shoot dry weight (g pot^{-1})	Root fresh weight (g pot^{-1})	Root dry weight (g pot^{-1})	Root dry weight: shoot dry weight	Chlorophyll content (SPAD values)
Non-inoculated								
−P	38.47 (±3.78)^{c}	19.47 (±1.79)^{c}	61.93 (±2.07)^{c}	17.00 (±1.43)^{b}	19.25 (±2.04)^{d}	8.36 (±1.47)^{c}	0.49 (±0.03)^{c}	27.39 (±1.88)^{b}
+P	50.05 (±2.48)^{b}	28.14 (±1.95)^{ab}	74.55 (±3.40)^{b}	25.42 (±1.58)^{a}	37.15 (±1.22)^{b}	14.82 (±1.26)^{b}	0.58 (±0.02)^{b}	27.7 (±1.15)^{b}
Inoculated								
−P	39.73 (±4.02)^{c}	26.15 (±1.91)^{bc}	73.03 (±3.76)^{b}	24.41 (±2.39)^{a}	31.33 (±2.73)^{c}	13.63 (±1.49)^{b}	0.55 (±0.02)^{bc}	28.79 (±2.64)^{b}
+P	67.91 (±3.32)^{a}	34.76 (±1.55)^{a}	87.10 (±2.50)^{a}	22.87 (±2.19)^{a}	55.06 (±2.12)^{a}	21.29 (±1.34)^{a}	0.93 (±0.03)^{a}	38.89 (±1.77)^{a}

Table 2
Nutrient content of chickpea leaves as influenced by P and *P. putida* inoculation. Values exhibit means ± standard error of three replicates, whereas case letters indicate significant differences among the treatments at $p \leq 0.05$ level.

Treatments	mg g^{-1} DW				
	N	P	K	Ca	Mg
Non-inoculated					
−P	23.76 (±1.46)^{b}	1.47 (±0.13)^{d}	22.08 (±1.27)^{b}	12.56 (±2.25)^{b}	3.11 (±0.15)^{a}
+P	24.87 (±1.94)^{b}	4.73 (±0.84)^{b}	20.50 (±1.94)^{b}	18.13 (±1.28)^{a}	2.64 (±0.40)^{ab}
Inoculated					
−P	29.90 (±1.14)^{a}	2.10 (±0.23)^{c}	26.68 (±2.95)^{a}	17.49 (±1.13)^{a}	2.12 (±0.16)^{b}
+P	32.12 (±2.21)^{a}	5.91 (±0.71)^{a}	27.42 (±3.56)^{a}	21.29 (±1.06)^{a}	2.28 (±0.19)^{b}

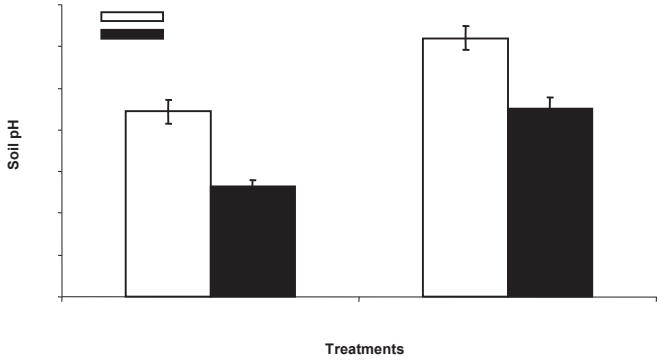

Fig. 1. Influence of *P. putida* seed inoculation on soil pH under P-deficiency. Values exhibit means ± standard error of three replicates, whereas case letters indicate significant differences among the treatments at $p \leq 0.05$ level.

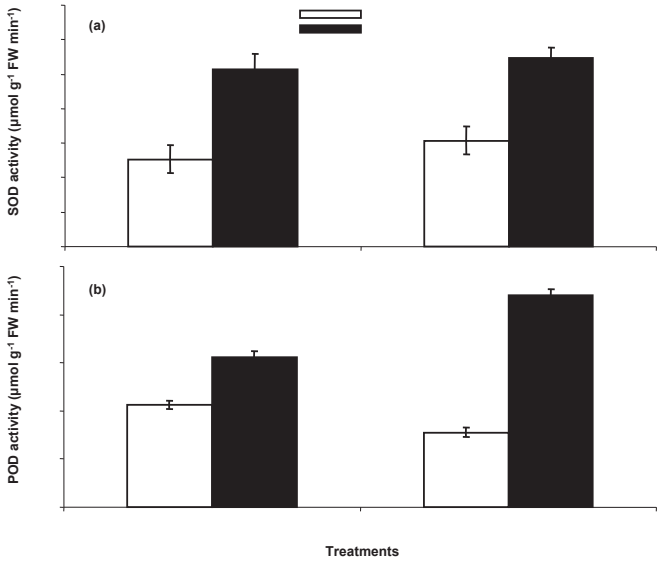

Fig. 2. Influence of *P. putida* seed inoculation on antioxidant enzymes activity of (a) SOD, and (b) POD in chickpea leaves under P-deficiency. Values exhibit means ± standard error of three replicates, whereas case letters indicate significant differences among the treatments at $p \leq 0.05$ level.

3.5. Organic acid concentrations in root exudates as influenced by P-deficiency and P. putida seed inoculation

Organic acids concentrations in root exudates of chickpea have been presented in Fig. 3. In non-inoculated conditions, application of P increased the citric acid and oxalic acid concentrations when compared with the P-deficiency. PGPR inoculation in any treatment combinations, particularly in combination with +P enhanced the concentrations of citric acid (0.0357 μmol g^{-1} root FW), malic acid (0.0257 μmol g^{-1} root FW) and oxalic acid (0.0190 μmol g^{-1} root FW) as compared to their non-inoculated respective. Overall, interaction between 'PGPR' and '+P' increased the citric acid and oxalic acid concentrations in root exudates as compared to the 'PGPR' alone.

