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INTEGRATED USE OF *Rhizobium leguminosarum*, PLANT GROWTH PROMOTING RHIZOBACTERIA AND ENRICHED COMPOST FOR IMPROVING GROWTH, NODULATION AND YIELD OF LENTIL (*Lens culinaris* Medik.)

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Maintenance of high bacterial population in the rhizosphere improves the efficiency of these organisms. This high bacterial population can be maintained by the application of enriched compost which supports their growth and activities. Thus integrated use of *Rhizobium*, plant growth promoting rhizobacteria (PGPR) containing 1-aminocyclopropane-1-carboxylate deaminase (ACC-deaminase) and P-enriched compost (PEC) could be highly effective for promoting growth, nodulation, and yield of lentil (*Lens culinaris* Medik.). A field study was conducted to evaluate the potential of *Rhizobium*, PGPR containing ACC-deaminase and PEC for promoting growth of lentil. For this study, the soil type was sandy clay loam soil having pH 7.6; EC (electrical conductivity) 2.8 dS m^{-1}; organic matter (OM) 0.59%; total N 0.032%; available P 7.9 mg kg^{-1}, and extractable K 129 mg kg^{-1}. Treatments were replicated thrice, using randomized complete block (RCB) design. Results showed that the integrated use of *R. leguminosarum* with *Pseudomonas* spp. containing ACC-deaminase along with PEC was highly effective and caused up to 73.5, 73.9, 74.4, 67.5, 73.3, 65.8, 40.5, and 52.5% increase in fresh biomass, grain yield, straw yield, pods $plant^{-1}$, nodule $plant^{-1}$, nodule dry weight $plant^{-1}$, 1000-grain weight, and N content in grain of lentil, respectively, as compared to respective control. It is concluded that integrated use of *R. leguminosarum* with *Pseudomonas* spp. having trait ACC-deaminase plus PEC would be an effective approach for better nodulation which consequently improved yield of lentil under natural conditions.

Key words: Co-inoculation, biological N fixation, *Pseudomonas* spp., rhizobium, ACC-deaminase, P-enriched compost, nodulation, yield, *Lens culinaris*.

Plant growth promoting bacteria are the natural potential resource which colonize roots of plants and stimulates growth and yield directly and indirectly (Afzal and Bano, 2008). Plant growth promoting bacteria are those present in the rhizosphere and improve the growth of the plant directly or indirectly. A considerable number of bacterial species from the rhizosphere have been isolated and their efficiency for improving plant growth has been assessed (Kloepper *et al*., 1989; Amor *et al*., 2008).

Ethylene is the plant hormone that regulates different plant responses (Arshad and Frankenberger, 2002; Babar *et al*., 2007). Moreover, it also affects several aspects of root development and nodule formation (Ligero *et al*., 1991; Babar *et al*., 2007). At low levels ethylene stimulate plant growth, but when it is present in high levels it can be damaging for plants, leading to epinasty, shorter root, and premature senescence.

Higher levels of ethylene are produced due to biotic and abiotic stresses which inhibit plant growth. Any change in the ethylene level of stressed plants may improve the growth and development (Arshad and Frankenberger, 2002; Banchio *et al*., 2008; Reid *et al*., 2011a). Inoculation with plant growth promoting rhizobacteria (PGPR) having trait 1-aminocyclopropane-1-carboxylate ACC-deaminase has been studied to lower ethylene, which subsequently improved the plant growth. Recent findings indicate that there are several bacteria that have an enzyme ACC-deaminase. This enzyme inhibits the ethylene production by breaking its precursor ACC (1-aminocyclopropane-1-carboxylic acid) into ammonia and α-ketobutyrate (Shaharoona *et al*., 2006; Arshad *et al*., 2007; Reid *et al*., 2011b).

