Pak. J. Bot., 40(4): 1735-1441, 2008.

INTEGRATED USE OF PLANT GROWTH PROMOTING BACTERIA AND P-ENRICHED COMPOST FOR IMPROVING GROWTH, YIELD AND NODULATION OF CHICKPEA

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Abstract

The plant growth promoting bacteria (PGPB) may improve growth and nodulation of leguminous crops through their ACC-deaminase activity. Their plant growth promoting activity can further be improved by adding recycled organic material which could act as a carrier to enhance microbial activities under field conditions. Three bacterial strains with ACC-deaminase were evaluated for their potentials to improve growth, yield and nodulation of chickpea (*Cicer arientum* L.) in the presence and absence of composted organic material enriched with P, both under pot and field conditions. The integrated use of PGPB and P-enriched compost resulted in a highly significant increase in fresh biomass (84%), number of pods plant^{-1} (97%), grain yield (79%) and number of nodules plant^{-1} (87%) compared to uninoculated control (without compost). The findings imply that inoculation with PGPB containing ACC-deaminase in the presence of P-enriched compost could be highly effective in improving growth, yield and nodulation of chickpea than sole application of either PGPB or P-enriched compost.

Introduction

The soil bacteria influencing the plant growth positively by any metabolic process are referred to as plant growth-promoting bacteria (PGPB) (Reid, 1995**;** Arshad & Frankenberger, 1998). PGPB use different mechanisms of action to improve plant growth and health which could be active either simultaneously or sequentially at different stages of plant growth. Some bacteria have been found to stimulate plant growth through their 1-aminocyclopropane-1-carboxylate (ACC)-deaminase activities (Glick *et al.,* 1998; Shaharoona *et al.,* 2006a, b). ACC-deaminase lowers ethylene levels in plants by converting ACC into α-ketobutyrate and ammonia, which is in contrast to activity of the enzyme ACC-synthase or ACC-oxidase, the latter are known to promote ethylene synthesis. Since higher concentrations of ethylene have been reported to inhibit root growth and nodulation (Nukui *et al.,* 2000**;** Arshad & Frankenberger, 2002), the bacteria containing ACC-deaminase may completely or partially eliminate potential inhibitory effects of higher ethylene concentrations in plants (Glick *et al.,* 1998).

Low C: N ratio organic material in the plant sphere can provide a niche for bacteria to enhance their growth and activities. Thus application of humus like organic material in the soil could have positive effect on plant root growth by supporting bacterial growth (Ahmad *et al.*, 2007; Zahir *et al.*, 2007). In addition, microbial activities can be made more efficient by maintaining high bacterial populations in the rhizosphere of a plant throughout the life cycle (Pooran *et al.,* 2002; Bajpai *et al.,* 2002; Cheuk *et al.,* 2003).

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Thus integrated use of PGPB with ACC-deaminase and value-added composted organic waste could be highly effective in improving yield and nodulation of chickpea crop.

The present study illustrates the effectiveness of inoculation with PGPB containing ACC-deaminase in the presence and absence of compost enriched with P in improving growth, yield and nodulation of chickpea under net house (pot) and field conditions.

Materials and Methods

Preparation of inoculum: Test tubes containing 150 mL broth of DF salt minimal medium containing ACC as a sole source of N were inoculated with three pre-isolated strains of PGPB such as *Enterobactor gergoviae* (J107), *Pantoea dispersa* (J112), and *Serratia oderifera* (J118). Inoculated tubes were incubated at 28±1 ^{o}C for 72 h with continuous shaking at 100 rpm. After incubation, uniform population (10^8-10^9 CFU mL^{-1}) of each strain was achieved by dilution with sterilized distilled water after measuring optical density at 600 nm.