3.6. Correlations among the studied parameters

Pearson's correlation coefficients were obtained for the conclusive relationship among the analyzed parameters at $^{*}p \leq 0.05$ and $^{**}p \leq 0.01$ levels (Table 3). Without PGPR, soil pH was correlated with leaf nutrient acquisition, particularly P (r = 0.947) and oxalic acid (r = 0.974), which then improved the plant growth parameters.

Similarly, under PGPR supply, soil pH was correlated with leaf N (r = 0.883), leaf P (r = 0.910) and citric acid (r = 0.838), which in turn enhanced the plant growth. Moreover, increased production of antioxidants, particularly POD activity was also correlated with improved plant growth under PGPR supply.

4. Discussion

4.1. P. putida seed inoculation enhances plant growth under P-deficiency

The current study revealed that PGPR inoculation under P-deficiency improved the growth of chickpea, which was comparable with individual factor '+P'. In the absence of *P. putida* seed inoculation, '+P' enhanced the root and shoot attributes as compared to the P-deficiency (Table 1). This confirms the essentiality of P in plant nutrition, as P plays a key role in plant growth functions like ATP synthesis and phosphorylation of photosynthetic proteins and enzymes (Zer and Ohad, 2003). Furthermore, growth enhancement by factor 'PGPR' was achieved due to the role of *P. putida* in releasing nutrients including P, however, its activity can be influenced where P is omitted or applied at normal rates. Alternatively, increased root and shoot weight could be attributed to the increased root proliferation (Tomar et al., 2004), which could thus enhance nutrient and water uptake of plants, as root exploration is dependent on succulence. Improved root and shoot biomass by *Pseudomonas* sp. have been reported previously (Mehnaz and Lazarovits, 2005; Yadav et al., 2010; Gopalakrishnan et al., 2014). Similarly, other studies also indicated that use of PGPR (Asghar et al., 2002) or PSB in combination with rock phosphate improved the plant growth (Han et al., 2006), however, PGPR response may vary to different plant species (Noumavo et al., 2013).

This is well known that bacteria inoculation may increase P-availability in soils through the root exudation of organic acids, which thus stimulate plant growth and enhance mineral uptake by plants (Park et al., 2003). The increase in leaf chlorophyll contents

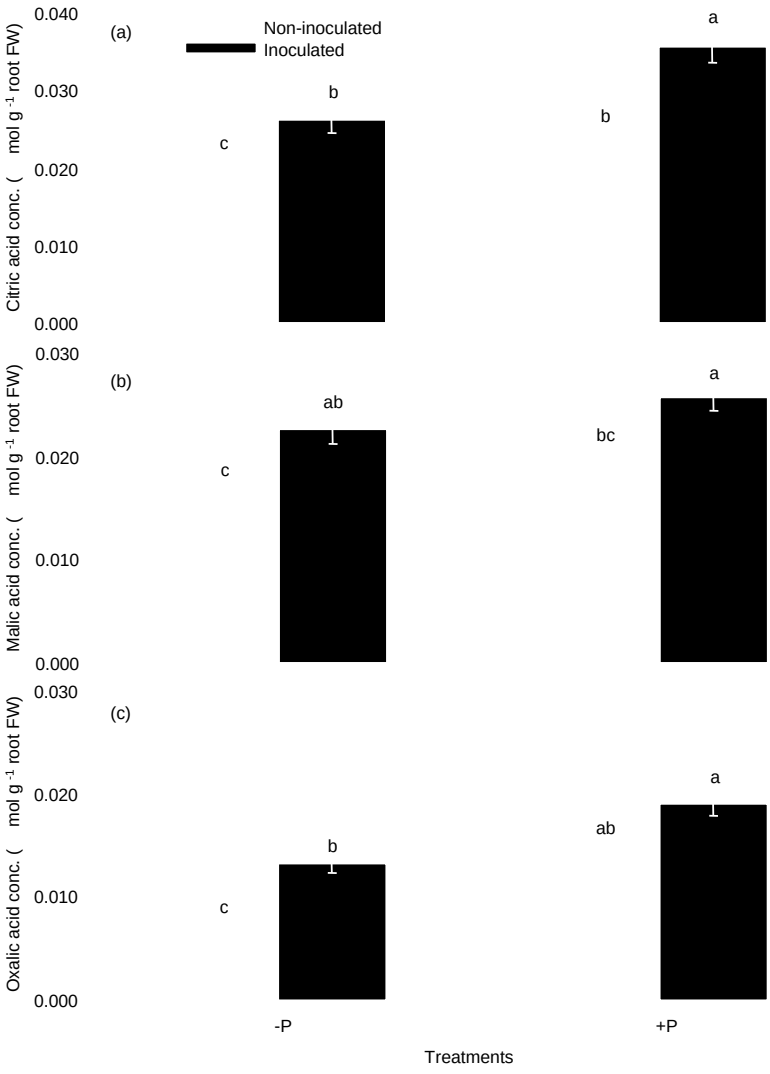

Fig. 3. In uence of P. putida seed inoculation on (a) citric acid (b) malic acid, (c) oxalic acid concentrations in root exudates under P-de ciency. Values exhibit means ± standard error of three replicates, whereas case letters indicate signi cant differences among the treatments at p 0.05 level.

by P. putida seed inoculation was correlated with the increased uptake of nutrients like N (r ¼ 0.812), P (r ¼ 0.956) and Ca (r ¼ 0.936). This happened mainly due to the reason that PGPR have N- xing capabilities, which can promote N uptake by plants and lead to the increase in leaf chlorophyll contents (Yildirim et al. (2011). Our results are in lines with Bashan et al. (2006) who observed an increase in leaf chlorophyll contents of wheat seedlings when inoculated with Azosprillium brasilense.