The researchers are focusing on co-inoculation with PGPR and *Rhizobium* in recent years and it is becoming the most popular approach for improving the growth in legumes. PGPR may increase the efficiency of *Rhizobium* inoculation in legumes through the production of

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antibiotics, siderophore, and certain enzymes. They also enhance the infection sites for *Rhizobium* by colonizing the root surface (Parmar and Dadarwal, 1999; Contesto *et al*., 2008). These PGPR may also compete with the *Rhizobium* for food and other nutrients thus may also inhibit nodule formation and growth in a given symbiotic relationship, depending upon the nature and concentration of secondary metabolites released by these bacteria (Contesto *et al*., 2008).

Moreover, maintenance of high bacterial population in the rhizosphere improves the efficiency of these organisms (Cheuk *et al*., 2003; Ahmad *et al*., 2008a; 2008b). This high bacterial population can be maintained by the application of enriched compost which supports their growth and activities (Cheuk *et al*., 2003). Thus integrated use of *Rhizobium* and PGPR containing ACC-deaminase along with enriched compost could be extremely useful approach in improving yield and nodulation of lentil crop.

Keeping in view the above discussion, a field study was planned to evaluate the potential of *Rhizobium* and PGPR containing ACC-deaminase in the presence of P-enriched compost (PEC) for growth, nodulation and yield of lentil (*Lens culinaris* Medik.).

MATERIALS AND METHODS

A field trial was conducted to evaluate the effectiveness of *Rhizobium* and PGPR containing ACC-deaminase along with PEC for improving growth, nodulation and yield of lentil. For this, two pre-characterized and identified strains (i.e. *Pseudomonas fragi* [P5], *Pseudomonas jesseni* [P10]) of PGPR and one *Rhizobium* (i.e. *Rhizobium leguminosarum* [Z22]) were used.

Preparation of inoculum

The inoculum of PGPR and *Rhizobium* strains were prepared by using Dworkin-Foster (DF) salt minimal media having ACC as sole N source and yeast extract mannitol medium, respectively. For this purpose, 250 mL flasks autoclaved DF-salt minimal medium and yeast extract mannitol medium were taken in autoclaved 250 mL flasks and inoculated with respective strains. All the flasks were incubated at 28 ± 1 °C for 3 d in the orbital shaking incubator at 100 rpm. After incubation, uniform population (OD_{550} = 0.45; 10^7 to 10^8 cfu mL) of each bacterial strain was achieved.

Seed disinfection and inoculation

For surface sterilization seeds were momentary dipped in ethanol (95%) and then in solution of $HgCl_2$ (0.2%) for 5 min, followed by rinsing four times with autoclaved distilled water to eliminate disinfectant (Russell *et al*., 1982).

For inoculation, the cell suspension of each bacterial strain was mixed with sterile peat for seed coating. Seed coating was carried out by inoculated peat mixed (seed to peat ratio 1:1) with 10% sugar solution. In case of control, seeds were coated by sterilized peat treated with sterilized broth plus 10% sugar solution. For co-inoculation, *Rhizobium* and PGPR strains were mixed in 1:1 ratio for attaining all possible combinations of co-inoculants. After inoculation seeds were spread over night for drying.

Field experiment

A field experiment was conducted at Post Graduate Agricultural Research Station, University of Agriculture, Faisalabad, Pakistan, to assess the effectiveness of co-inoculation of *Rhizobium* with PGPR containing ACC-deaminase both in the absence and presence of PEC for improving growth, nodulation and yield of lentil.

Organic waste materials were composted as described by Ahmad *et al*. (2008a). From the composted organic waste materials, 50% PEC was prepared by enrichment with 50% of full dose of phosphatic fertilizer using single super phosphate (SSP) as the source of P while remaining 50% P was applied at the time of sowing. NPK fertilizers were applied at 25:60:25 kg ha^{-1} of each in form of urea and sulfate of potash, respectively. The PEC was applied at 350 kg ha^{-1}. All the bacterial strains were tested both in the absence and presence of P-enriched compost.