Seed disinfection and inoculation: Seeds were surface-disinfected by immersing them first in 95 % ethanol for 30 s and then in 0.2 % solution of $HgCl_2$ for 3 min, following several rinses with sterilized water to remove disinfectant (Russell *et al.,* 1982). For inoculation, the cell suspension of each strain was mixed with sterile peat for seed coating. Seed coating was carried out with inoculated peat mixed (seed to peat ratio 4: 1, w/w) with 10% sugar solution. In the case of uninoculated control, seeds were coated with the sterilized peat treated with sterilized broth and 10% sugar solution. Inoculated seeds were placed over night for drying.

Pot experiment: Pot experiment was conducted to assess the potential of combined application of PGPB containing ACC-deaminase both in the presence and absence of P-enriched compost for promoting growth, yield and nodulation of chickpea. Fruit and vegetable wastes were composted according to the method described by Ahmad *et al*. (2008a, b). The composted material was enriched with 50% of full dose of P fertilizer using single super phosphate as the source of P while 50% P was applied directly at the time of sowing. The full dose of P in this experiment was 60 kg ha^{-1}. Fertilizers N & K were applied at the rate 25 & 25 kg ha^{-1} in the form of urea and sulfate of potash, respectively. The P-enriched compost was applied at the rate of 300 kg ha^{-1}. All the three PGPB strains were tested with and without application of P-enriched compost. Treatments were replicated six times by using complete randomized design (CRD). Three treatments were harvested at flowering stage for nodulation data and the remaining three were harvested at maturity stage. Canal water was used for irrigation. Inoculated and uninoculated seeds were sown in pot with sandy clay loam soil, having physico-chemical properties such as pH 7.4; electrical conductivity, 2.6 dS m^{-1}; organic matter, 0.63%; total nitrogen, 0.03%; available phosphorus, 7.8 mg kg^{-1} and extractable potassium, 118 mg kg^{-1}.

Field experiment: An identical field experiment was conducted in the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. In this study again all the three PGPB strains were tested with and without application of P-enriched compost. Treatments were replicated thrice by using randomized complete block design (RCBD) with same agronomic practices as described above. Canal water was used for irrigation. Crop was harvested at maturity and parameters like number of pods plant^{-1}, fresh biomass, grain yield and number and weight of nodules plant^{-1} were recorded. The data were analyzed statistically (Steel *et al.,* 1997) and means were compared by Duncan's multiple range tests (Duncan, 1955).

Results

All the tested strains of PGPB showed ability to promote fresh biomass both in the presence and absence of P-enriched compost, compared to their respective uninoculated controls (Table 1). *Serratia oderifera* (J118) caused maximum increase of 53.4% in fresh biomass over uninoculated control in the presence of chemical fertilizer while 84% increase was observed when the inoculation was done in the presence of P-enriched compost. Inoculation with *Pantoea dispersa* (J112) and *Enterobactor gergoviae* (J107) yielded 69 and 51% increases in fresh biomass compared to control. A significant increase (up to 48%) in grain yield was also exhibited by inoculation with PGPB in the absence of enriched compost, while increase in grain yields reached up to 79% when the bacteria were used in combination with P-enriched compost. *Pantoea dispersa* (J112) was the most effective bacterium among the tested PGPB.

In the absence of P-enriched compost, the maximum number of pods per plant (58% increases over control) were recorded with *Pantoea dispersa* (J112) and, pods were increased up to 96.6% when inoculation was combined with enriched compost (Table 2). Next effective strain *Serratia oderifera* (J118) had also significant increasing effect that ranged from 50.4 to 61.3% over control, in the absence and presence of P-enriched compost. Once again, combined application of *Pantoea dispersa* (J112) and P-enriched

Table 1: Effect of plant growth promoting bacteria with ACC-deaminase activity in the presence and absence of P-enriched compost on fresh biomass and grain yield of chickpea (pot trial)