4.2. P. putida seed inoculation enhances nutrient content of chickpea leaves under P-de ciency

Numerous researchers have reported that PGPR inoculation increases bioavailability of macro- and micro-nutrients in various crops like chickpea (Elkoca et al., 2008), barley (Cakmakci et al., 2007), tomato (Adesemoye et al., 2010), lettuce (Lai et al., 2008), strawberry (Günes et al., 2009), and broccoli (Yildirim et al., 2011), however, mechanism is not completely known. For example, some researchers have suggested that PGPR-mediated enhancement in nutrient uptake by plants is attributed to the increased plant water uptake (Dey et al., 2004), which mediates the solubility of nutrients in soils (Dodd and Perez-Alfocea, 2012). Other studies reported that increase in nutrient uptake by plants is related to the increased root surface area (Yildirim et al., 2011), which enhances root exudation and microbial activity, thereby resulting in higher acquisition of nutrients by plant roots (Adesemoye et al., 2008).

In recent years, Ndakidemi et al. (2011) has suggested that soil micro-organisms greatly in uence the soil pH, which helps in maximum nutrient uptake by plants. In the present study, PGPR inoculation decreased the soil pH and correlated with the increase in nutrient uptake (Leaf N; r ¼ 0.883, Leaf P; r ¼ 0.910, and Leaf Ca; r ¼ 0.975), as higher pH both under e P and þ P resulted in the decreased leaf N, P, K and Ca contents as compared to their inoculated respective (Table 2). Higher uptake of P and Ca by chickpea plants with the application of P as compared to e P might involve Ca-P interactions in soil, as P supply enhanced the passive uptake of both elements. In this regard, Li et al. (2010) conducted a greenhouse experiment with celery and observed a decrease in Ca and Mg concentrations under low P and low Ca and Mg supply conditions as compared to suf cient levels of P. According to Valentine et al. (2001) , change in P-availability pattern or supply is coordinated with the alterations in nutrient uptake pattern of other essential elements, as P-availability in soils increases N uptake and its utilization in plants (Kim et al., 2003). If this is true, then

Table 3
Pearson's correlation coef cients exhibiting signi cant relationship among the measured parameters at * p 0.05 and **p 0.01 levels.

	pH	Shoot length	Root length	Shoot FW	Shoot DW	Root FW	Root DW	Root: Shoot	Leaf chlorophyll	Leaf N	Leaf P	Leaf K	Leaf Ca	Leaf Mg	SOD	POD	Citric acid	Malic acid	Oxalic acid
Non-inoculated																			
pH	1																		
Shoot length	0.826*	1																	
Root length	0.970**	0.803	1																
Shoot FW	0.998**	0.816*	0.978**	1															
Shoot DW	0.996**	0.866*	0.971**	0.994**	1														
Root FW	0.978**	0.887*	0.921**	0.973**	0.987**	1													
Root DW	0.994**	0.846*	0.987**	0.995**	0.996**	0.969**	1												
Root:Shoot	0.962**	0.703	0.980**	0.967**	0.948**	0.889*	0.968**	1											
Leaf chlorophyll	0.331	0.08	0.518	0.357	0.308	0.15	0.388	0.562	1										
Leaf N	0.661	0.408	0.801	0.682	0.644	0.513	0.708	0.828*	0.926**	1									
Leaf P	0.947**	0.921**	0.881*	0.940**	0.964**	0.992**	0.939**	0.830*	0.056	0.428	1								
Leaf K	0.185	0.375	0.02	0.158	0.212	0.366	0.127	0.077	0.865*	0.611	0.451	1							
Leaf Ca	0.989**	0.830*	0.994**	0.992**	0.990**	0.954**	0.998**	0.978**	0.439	0.746	0.919**	0.071	1						
Leaf Mg	0.507	0.471	0.305	0.485	0.512	0.635	0.44	0.293	0.614	0.29	0.675	0.900*	0.392	1					
SOD	0.668	0.45	0.816*	0.691	0.658	0.529	0.72	0.826*	0.915*	0.996**	0.452	0.592	0.757	0.286	1				
POD	0.689	0.725	0.524	0.668	0.709	-0.812*	0.646	0.48	0.449	0.08	-0.854*	0.835*	0.602	0.948**	0.062	1			
Citric acid	0.793	0.745	0.857*	0.786	0.792	0.712	0.826*	0.846*	0.656	0.842*	0.672	0.256	0.845*	0.036	0.847*	0.24	1		
Malic acid	0.747	0.907*	0.816*	0.763	0.792	0.769	0.796	0.688	0.319	0.571	0.793	0.09	0.796	0.191	0.628	0.466	0.742	1	
Oxalic acid	0.974**	0.816*	0.940**	0.980**	0.972**	0.968**	0.963**	0.910*	0.246	0.583	0.949**	0.261	0.954**	0.562	0.6	0.721	0.695	0.779	1
Inoculated																			
pH	1																		
Shoot length	0.902*	1																	
Root length	0.990**	0.843*	1																
Shoot FW	0.997**	0.927**	0.977**	1															
Shoot DW	0.117	0.469	0.004	0.197	1														
Root FW	0.960**	0.972**	0.918**	0.979**	0.39	1													
Root DW	0.998**	0.925**	0.981**	0.999**	0.173	0.974**	1												
Root:Shoot	0.947**	0.962**	0.905*	0.970**	0.425	0.998**	0.963**	1											
Leaf chlorophyll	0.991**	0.947**	0.965**	0.998**	0.249	0.989**	0.997**	0.981**	1										
Leaf N	0.883*	0.629	0.926**	0.841*	0.363	0.716	0.855*	0.687	0.812*	1									
Leaf P	0.910*	0.961**	0.857*	0.941**	0.515	0.988**	0.931**	0.995**	0.956**	0.61	1								
Leaf K	0.616	0.27	0.697	0.549	0.711	0.371	0.57	0.333	0.504	0.914*	0.236	1							
Leaf Ca	0.975**	0.803	0.989**	0.953**	0.106	0.874*	0.961**	0.853*	0.936**	0.965**	0.796	0.775	1						
Leaf Mg	0.484	0.65	0.457	0.48	0.122	0.5	0.506	0.454	0.507	0.414	0.433	0.259	0.476	1					
SOD	0.651	0.285	0.744	0.589	0.644	0.42	0.606	0.395	0.545	0.913*	0.301	0.970**	0.793	0.174	1				
POD	0.883*	0.893*	0.838*	0.915*	0.508	0.957**	0.900*	0.975**	0.925**	0.584	0.982**	0.219	0.766	0.264	0.318	1			
Citric acid	0.838*	0.837*	0.773	0.869*	0.45	0.897*	0.850*	0.908*	0.871*	0.567	0.917**	0.231	0.731	0.154	0.27	0.932**	1		
Malic acid	0.734	0.799	0.729	0.73	0.155	0.73	0.743	0.697	0.743	0.621	0.67	0.401	0.706	0.805	0.405	0.57	0.458	1	
Oxalic acid	0.698	0.822*	0.659	0.738	0.65	0.827*	0.725	0.844*	0.765	0.344	0.869*	0.024	0.55	0.369	0.106	0.871*	0.717	0.715	1