Seeds were sown in the field with sandy clay loam soil having pH 7.6; EC (electrical conductivity) 2.8 dS m^{-1}; organic matter (OM) 0.59% (Walkley, 1947); total N 0.032% (AOAC, 1999); available P 7.9 mg kg^{-1} (Olsen *et al*., 1954) and extractable K 129 mg kg^{-1} (Method 11a of U.S. Salinity Laboratory Staff, 1954). Treatments were replicated thrice, using randomized complete block (RCB) design. Canal water was used for irrigation. Data regarding nodulation were collected at flowering. The crop was harvested and data concerning yield and yield contributing parameters were recorded. Nitrogen content in grain and straw samples were determined through chemical analysis. R-Stat program was applied to analyze the data (Steel *et al*., 1997) and means were compared by using Duncan's multiple range tests (Duncan, 1955).

RESULTS

Inoculation significantly enhanced the plant height of lentil over uninoculated control (Table 1). Although single inoculation caused a significant increase in plant height in the absence and presence of compost amended soil as compared to control. Up to 18.7% increase in plant height was observed in case of single inoculation as compared to control; however, the effect was more pronounced (25.3% higher than control) when the plant were grown in soil amended with PEC. Co-inoculation of *R. leguminosarum* (Z22) with *P. jesseni* (P10) having trait ACC-deaminase resulted in highly significant effect as 24.9% increase in plant height was recorded as compared to control. In case of PEC, co-inoculation with all the two combination of

Table 1. Effect of co-inoculation of *Rhizobium* with plant growth promoting bacteria containing ACC-deaminase on growth parameters of lentil in the absence and presence of P-enriched compost (mean of three replicates).

	Plant height		Fresh biomass		Root length		Root dry weight	
Treatments	No compost	Compost	No compost	Compost	No compost	Compost	No compost	Compost
	cm		t ha^{-1}		cm		g plant^{-1}	
Control	35.17e	37.17e	6.62d	7.07d	17.25d	18.58d	2.27d	2.40c
Pseudomonas fragi (P5)	38.75d	40.29d	9.23c	9.33c	20.17c	21.00c	2.59c	2.77b
Pseudomonas jesseni (P10)	40.42c	42.00c	8.93c	9.93b	20.75bc	21.67c	2.64c	2.99b
Rhizobium leguminosarum (Z22)	41.75b	44.08b	9.67b	10.19b	20.33c	21.42c	2.73c	2.92b
R. leguminosarum (Z22) × *P. fragi* (P5)	43.42a	45.25a	10.93a	11.50a	22.08a	24.58a	3.19b	3.50a
R. leguminosarum (Z22) × *P. jesseni* (P10)	43.92a	44.67ab	10.55a	11.34a	21.67ab	23.08b	3.33a	3.58a
LSD value	1.091		0.393		0.965		1.060	

Mean values sharing similar letter(s) in a parameter are non-significant at $P < 0.05$, according to Duncan's multiple range tests.
ACC-deaminase: 1-aminocyclopropane-1-carboxylate deaminase; LSD: least significant difference.

Rhizobium and rhizobacteria resulted in 27% increase in plant height than control.

Fresh biomass results revealed that all inoculation/co-inoculation treatments significantly increased fresh biomass both in the presence and absence of PEC (Table 1). The maximum increase of 73.5% in fresh biomass was observed in case of co-inoculation of *R. leguminosarum* (Z22) with *P. fragi* (P5) followed by *R. leguminosarum* (Z22) with *P. jesseni* (P10) plus PEC as compared to uninoculated control. In absence of PEC, up to 64.9% increase in fresh biomass was recorded because of *R. leguminosarum* (Z22) with *P. fragi* (P5) as compared to control. In case of single inoculation, *R. leguminosarum* (Z22) was the most effective as compared to rhizobacteria both in the compost amended and unamended soils. While, the root dry weight and root length was also improved by co-inoculation in the presence of PEC.