Treatments	Fresh biomass (t ha^{-1})		Grain yield (t ha^{-1})	
	Without compost	With compost	Without compost	With compost
Control	16.9d (±1.87)	19.9d (±1.79)	5.7d (±0.91)	6.3d (±1.02)
Enterobactor gergoviae (J107)	26.1c (±2.13)	27.0c (±3.12)	7.8c (±1.02)	8.3bc (±0.78)
Pantoea dispersa (J112)	26.9c (±2.37)	30.7ab (±2.26)	8.8bc (±0.89)	10.8a (±1.21)
Serratia oderifera (J118)	27.5bc (±1.92)	33.6a (±2.89)	8.4bc (±1.13)	9.3b (±0.96)

Values sharing similar letter(s) in a parameter are non-significant at $P< 0.05$, according to Duncan's multiple range tests. ±: [Standard error of means]

Table 2: Effect of plant growth promoting bacteria with ACC-deaminase activity in the presence and absence of P-enriched compost on number of pods per plant and 100-grain weight of chickpea (pot trial)

Treatments	Number of pods plant^{-1}		100-grain weight (g)	
	Without compost	With compost	Without compost	With compost
Control	36.7e (±3.12)	39.7e (±5.12)	29.5e (±2.16)	31.2de (±2.59)
Enterobactor gergoviae (J107)	46.3d (±2.67)	51.7cd (±3.49)	32.4cd (±3.31)	34.0bc (±3.07)
Pantoea dispersa (J112)	56.7bc (±4.32)	75.0a (±4.31)	33.5bc (±2.05)	36.0a (±3.87)
Serratia oderifera (J118)	59.7b (±5.34)	61.0b (±3.59)	32.4cd (±3.27)	34.7ab (±2.02)

Values sharing similar letter(s) in a parameter are non-significant at $P< 0.05$, according to Duncan's multiple range tests. ±: [Standard error of means]

Table 3: Effect of plant growth promoting bacteria with ACC-deaminase activity in the presence and absence of P-enriched compost on number of nodules per plant, nodule dry weight per plant and root dry weight chickpea (pot trial)

Treatments	Number of nodules $plant^{-1}$		Nodule dry $plant^{-1}$ (g)		Root dry weight $plant^{-1}$ (g)	
	Without compost	With compost	Without compost	With compost	Without compost	With compost
Control	30.3e (±3.59)	34.3e (±4.31)	1.13d (±0.06)	1.21cd (±0.08)	1.13d (±0.02)	1.21cd (±0.02)
Enterobacter gergoviae (J107)	40.0d (±4.51)	42.4cd (±4.79)	1.25cd (±0.08)	1.37b (±0.09)	1.25bcd (±0.01)	1.37b (±0.03)
Pantoea dispersa (J112)	43.7cd (±3.69)	58.7a (±4.35)	1.29bc (±0.05)	1.61a (±0.11)	1.28bc (±0.03)	1.61a (±0.04)
Serratia oderifera (J118)	47.7bc (±4.19)	52.0b (±3.86)	1.34bc (±0.12)	1.68a (±0.12)	1.34bc (±0.04)	1.68a (±0.04)

Values sharing similar letter(s) in a parameter are non-significant at $P< 0.05$, according to Duncan's multiple range tests. ±: [Standard error of means]

Table 4: Effect of plant growth promoting bacteria with ACC-deaminase activity in the presence and absence of P-enriched compost on fresh biomass and grain yield of chickpea (Field trial)

Treatments	Fresh biomass (t ha^{-1})		Grain yield (t ha^{-1})	
	Without compost	With compost	Without compost	With compost
Control	6.1c (±1.12)	6.9bc (± 1.02)	1.8d (±0.18)	2.1cd (±0.27)
Enterobacter gergoviae (J107)	7.3bc (±1.08)	7.6bc (±1.49)	2.3cd (± 0.29)	2.6bc (±0.23)
Pantoea dispersa (J112)	7.8b (±1.32)	11.2a (± 1.38)	2.8bc (±0.14)	3.5a (±0.31)
Serratia oderifera (J118)	8.2b (±1.17)	10.4a (± 1.59)	2.5cd (±0.37)	3.1ab (±0.29)

Values sharing similar letter(s) in a parameter are non-significant at $P< 0.05$, according to Duncan's multiple range tests. ±: [Standard error of means]

compost resulted in maximum increase of up to 21% over control in case of 100-grain weight, which was followed by *Serratia oderifera* (J118) (16.6%). Both treatments were non-statistically significant with each other.