increased leaf N content would be expected in non-inoculated þP-treatment. This happened only in inoculated treatments (Table 2), which suggest that PGPR strain used in our study has the potential to fix atmospheric N even under P-deficiency, however, P-supply may enhance this mechanism. This is well supported by the findings of Han et al. (2006) who reported an increase in N uptake by pepper and cucumber plants was related to the plant inoculation with PSB along with rock phosphate addition.

Combined use of P. putida and P were effective in increasing P, Ca, and K content in whole leaf when compared with the un-inoculated þP treatment. Similar to þP treatment, PGPR also produced similar results under eP conditions. Higher uptake of nutrients by chickpea plants was achieved due to mobilization of elements, particularly Ca and P from calcium-phosphate minerals. Furthermore, PGPR might have similar mechanism of action for the release of K and other minerals. Similar results have been reported previously by Mia et al. (2010), who treated banana plants with chemical fertilizers and PGPR and observed positive effects on Ca, Mg, K uptake by plants. Accordingly, Liu et al. (2013) reported an increase in the uptake of N, P and K by PGPR inoculation of Fraxinus americana seeds as compared to the control or fertilizers treated plants. As PGPR are good mobilizers of nutrients, they enhance nutrient uptake by plants, subsequently, accumulate in their tissues. On the other hand, decrease in leaf Mg in un-inoculated þP treatment as compared to P-deficiency might have resulted due to competition mechanism of cation uptake. PGPR inoculation further increased the leaf Ca content, whereas decreased leaf Mg in any treatment combination (Table 2). This shows that Ca-P interactions are pre-dominant in soil and plant systems, which could thus compete with other cations like Mg. Several researchers have suggested competition mechanism between Ca and Mg for their uptake (Carvajal et al., 1999; Paiva et al., 1998; Hao and Papadopoulos, 2003).

4.3. Decrease in soil pH enhances P uptake of inoculated chickpea under P-deficiency

This is the first study to investigate the effects of PGPR on P uptake in response to decrease in soil pH and root exudation of organic acids. Studies on P-deficiency in plants have determined the effects of PGPR inoculation on P uptake either in relation to the soil pH or root exudation of various organic acids, but not together. For example, study by Cakmakci et al. (2007) observed higher uptake of P with the decrease in soil pH when barley plants were inoculated with Bacillus megaterium RC01 and Bacillus M-13. In other studies, phosphate solubilizing microbes enhanced the P uptake of different plant species through the production of low molecular weight organic acids (Kim et al., 1997; Jones, 1998; Khan et al., 2006), which can lower the soil pH and thus influence the solubility of insoluble phosphate.

In general, solubility of Ca-P increases with the decrease in soil pH (Gahoonia et al., 1992). In the current study, lowest value of soil pH was observed in inoculated P-deficient treatment as compared to the other treatments (Fig. 1). The decrease in soil pH was correlated with the enhanced production of organic acids, particularly, oxalic acid ($r = 0.974$; Table 3). This is evident from the literature that P-solubilization in soil can be enhanced by bacteria or PGPR via root exudation of organic acids (de Freitas et al., 1997; Pal, 1998; Vessey, 2003). Further, these organic acids may chelate phosphates bonded cations and solubilize insoluble phosphates after releasing their hydroxyl or carboxyl groups (Kpomblekou and Tabatabai, 1994). We observed highest value of pH in non-inoculated þP treatment as compared to all other treatments. This might be due to the fact that P application reacted with Ca in soils and adsorbed onto the surface of soil particles. The opposite was observed when þP was combined with PGPR inoculation. This is an indicative of Ca-P solubility in soils through the induced secretion of root exudates, which could further reduce the soil pH.

4.4. P. putida seed inoculation enhances antioxidant enzymes activities in chickpea leaves under P-deficiency

The present study was carried out to determine whether PGPR inoculation contributes in antioxidative adjustments of plants or not, as deficiency of mineral elements in plants results in oxidative stress production. During oxidative stress, ROS cause impairment in mineral nutrition of plants, which results in photo-oxidative damage (Cakmak, 2005). We measured activity of major antioxidant enzymes like SOD and POD, as SOD is the first antioxidant enzyme that catalyses the dismutation of O_2 to H_2O_2 (Alscher et al., 2002), and H_2O_2 is further scavenged into H_2O and O_2 by peroxidases like APX and GPX (Wang et al., 2009). In the current study, non-inoculated P-deficient treatment induced POD activity, but not SOD as compared to the þP treatment. This is an indicative of H_2O_2 production under P-deficiency. Our results are in accordance with the findings of Wan et al. (2006) for POD, but not for SOD, who supplied different concentrations of P to tomato and observed an increase in antioxidative activity of SOD and POD enzymes. Similarly, Chen et al. (2015) and Tewari et al. (2007) also observed higher SOD activity under short-term and long-term P-starvation. These differences might occur due to genotypic variations in plants or quick conversion of O_2 to H_2O_2. Similar to non-inoculated treatments, inoculated treatments either P-deficient or þP remained insignificant for SOD activity, but enhanced the POD activity (Fig. 2a, b). Higher SOD and POD activities in both treatments in combination with PGPR suggest that PGPR have role in the adaptation of plants through the induced antioxidant production. There is increasing evidence that PGPR confer tolerance in plants to various abiotic stresses like boron toxicity either alone (Sirajuddin et al., 2016) or in combination with salinity (Khan et al., 2016). Hence, PGPR can use similar strategy and enhance the antioxidative activity of various enzymes.