Maximum (65.8% over untreated control) increase in straw yield was observed when *R. leguminosarum* (Z22) was co-inoculated with *P. fragi* (P5) (Table 2). It was followed by co-inoculation with *R. leguminosarum* (Z22) × *P. jesseni* (P10), which gave increase up to 57.2% in straw yield over control. In case of sole inoculation, maximum increase (47%) in straw yield was recorded with *R. leguminosarum* (Z22) and it was further increased up to 52.5% when the same isolate was tested in the presence of compost amended soil. Co-inoculation with PEC yielded highest increase in straw yield which was 74.3% higher than control. In general, inoculation/co-inoculation treatments showed better performance in soil amended with PEC than soil not amended with PEC.

The results regarding grain yield showed that maximum grain yield (73.9% more over control) was obtained in case of co-inoculation of *R. leguminosarum* (Z22) with *P. jesseni* (P10) in the presence of PEC and next effective treatment was *R. leguminosarum* (Z22) × *P. fragi* (P5) plus PEC (Table 2). In the absence of PEC, the increase in grain yield was recorded up to 66% due to application of *R. leguminosarum* (Z22) with *P. jesseni* (P10) compared to control. While in case of single inoculation *R. leguminosarum* (Z22) was the most effective both in the presence and absence of PEC.

Maximum number of pods per plant were recorded when *R. leguminosarum* (Z22) was co-inoculated with *P. jesseni* (P10), there was 39% number of pods than respective control (Table 2). It was followed by co-inoculation with *R. leguminosarum* (Z22) × *P. fragi* (P5), which gave increase up to 29.5% in number of pods per plant over control. In case of single inoculation, maximum increase (32.7%) in number of pods was recorded with *R. leguminosarum* (Z22) and it was further increased up to 43.8% when the same isolate was tested in the presence of compost amended soil. Co-inoculation with PEC yielded highest increase in number of pods per plant which was 67.5% more than control. Overall, inoculation/co-inoculation treatments showed better performance in the presence of PEC than soil not amended with PEC.

In case of 1000-grain weight of lentil, all inoculation/co-inoculation treatments showed significant increase over control (Table 2). Inoculation/co-inoculation caused a maximum increase in 1000-grain weight of 15% over uninoculated control. But inoculation/co-inoculation along with PEC yielded highest of 40.9% in 1000-grain weight followed by 31.2% increase due to application *R.*

Table 2. Effect of co-inoculation of *Rhizobium* with plant growth promoting bacteria containing ACC-deaminase on yield and yield contributing parameters of lentil in the absence and presence of P-enriched compost (mean of three replicates).

	Straw yield		Grain yield		N° of pods plant^{-1}		1000-grain weight	
Treatments	No compost	Compost	No compost	Compost	No compost	Compost	No compost	Compost
	t ha^{-1}						g	
Control	5.09g	5.35g	1.54c	1.73d	102.67d	115.00e	18.80c	19.48d
Pseudomonas fragi (P5)	7.09ef	7.18cde	2.05b	2.24c	118.33c	138.00d	20.22b	21.06c
Pseudomonas jesseni (P10)	6.77f	7.61abc	2.16b	2.35bc	129.67b	139.00cd	20.28b	21.32c
Rhizobium leguminosarum (Z22)	7.48def	7.76bcd	2.19b	2.44b	136.33ab	146.57c	20.67b	21.47c
R. leguminosarum (Z22) × *P. fragi* (P5)	8.44ef	8.87a	2.48a	2.63a	132.00b	161.00b	21.48a	24.66b
R. leguminosarum (Z22) × *P. jesseni* (P10)	8.00de	8.66ab	2.55a	2.68a	142.67a	172.33a	21.61a	26.49a
LSD value	0.675		0.169		7.867		0.522	

Mean values sharing similar letter(s) in a parameter are non-significant at $P < 0.05$, according to Duncan's multiple range tests.
ACC-deaminase: 1-aminocyclopropane-1-carboxylate deaminase; LSD: least significant difference.