All the PGPB strains exhibited significant increase in number of nodules alone as well as in combination with P-enriched compost (Table3). The highest increase of 82.5% in number of nodules compared with control was recorded in case of inoculation with *Pantoea dispersa* (J112), followed by *Serratia oderifera* (J118). Strain J107, *Enterobacter gergoviae* caused up to 23.4% increases in number of nodules respectively compared to control. Likewise, significant increases in nodule dry weights were also recorded when inoculation was integrated with P-enriched compost. The maximum increase (46.6%) in weight of nodules over control was observed upon inoculation with *Serratia oderifera* (J118) which was further raised up to 63.7% when it was used in combination with P-enriched compost. Next to it, *Pantoea dispersa* (J112) in combination with P-enriched compost showed an increase of 49% in nodule weight compared with control. The maximum increases in root dry weight were up to 45.7% compared with control in response to inoculation with *Serratia oderifera* (J118) in the presence of P-enriched compost. It was followed by *Pantoea dispersa* (J112) plus P-enriched compost which caused an increase of 40% over control.

The results of field trial further revealed the effectiveness of inoculation with ACC-deaminase PGPB both in the presence and absence of compost enriched with P. Sole applications of *Pantoea dispersa* (J112) and *Serratia oderifera* (J118) were found to increase fresh biomass for up to 29.5% as compared to control (Table 4). However, inoculation effects were further improved (73% increase over control) when it was used in combination with P-enriched compost. Similarly, up to 44.5% increase in grain yield compared to control was recorded in response to inoculation with *Serratia oderifera* (J118) and *Pantoea dispersa* (J112) when applied alone (without compost). The increase in yield was up to 77.8% when PGPB were used in combination with P-enriched compost.

Table 5: Effect of plant growth promoting bacteria with ACC-deaminase activity in the presence and absence of P-enriched compost on number of pods per plant and 100-grain weight of chickpea (Field trial)

Treatments	No. of pods $plant^{-1}$		100-grain weight (g)	
	Without compost	With compost	Without compost	With compost
Control	37.3d (±3.12)	41.3d (±4.57)	31.5 (±2.56)	32.4 (±1.73)
Enterobactor gergoviae (J107)	49.3c (±4.29)	50.7c (±6.39)	33.0 (±2.34)	34.6 (±4.41)
Pantoea dispersa (J112)	61.3b (±5.78)	76.7a (±6.18)	33.5 (±2.14)	36.2 (±3.89)
Serratia oderifera (J118)	59.0b (±4.97)	64.7b (±5.61)	33.3 (±3.56)	35.5 (±3.32)

Values sharing similar letter(s) in a parameter are non-significant at $P < 0.05$, according to Duncan's multiple range tests. ±: [Standard error of means]

Table 6: Effect of plant growth promoting bacteria with ACC-deaminase activity in the presence and absence of P-enriched compost on number of nodules per plant, nodule dry weight per plant and root dry weight chickpea (Field trial)

Treatments	No. of nodules $plant^{-1}$		Nodule dry weight (g $plant^{-1}$)		Root dry weight (g $plant^{-1}$)	
	Without compost	With compost	Without compost	With compost	Without compost	With compost
Control	32.0e (±6.89)	38.67de (±5.65)	0.29c (±0.04)	0.33bc (±0.04)	1.26c (±0.04)	1.33bc (± 0.04)
Enterobactor gergoviae (J107)	42.33d (±7.12)	44.67cd (±5.16)	0.39abc (±0.05)	0.45abc (±0.04)	1.38bc (±0.02)	1.52b (±0.02)
Pantoea dispersa (J112)	50.33bc (±6.34)	65.67a (±6.49)	0.43abc (±0.03)	0.501 (±0.04)	1.48bc (±0.04)	1.86a (±0.05)
Serratia oderifera (J118)	45.33cd (±4.56)	53.33b (±6.39)	0.39abc (±0.05)	0.46ab (±0.06)	1.42bc (±0.04)	1.78a (±0.03)