4.5. P. putida seed inoculation enhances root exudation under P-deficiency

Root exudation of organic acids increases the mobility of nutrients such as P, Fe, Zn and Mn (Zhang et al., 1997). Among the root exudates, citric acid has been detected frequently that mobilizes P in soils mainly by ligand exchange, dissolution and occupation of P sorption sites (Fox et al., 1990; Gerke, 1995). Without PGPR inoculation, P-deficiency remained insignificant for citric acid and malic acid concentrations in root exudates even though it decreased the oxalic acid concentration as compared to the þP (Fig. 3). In general, þP exhibited correlations between citric acid and soil pH ($r = 0.838$; Table 3). It seems that P-deficiency does not stimulate plant roots to excrete organic acids rather can be responsive to additional P. On the other hand, this could be resulted due to the fact that net release of protons in roots is compensated with excess uptake of cations (Dinkelaker et al., 1989; Le Bot et al., 1990) and net concomitant root exudation (Neumann and Römheld, 1999). Several authors have reported similar results where P-starvation did not contribute in rhizosphere acidification and exudation of carboxylic acids (Neumann and Römheld, 1999; Zhang et al., 1997) even decreased the concentration of organic acids in tomato (Neumann and Römheld, 1999). Contrary, researchers have suggested that increased uptake of P in plants is due to root exudation of low molecular weight organic acids (Kim et al., 1997; Jones, 1998; de Freitas et al., 1997; Pal, 1998; Vessey, 2003), but they did not report organic acids concentrations. This is the first study to detect

organic acids concentration in root exudates of inoculated plants grown under P-de cient or þ P conditions. In our study, PGPR alone or in combination with þ P contributed in rhizosphere acidi cation through the enhanced root exudation of organic acids, especially oxalic acid (r ¼ 0.974; Table 3). This happened mainly because of root-induced chemical modi cations in rhizosphere in the presence of PGPR. Our results are in agreement with the ndings of Hoberg et al. (2005) who observed 3 e 10 times increase in organic acids concentrations in the media of P. ourescenswhen compared with the Gordonia sp. It is expected that PGPR strain used in our study also operated similar mechanism in rhizosphere, and enhanced the root exudation.

5. Conclusions

Individually, both 'þ P' and 'PGPR' improved the plant growth, but with different mechanisms. As increased root exudation of citric acid and oxalic acid in þ P treatment was not effective for rhizosphere acidi cation, growth enhancement occurred only due to increased uptake of nutrients like P and Ca. Factor 'PGPR' either alone or in combination with 'þ P' improved the plant growth, plant uptake of N, P, and K, antioxidative activity of SOD and POD enzymes and concentrations of organic acids, whereas reduced the rhizosphere soil pH. Therefore, growth enhancement by 'PGPR' was related to higher plant antioxidation activity as well as nutrient uptake of chickpea including P as a result of increased root exudation and rhizosphere acidi cation. Overall, interaction between 'PGPR' and 'þ P' resulted in higher exudation, which enhanced the P uptake and increased maximum of the growth attributes as compared to the 'þ P'. We suggest that particular PGPR strain not only enhances P uptake, but also improves the N and K uptake by plants grown on high pH soils, which can thus reduce the application of chemical fertilizers.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.plaphy.2016.07.023 .

Con ict of interest

The authors declare that there is no con ict of interest.

Author contribution statement

DI and SM designed and conducted the experiments. GM contributed in soil and plant nutrient analyses. MS and SM analyzed organic acids concentration. KSK and NA critically reviewed the manuscript. Moreover, KSK provided laboratory facilities for basic soil analysis. All the authors read and approved the manuscript.