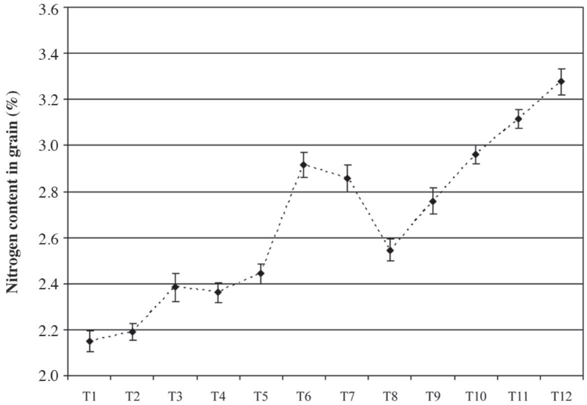
leguminosarum (Z22) × *P. fragi* (P5) in the presence of PEC over uninoculated control. Rest of treatments also showed significant increase in 1000-grain weight ranged from 7.6 to 14.2% compared to control.

All the inoculation/co-inoculation treatments showed an increase in number of nodules per plant significantly over control both in the presence and absence of PEC (Table 3). The maximum increase (up to 53.3%) in number of nodules per plant was observed in response to *R. leguminosarum* (Z22) with *P. jesseni* (P10) over control but this increase was reached up to 73.3% when the same inoculants were applied in soil amended with PEC. In case of sole application, highest increase in number of nodules was recorded up to 27.8%, due to inoculation of *R. leguminosarum* (Z22) over control. The increase of nodules per plant was up to 43.3% when the same isolate was used with PEC, in comparison with control.

The nodule fresh and dry weight was also enhanced by inoculation. But the results were more pronounced in case of co-inoculation in the presence of PEC (Table 3). The maximum nodule fresh and dry weight was found in case of co-inoculation of *R. leguminosarum* (Z22) with *P. jesseni* (P10) in the presence of PEC.

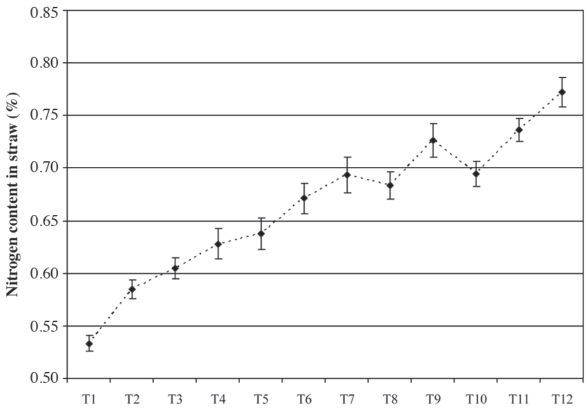
In case of sole inoculation, maximum N content in grain was observed in case of inoculation with *R. leguminosarum* (Z22) (13.6% more over control) followed by *P. fragi* (P5) which showed an increase up to 10.8% over control (Figure 1). In case of co-inoculation, maximum increase 37.7% N content in grain was observed in response to co-inoculation of *R. leguminosarum* (Z22) with *P. jesseni* (P10), over control. The same co-inoculation strains caused an increase of 52.5% in N content when applied in soil amended with PEC.

In case of sole inoculation maximum N content in straw was recorded in case of inoculation with *R. leguminosarum* (Z22) (20.3% increase over control) followed by *P. jesseni* (P10) which showed increase up to 18.5% over control (Figure 2). In case of co-inoculation maximum increase (37%) in N content in straw was observed in response to *R. leguminosarum* (Z22) × *P. fragi* (P5) over control. When co-inoculation was tested in the presence of PEC, maximum increase in N content in straw was recorded 45.6% in case of *R. leguminosarum* (Z22) with *P. jesseni* (P10) as compared to uninoculated control.