Values sharing similar letter(s) in a parameter are non-significant at $P < 0.05$, according to Duncan's multiple range tests. ±: [Standard error of means]

The maximum increase (95% over control) in pods per plant was observed in response to inoculation with *Pantoea dispersa* (J112) in the presence of P-enriched compost (Table 5). *Serratia oderifera* (J118) was the second most effective bacterium that increased number of pods by 66.1% compared to control when applied with P-enriched compost. Strain *Enterobactor gergoviae* (J107) remained the least effective among the tested bacteria in both the cases when used alone or in combination with P-enriched compost. All the PGPB strains in combination with P-enriched compost significantly increased 100 grain weight of chickpea compared to uninoculated/untreated control. *Pantoea dispersa* strain J112 along with P-enriched compost showed an increase in 100-grain weight of 14.4% compared with control. It was followed by *Serratia oderifera* (J118) plus P-enriched compost with an increase of 12.4% compared with control.

Numbers of nodules were also significantly improved in response to inoculation with PGPB strains when applied alone or in combination with P-enriched compost (Table 6). The greatest numbers of nodules were recorded with *Pantoea dispersa* (J112) and an increase of 87% was observed compared to control. *Serratia oderifera* (J118) also significantly increased number of nodules (55.2%) in the presence of P-enriched compost compared with control. However without P-enriched compost, inoculation promoted number of nodules up to 47.4% compared with control. Almost similar trend was observed in the case of nodule weight and the maximum weight of nodules was observed with the strain J112, followed by J118. The increase in root dry weight compared to control was up to 17% in response to inoculation with *Pantoea dispersa* (J112) and *Serratia oderifera* (J118), however, it increased to 44.5% when used in combination with P-enriched compost.

Discussion

The results illustrate the effectiveness of integrated use of PGPB containing ACC-deaminase and compost enriched with P for increasing growth, yield and nodulation of chickpea both under pot and field conditions. Although inoculation with bacteria containing ACC-deaminase trait had promising effects on growth, yield and nodulation of chickpea, but inoculation was highly effective in the presence of P-enriched compost. This implies that bacteria might have positively affected nodulation and yield through their ACC-deaminase activity, in addition to enhanced P-availability to the plants. Organic material served as substrate to maintain high populations of bacteria in the rhizosphere that resulted in better root system due to lowering of ethylene levels in plant as a result of ACC-deaminase activity. This premise is supported by the data given in Table 3 & 6 which reveal greater root biomass in response to inoculation with PGPB. The findings of other researchers also support our premise that bacteria with ACC-deaminase activity promote root system (Glick *et al.,* 1998; Belimove *et al.,* 2002).

Since higher concentration of ethylene also inhibits nodulation process, thus inoculation with PGPB containing ACC-deaminase could improve number of nodules and nodule weight in chickpea. Moreover, better root system would increase the probability of infection for rhizobia. Compost is also source of macro and micronutrients (Pooran *et al.,* 2002; Cheuk *et al.,* 2003; Ahmad *et al*., 2008a, b), which affect plant growth through improved nutrient availability. Thus, the presence of PGPB containing ACC- deaminase and P-enriched compost on the roots vicinity could promote root growth, nodulation, and yield of chickpea by lowering ethylene concentration and improving nutrient availability. Ma *et al.* (2004) have reported that ACC deaminase promote nodulation by adjusting ethylene levels in legumes. Similar kinds of results have been reported by Shaharoona *et al.* (2006) that ACC-deaminase improved nodulation of mungbean.

In conclusion, use of PGPB containing ACC-deaminase in the presence of P-enriched compost could be very effective for improving nodulation and yield of chickpea under field conditions.

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(Received for publication 21 April, 2008)