References

Abel, S., Ticconi, C.A., Delatorre, C.A., 2002. Phosphate sensing in higher plants. Physiol. Plant 115, 1e8.
Adesemoye, A.O., Torbert, H.A., Kloepper, J.W., 2010. Increased plant uptake of nitrogen from ^{15}N-depleted fertilizer using plant growth promoting rhizobacteria. Appl. Soil Ecol. 46, 54e58.
Adesemoye, A., Torbert, H., Kloepper, J., 2008. Enhanced plant nutrient use ef - ciency with PGPR and AMF in an integrated nutrient management system. Can. J. Microbiol. 54, 876e886.
Afzal, A., Bano, A., Fatima, M., 2010. Higher soybean yield by inoculation with N- xing and P-solubilizing bacteria. Agron. Sustain. Dev. 30, 487 e495.
Alscher, R.G., Erturk, N., Heath, L.S., 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J. Exp. Bot. 53, 1331 e1341.
Asghar, H., Zahir, Z., Arshad, M., Khaliq, A., 2002. Relationship between in-vitro production of auxins by rhizobacteria and their growth promoting activities in Brassica junceaL. Biol. Fert. Soils 35, 231e237.
Bargaz, A., Faghire, M., Farissi, M., Drevon, J.J., Ghoulam, C., 2013. Oxidative stress in the root nodules of Phaseolus vulgarisis induced under conditions of phosphorus de ciency. Acta Physiol. Plant 35, 1633e1644.
Bashan, Y., Holguin, G., de-Bashan, L.E., 2006.Azospirillum-plant relationships: physiological, molecular, agricultural, and environmental advances. Can. J. Microbiol. 50, 521 e577.
Beauchamp, C., Fridovich, I., 1971. Superoxide dismutase: improved assay and an assay applicable to acrylamide gels. Anal. Biochem. 44, 276 e287.
Cakmakci, R., Donmez, M.F., Erdogan, U., 2007. The effect of plant growth promoting rhizobacteria on barley seedling growth, nutrient uptake, some soil properties, and bacterial counts. Turk. J. Agric. 31, 189e199.
Cakmak, I., 2005. Role of mineral nutrients in tolerance of crop plants to environmental stress factors. In: Proceedings from the International Symposium on Fertigation-optimizing the Uilization of Water and Nutrients. International Potash Institute, Horgen, pp. 35 e48.
Carvajal, M., Martinez, V., Cerda, A., 1999. In uence of magnesium and salinity on tomato plants grown in hydroponics culture. J. Plant Nutr. 22, 177 e190.
Chen, S., Zhao, H., Ding, G., Xu, F., 2015. Genotypic differences in antioxidant response to phosphorus de ciency in Brassica napus Plant Soil 391, 19e32.
de Freitas, J.R., Banerjee, M.R., Germida, J.J., 1997. Phosphatesolubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (Brassica napusL.). Biol. Fert. Soils 24, 358e364.
Del-Rio, L.A., Corpas, F.J., Sandalio, L.M., Palma, J.M., Barroso, J.B., 2003. Plant peroxisomes, reactive oxygen metabolism and nitric oxide. IUBMB Life 55, 71 e81.
Deubel, A., Gransee, A., Merbach, W., 2000. Transformation of organic rhizodeposits by rhizoplane bacteria and its in uence on the availability of tertiary calcium phosphate. J. Plant Nutr. Soil Sci. 163, 387e392.
Dey, R., Pal, K., Bhatt, D., Chauhan, S., 2004. Growth promotion and yield enhancement of peanut (Arachis hypogaeaL.) by application of plant growth promoting rhizobacteria. Microbiol. Res. 159, 371 e394.
Dinkelaker, B., Römheld, V., Marschner, H., 1989. Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (Lupinus albus L.). Plant Cell Environ. 12, 285 e292.
Dodd, I.C., Perez-Alfocea, F., 2012. Microbial amelioration of crop salinity stress. J. Exp. Bot. 63, 3415e3428.
Elkoca, E., Kantar, F., Sahin, F., 2008. Inuence of nitrogen xing and phosphorus solubilizing bacteria on the nodulation, plant growth, and yield of chickpea. J. Plant Nutr. 31, 157e171.
Elliott, A.C., Woodward, W.A., 2007. Statistical Analysis Quick Reference Guide Book with SPSS Examples.
El-Tarabily, K.A., 2008. Promotion of tomato (Lycopersicon esculentumMill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing Streptomycete actinomycetes Plant Soil 308, 161e174.
Figueiredo, M.V.B., Martinez, C.R., Burity, H.A., Chanway, C.P., 2008. Plant growth-promoting rhizobacteria for improving nodulation and nitrogen xation in the common bean (Phaseolus vulgarisL.). World J. Microbiol. Biotechnol. 24, 1187e1193.
Fox, T.R., Comerford, N.B., McFee, W.W., 1990. Phosphorus and aluminium release from a spodic horizon mediated by organic acids. Soil Sci. Soc. Am. J. 54, 1763e1767.
Gahoonia, T.S., Claassen, N., Jungk, A., 1992. Mobilization of phosphate in different soils by ryegrass supplied with ammonium or nitrate. Plant Soil 140, 241 e248.
Gaind, S., Gaur, A.C., 1991. Thermotolerant phosphate solubilizing microorganisms and their interaction with mung bean. Plant Soil 133, 141 e149.
Gaur, A.C., 1990. Physiological functions of phosphate solubilizing microorganisms. In: Gaur, A.C. (Ed.), Phosphate Solubilizing Micro-organisms as Biofertilizers. Omega Scienti c Publishers, New Delhi, pp. 16 e72.
Gerke, J., 1995. Chemische Prozesse der Nährstoffmobilisierung in der Rhizosph äre und ihre Bedeutung für den Übergang vom Boden in die P anze. Cuvillier Verlag, Göttingen .
Gopalakrishnan, S., Vadlamudi, S., Apparla, S., Bandikinda, P., Vijayabharathi, R., Bhimineni, R., Rupela, O., 2013. Evaluation of Streptomyces sp. for their plant growth promotion traits in rice. Can. J. Microbiol. 59, 534 e539.
Gopalakrishnan, S., Srinivas, V., Prakash, B., Sathya, A., Vijayabharathi, R., 2014. Plant growth-promoting traits of Pseudomonas geniculata isolated from chickpea nodules. 3 Biotech. 1e9.
Grossman, A., Takahashi, H., 2001. Macronutrient utilization by photosynthetic eukaryotes and the fabric of interactions. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52, 163e210.
Günes, A., Atatoglu, N., Turan, M., Esitken, A., Ketterings, Q.M., 2009. Effects of phosphate solubilizing microorganisms on strawberry yield and nutrient concentrations. J. Plant Nutr. Soil Sci. 172, 385e392.
Gyaneshwar, P., Kumar, G.N., Parekh, L.J., Poole, P.S., 2002. Role of soil microorganisms in improving P nutrition of plants. Plant Soil 245, 83 e93.
Han, H.S., Supanjani, Lee, K.D., 2006. Effect of co-inoculation with phosphate and

potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant Soil Environ. 52, 130 e136.
Hao, X., Papadopoulos, A.P., 2003. Effects of calcium and magnesium on growth, fruit yield and quality in a fall greenhouse tomato crop grown on rockwool. Can. J. Plant Sci. 83, 903e912.
Hao, X., Cho, C.M., Racz, G.J., Chang, C., 2002. Chemical retardation of phosphate diffusion in an acid soil as affected by liming. Nutr. Cycl. Agroecosys 64, 213e224.
Hoberg, E., Marschner, P., Lieberei, R., 2005. Organic acid exudation and pH changes by Gordonia sp. and Pseudomonas uorescens grown with P adsorbed to goethite. Microbiol. Res. 160, 177 e187.
Jones, D.L., 1998. Organic acids in the rhizosphere -a critical review. Plant Soil 205, 25e44.
Juszczuk, I., Malusa, E., Rychter, A.M., 2001. Oxidative stress during phosphate de ciency in roots of bean plants (Phaseolus vulgaris L.). J. Plant Physiol. 158, 1299e1305.
Kandlbinder, A., Finkemeier, I., Wormuth, D., 2004. The antioxidant status of photosynthesizing leaves under nutrient de ciency: redox regulation, gene expression and antioxidant activity in Arabidopsis thaliana. Physiol. Plant 120, 63e73.
Khan, M.S., Zaidi, A., Wani, P.A., 2006. Role of phosphate solubilizing microorganisms in sustainable agriculture-A review. Agron. Sustain. Dev. 26, 1 e15.
Khan, A., Jilani, G., Akhtar, M.S., Naqvi, S.M.S., Rasheed, M., 2009. Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. J. Agric. Biol. Sci. 1, 48e58.
Khan, A., Sirajuddin, Zhao, X.Q., Javed, M.T., Khan, K.S., Bano, A., Shen, R.F., Masood, S., 2016.Bacillus pumilus enhances the tolerance in rice (Oryza sativa L.) to combined stresses of NaCl and high boron due to limited uptake of Na $^{+}$. Environ. Exp. Bot. 124, 120e129.
Kim, K.Y., Jordan, D., McDonald, G.A., 1997. Solubilization of hydroxyapatite by Enterobacter agglomerans and cloned Escherichia coli in culture medium. Biol. Fert. Soils 24, 347e352.
Kim, T., Jung, W., Lee, B., Yoneyama, T., Kim, H., Kim, K., 2003. P effects on N uptake and remobilization during regrowth of Italian ryegrass (Lolium muti orum). Environ. Exp. Bot. 50, 233e242.
Kpomblekou, K., Tabatabai, M.A., 1994. Effect of organic acids on release of phosphorus from phosphate rocks. Soil Sci. 158, 442 e453.
Laheurte, F., Berthelin, J., 1988. Effect of phosphate solubilizing bacteria on maize growth and root exudation over four levels of labile phosphorus. Plant Soil 105, 11e17.
Lai, W.A., Rekha, P.D., Arun, A.B., Young, C.C., 2008. Effect of mineral fertilizer, pig manure, and Azospirillum rugosum on growth and nutrient contents of Lactuca sativa L. Biol. Fert. Soils 45, 155e164.
Le Bot, J., Alloush, G.A., Kirkby, E.A., Sanders, F.E., 1990. Mineral nutrition of chickpea plants supplied with NO_3 or NH_4-N. II. Ionic balance in relation to phosphorus stress. J. Plant Nutr. 13, 1591e1601.
Leinhos, V., Vacek, O., 1994. Biosynthesis of auxins by phosphate-solubilizing rhizobacteria from wheat (Triticum aestivum) and rye (Secale cereale). Microbiol. Res. 149, 31e35.
Li, Y., Wang, T., Li, J., Ao, Y., 2010. Effect of phosphorus on celery growth and nutrient uptake under different calcium and magnesium levels in substrate culture. Hort. Sci. (Prague) 37, 99e108.
Liu, F., Xing, S., Ma, H., Du, Z., Ma, B., 2013. Plant growth-promoting rhizobacteria affect the growth and nutrient uptake of Fraxinus americana container seedlings. Appl. Microbiol. Biotechnol. 97, 4617 e4625.
Lynch, J.P., Brown, K.M., 2008. Root strategies for phosphorus acquisition. Plant Ecophysiol. 7, 83e116.
Masood, S., Saleh, L., Witzel, K., Plieth, C., Mühling, K.H., 2012. Determination of oxidative stress in wheat leaves as in uenced by boron toxicity and NaCl stress. Plant Physiol. Biochem. 56, 56e61.
Mehnaz, S., Lazarovits, G., 2005. Inoculation effects of Pseudomonas putida, Gluconacetobacter azotocaptans, and Azospirillum lipoferum on corn plant growth under greenhouse conditions. Microb. Ecol. 51, 326 e335.
Mia, M.A.B., Shamsuddin, Z.H., Wahab, Z., Marziah, M., 2010. Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue-cultured Musa plantlets under nitrogen-free hydroponics condition. Aus. J. Crop Sci 4, 85e90.
Moodie, C.E., Smith, H.W., MacGreery, R.A., 1959. Laboratory Manual of Soil Fertility. State College Washington, Washington (DC), p. 175. Mimeograph, Pullman .
Nassar, A.H., El-Tarabily, K.A., Sivasithamparam, K., 2003. Growth promotion of bean (Phaseolus vulgaris L.) by a polyamine producing isolate of Streptomyces griseoluteus Plant Growth Regul. 40, 97 e106.
Ndakidemi, P.A., Bambara, S., Makoi, J.H.J.R., 2011. Micronutrient uptake in common bean (Phaseolus vulgaris L.) as affected by rhizobium inoculation, and the supply of molybdenum and lime. Plant Omics 4, 40 e52.
Neumann, G., Römheld, V., 1999. Root excretion of carboxylic acids and protons in phosphorus-de cient Plants. Plant Soil 211, 121 e130.
Noumavo, P.A., Kochoni, E., Didagbe, Y.O., Adjanohoun, A., Allagbe, M., Sikirou, R., Gachomo, E.W., Kotchoni, S.O., Baba-Moussa, L., 2013. Effect of different plant growth promoting rhizobacteria on maize seed germination and seedling development. Am. J. Plant Sci. 4, 1013e1102.