ACC-deaminase: 1-aminocyclopropane-1-carboxylate deaminase.
T1: Control (untreated); T2: P-enriched compost (PEC); T3: *Pseudomonas fragi* (P5); T4: *Pseudomonas jesseni* (P10); T5: *Rhizobium leguminosarum* (Z22), T6: *P. fragi* (P5) + PEC; T7: *P. jesseni* (P10) + PEC; T8: *R. leguminosarum* (Z22) + PEC; T9: *R. leguminosarum* (Z22) × *P. fragi* (P5); T10: *R. leguminosarum* (Z22) × *P. jesseni* (P10); T11: *R. leguminosarum* (Z22) × *P. fragi* (P5) + PEC; T12: *R. leguminosarum* (Z22) × *P. jesseni* (P10) + PEC.

Figure 1. Effect of co-inoculation with *Rhizobium* and plant growth promoting rhizobacteria (PGPR) containing ACC-deaminase on N content in grain of lentil in the absence and presence of P-enriched compost.



ACC-deaminase: 1-aminocyclopropane-1-carboxylate deaminase.
T1: Control (untreated); T2: P-enriched compost (PEC); T3: *Pseudomonas fragi* (P5); T4: *Pseudomonas jesseni* (P10); T5: *Rhizobium leguminosarum* (Z22), T6: *P. fragi* (P5) + PEC; T7: *P. jesseni* (P10) + PEC; T8: *R. leguminosarum* (Z22) + PEC; T9: *R. leguminosarum* (Z22) × *P. fragi* (P5); T10: *R. leguminosarum* (Z22) × *P. jesseni* (P10); T11: *R. leguminosarum* (Z22) × *P. fragi* (P5) + PEC; T12: *R. leguminosarum* (Z22) × *P. jesseni* (P10) + PEC.

Figure 2. Effect of co-inoculation with *Rhizobium* and plant growth promoting rhizobacteria (PGPR) containing ACC-deaminase on N content in straw of lentil in the absence and presence of P-enriched compost.

Table 3. Effect of co-inoculation with *Rhizobium* and plant growth promoting rhizobacteria containing ACC-deaminase on nodulation of lentil in the absence and presence of P-enriched compost (mean of three replicates).

	Nodule plant^{-1}		Nodule fresh weight		Nodule dry weight	
Treatments	No compost	Compost	No compost	Compost	No compost	Compost
			g plant^{-1}			
Control	30.00e	32.33e	0.52e	0.55e	0.14d	0.14d
Pseudomonas fragi (P5)	36.00d	40.33d	0.69d	0.72d	0.18c	0.19c
Pseudomonas jesseni (P10)	37.67c	41.33d	0.72c	0.76c	0.19bc	0.21bc
Rhizobium leguminosarum (Z22)	38.33c	43.00c	0.73c	0.77c	0.19bc	0.21bc
R. leguminosarum (Z22) × *P. fragi* (P5)	44.00b	47.33a	0.76b	0.79b	0.20ab	0.21ab
R. leguminosarum (Z22) × *P. jesseni* (P10)	46.00a	52.00b	0.79a	0.84a	0.21a	0.23a
LSD value	1.466		0.0169		0.0168	

Mean values sharing similar letter(s) in a parameter are non-significant at $P < 0.05$, according to Duncan's multiple range tests.
ACC-deaminase: 1-aminocyclopropane-1-carboxylate deaminase; LSD: least significant difference.

DISCUSSION

The use of *Rhizobium* and plant growth promoting bacteria containing ACC-deaminase for increasing yield of legumes is becoming more popular in recent years. Therefore, researchers are focusing on understanding the mechanisms responsible for growth promotion by PGPB. Therefore, a study was conducted to demonstrate the effectiveness of integrated use of *Rhizobium* and PGPR containing ACC-deaminase in the presence of PEC for growth promotion and yield of lentil under field conditions. It is highly likely that the stability of PGPR isolate to cleave the ACC was the responsible mechanism of action for promoting root and shoot growth which might have resulted in lowering of the endogenous ACC level, thus decreasing the endogenous ethylene production by plants. This premise is strongly supported by the work reported by several researchers (Glick *et al*., 1998; Mayak *et al*., 2004; Reid *et al*., 2011b).

Other possibility of this growth promotion could be that the rhizobacterial isolates might have better ability to colonize the roots of lentil, phosphate solubilization, chitinase activity, and the production of other biologically active substances, which made them more competitive under field conditions. Ability of these strains to inhibit pathogens may also be one of the factors that increased the ability of these strains to survive and increase the growth of lentil under field conditions. The production of antifungal metabolites by *Pseudomonas* spp. has been reported previously (Pal *et al*., 2001; Banchio *et al*., 2008; Contesto *et al*., 2008).

Inoculation with *Rhizobium* strains showed increase in the root nodulation, dry weight of nodules per plant, N-content of plant, grain yield, and straw yield as compared to control. The results are in line with Provorov (1998), who observed that due to inoculation, increase in plant height in mung bean was 15.2% higher over control. They also observed that the effect of inoculation with three different strains on total weight and recorded an increase in total dry weight up to 36.5% higher than control.

The possibility of this growth promotion could be that *Rhizobium* fixes atmospheric N_2 through legumes-*Rhizobium* association and hence enriches N-status of soil in addition to the benefit and improvement of growth and yield of the crop itself.

Inoculation with either PGPR containing ACC-deaminase or *Rhizobium* showed positive effect on plant biomass, nodule weight, and number of nodules plant^{-1} over control. Moreover, it was also observed that co-inoculation with PGPR containing ACC-deaminase activity and *Rhizobium* further improved the growth of lentil as compared to inoculation with either PGPR containing ACC-deaminase activity or *Rhizobium* alone. The presence of ACC-deaminase containing PGPR and *Rhizobium* on the roots would promote nodulation, root elongation and ultimately growth of lentil by reducing the synthesis of ethylene. However, ACC is the direct precursor of ethylene in plants (Yang and Hoffman, 1984). Thus ACC-deaminase promoted nodulation by adjusting ethylene levels in legumes. Rhizobacterial strains possessing ACC-deaminase activity may also promote growth of inoculated plants and could increase nodulation in legumes by co-inoculation with *Rhizobium* (Shaharoona *et al*., 2006; Babar *et al*., 2007).

The significant increase in nodulation is due to the result of co-inoculation with *Rhizobium* and PGPR containing ACC-deaminase. Co-inoculation with symbiotic and rhizosphere bacteria may enhance nodulation by a variety of mechanisms e.g. PGPR can reduce endogenous ethylene production by plants, antibiotics against pathogenic organisms, siderophores chelating insoluble cations and colonize root surfaces, thereby out competing pathogens (Parmar and Dadarwal, 1999; Banchio *et al*., 2008; Contesto *et al*., 2008).

It was also observed that inoculation with PGPR containing ACC-deaminase and *Rhizobium* along with PEC further showed positive effect on the growth, yield and number of nodules per plant over control. This argument is supported by the fact that compost was a source of OM containing macro and micro nutrients (Cheuk *et al*., 2003), which enhanced the fertility status with respect to nutrient availability and uptake, consequently resulted in higher growth and yield. The advantages of OM in terms of crop yield and nutrient uptake have also been observed by Johnston (1986). Similarly, McConnell *et al*. (1993) reported that compost applied at 18-146 t ha^{-1} produced a 6 to 163% increase in soil organic matter (SOM). Furthermore, OM maintains the dynamics and productive potential of the "rhizosphere". Organic matter improves soil structure, increases water retention capacity, stimulates soil microbes, and provides nutrients (Fontaine *et al*., 2003; Ahmad *et al*., 2008a; 2008b; Shahzad *et al*., 2008; Reid *et al*., 2011a; 2011b). The supplementary effect of compost may also be attributed to better supply of the organic C and energy required by heterotrophs, consequently, more and efficient establishment of PGPR producing better results.