Paiva, E.A.S., Sampaio, R.A., Martinez, H.E.P., 1998. Composition and quality of tomato fruit cultivated in nutrient solution containing different calcium concentrations. J. Plant Nutr. 21, 2653 e2661.
Pal, S.S., 1998. Interaction of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. Plant Soil 198, 169 e177.
Park, M., Singvilay, O., Seok, Y., Chung, J., Ahn, K., Sa, T., 2003. Effect of phosphate solubilizing fungi on P uptake and growth to tobacco in rock phosphate applied soil. Korean J. Soil. Sci. Fert. 36, 233e238.
Panhwar, Q.A., Othman, R., Rahman, Z.A., Meon, S., Ismail, M.R., 2012. Isolation and characterization of phosphate-solubilizing bacteria from aerobic rice. Afr. J. Biotechnol. 11, 2711 e2727.
Peix, A., Rivas-Boyero, A.A., Mateos, P.F., RodriguezBarrueco, C., Martinez-Molina, E., Velaquez, E., 2001. Growth promotion of chickpea and barley by a phosphate solubilizing strain of Mesorhizobium mediteraneum under growth chamber conditions. Soil Biol. Biochem. 33, 103 e110.
Ponmurugan, P., Gopi, C., 2006. Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. J. Agron. 5, 600e604.
Sadeghi, A., Karimi, E., Dahazi, P.A., Javid, M.G., Dalvand, Y., Askari, H., 2012. Plant growth promoting activity of an auxin and siderophore producing isolate of Streptomyces under saline soil condition. World J. Microbiol. Biotechnol. 28, 1503e1509.
Schachtman, D.P., Reid, R.J., Ayling, S.M., 1998. Phosphorus uptake by plants: from soil to cell. Plant Physiol. 116, 447 e453.
Sirajuddin, Khan, A., Ali, L., Chaudhary, H.J., Munis, M.F.H., Bano, A., Masood, S., 2016. Bacillus pumilus alleviates boron toxicity in tomato (Lycopersicum esculentum L.) due to enhanced antioxidant enzymatic activity. Sci. Hortic. 200, 178e185.
Tewari, R.K., Kumar, P., Sharma, P.N., 2007. Oxidative stress and antioxidant responses in young leaves of mulberry plants grown under nitrogen, phosphorus or potassium de ciency. J. Integr. Plant Biol. 49, 313e322.
Tokala, R.K., Strap, J.L., Jung, C.M., Crawford, D.L., Salove, H.M., Deobald, L.A., Bailey, J.F., Morra, M.J., 2002. Novel plant-microbe rhizosphere interaction involving Streptomyceslydicus WYEC108 and the pea plant (Pisum sativum). Appl. Environ. Microbiol. 68, 2161 e2171.
Tomar, S.S., Singh, R., Singh, S.P., 2004. Response of phosphorus, sulphur and Rhizobium inoculation on growth, yield and quality of soybean (Glycine max L.). Prog. Agric. 4, 72e73.
udDin, I., Bano, A., Masood, S., 2015. Chromium toxicity tolerance of Solanum nigrum L. and Parthenium hysterophorus L. plants with reference to ion patter, antioxidation activity and root exudation. Ecotoxicol. Environ. Safe 113, 271 e278.
Valentine, A.J., Osborne, B.A., Mitchell, D.T., 2001. Interactions between phosphorus supply and total nutrient availability on mycorrhizal colonization, growth and photosynthesis of cucumber. Sci. Hortic. 88, 177 e189.
Van-Assche, F., Cardinaels, C., Clijsters, H., 1988. Induction of enzyme capacity in plants as a result of heavy metal toxicity: dose-response relations in Phaseolus vulgaris L. treated with zinc and cadmium. Environ. Pollut. 52, 103 e115.
Vance, C.P., Uhde-Stone, C., Allan, D.L., 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol. 157, 423 e447.
Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255, 571e586.
Wahid, O.A.A., Mehana, T.A., 2000. Impact of phosphatesolubilizing fungi on the yield and phosphorus uptake by wheat and faba bean plants. Microbiol. Res. 155, 221e227.
Wan, Y., Zhu, L., Yang, S., Yang, Z., Zhu, W., 2006. Effects of P de ciency on protective enzymes activity and membrane lipid peroxidation in different tomato genotypes. In: ISHS Acta Horticulturae 856: International Symposium on Vegetable Safety and Human Health .
Wang, W.B., Kim, Y.H., Lee, H.S., Kim, K.Y., Deng, X.P., Kwak, S.S., 2009. Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. Plant Physiol. Biochem. 47, 570 e577.
Yadav, J., Verma, J.P., Tiwari, K.N., 2010. Effect of plant growth promoting rhizobacteria on seed germination and plant growth of chickpea (Cicer arietinum L.) under in Vitro conditions. Biol. Forum-An Int. J. 2, 15 e18.
Yasmin, H., Bano, A., 2011. Isolation and characterization of phosphate solubilizing bacteria from rhizospheric soil of weeds of Khewra Salt Range and Attock. Pak. J. Bot. 43, 1663e1668.
Yildirim, E., Karlidag, H., Turan, M., Dursun, A., Goktepe, F., 2011. Growth, nutrient uptake, and yield promotion of broccoli by plant growth promoting rhizobacteria with manure. Hort. Sci. 46, 932 e936.
Zhang, F.S., Ma, J., Cao, Y.P., 1997. Phosphorus de ciency enhances root exudation of low-molecular weight organic acids and utilization of sparingly soluble inorganic phosphate by radish (Raghanus sativus L.) and rape (Brassica napus L.) plants. Plant Soil 196, 261 e264.
Zer, H., Ohad, I., 2003. Light, redox state, thylakoid-protein phosphorylation and signalling gene expression. Trends Biochem. Sci. 28, 467 e470.