Hence it could be expected that the presence of plant growth promoting bacteria and enriched compost on the roots would promote nodulation, root elongation and ultimately growth of lentil by regulating the synthesis of ethylene and nutrient availability as reported by Ma *et al*. (2003) that ACC-deaminase promoted nodulation by adjusting ethylene levels in legumes. It was further confirmed by Shaharoona *et al*. (2006) who stated that integrated use of effective strains of plant growth promoting rhizobacteria containing ACC-deaminase with nitrogenous fertilizer increased the number of nodules, fresh and dry weight of nodules significantly as compared to PGPR and/or nitrogenous fertilizer alone, most likely by decreased C_2H_2 levels in the plant roots during early stages of development and concluded that PGPR containing ACC-deaminase could be used to increase nodulation in legumes.

Generally, it was observed that *P. jesseni* (P10) performed better in co-inoculation with *Rhizobium* in the absence and presence of PEC for improving nodulation and yield of lentil. Consequently, ACC-deaminase activity of PGPR along with other valuable characters was, most likely, responsible for improving nodulation and yield of lentil during co-inoculation of *Rhizobium* strain.

CONCLUSION

It is concluded that integrated use of *Rhizobium*, PGPR containing ACC-deaminase in the presence of P-enriched compost would be a suitable approach for improving growth, yield and nodulation in lentil.

Uso integrado de *Rhizobium leguminosarum*, rizobacterias promotoras de crecimiento vegetal y compost enriquecido para mejorar el crecimiento, nodulación y rendimiento de lenteja (*Lens culinaris* Medik.). La mantención de una alta población bacteriana en la rizósfera mejora la eficiencia de estos organismos. Esta población bacteriana puede ser mantenida por la aplicación de compost enriquecido que mantiene su crecimiento y actividades. Por lo tanto, el uso integrado de *Rhizobium*, rhizobacterias promotoras de crecimiento vegetal (PGPR) conteniendo 1-aminociclopropano-1-carboxilato desaminasa (ACC-desaminasa) y compost enriquecido con P (PEC) podría ser altamente efectivo en la promoción de crecimiento, nodulación, y producción de lenteja (*Lens culinaris* Medik.). Un estudio de campo se condujo para evaluar el potencial de *Rhizobium*, PGPR conteniendo ACC-desaminasa y PEC para promover el crecimiento de lenteja. Para este estudio, el tipo de suelo fue franco-arcillo arenoso con pH 7,6; conductividad eléctrica 2,8 dS m^{-1}; materia orgánica 0,59%; N total 0,032%; P disponible 7,9 mg kg^{-1}, y K extraíble 129 mg kg^{-1}. Los tratamientos se repitieron tres veces, usando diseño de bloques completos al azar. Los resultados mostraron que el uso integrado de *R. leguminosarum* con *Pseudomonas* spp. conteniendo ACC-desaminasa junto con PEC fue altamente efectivo y causaron hasta 73.5, 73.9, 74.4, 67.5, 73.3, 65.8, 40.5, y 52.5% de aumento en biomasa fresca, producción de grano, producción de paja, vainas $planta^{-1}$, peso seco de nódulo por planta, peso de 1000 granos, y contenido de N en grano de lenteja, respectivamente, comparado con el respectivo control. Se concluyó que el uso integrado de *R. leguminosarum* con *Pseudomonas* spp. portando el rasgo de ACC-desaminasa más PEC podría ser un apronte efectivo para mejor nodulación que consecuentemente mejoró producción de lenteja bajo condiciones naturales.

Palabras clave: Co-inoculación, fijación biológica de N, *Pseudomonas* spp., rhizobios, ACC-deaminasa, compost P-enriquecido, nodulación, rendimiento, *Lens culinaris*.

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