Soil Environ. 32(1): 79-86, 2013
www.se.org.pk
Online ISSN: 2075-1141
Print ISSN: 2074-9546

Short Communication

Synergistic effect of rhizobia and plant growth promoting rhizobacteria on the growth and nodulation of lentil seedlings under axenic conditions

Muhammad Zafar-ul-Hye*[1], Maqshoof Ahmad[2] and Sher Muhammad Shahzad[3]
[1]Department of Soil Science, Bahauddin Zakariya University Multan, Pakistan
[2]Department of Soil Science, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan
[3]Department of Soil and Environmental Sciences, University of Sargodha, Pakistan

Abstract

Plant growth promoting rhizobacteria (PGPR) containing ACC-deaminase in combination with rhizobia can improve the growth and nodulation in plants by suppressing the endogenous level of ethylene. In the present study, ten strains, each of PGPR and rhizobia from the previously screened cultures were tested for their effect as co-inoculants on growth and nodulation of lentil in growth pouches under axenic conditions. Results showed that most of the combinations improved the lentil growth as compared to the un-inoculated control. Maximum increase in shoot length (1.87 fold), root length (1.97 fold) and total biomass (1.98 fold) over the un-inoculated control was observed in the treatment where the lentil seedlings were inoculated with the combination Z24P10. Co-inoculation also improved the nodulation in lentil and the maximum number of nodules plant^{-1} (24 nodules) were observed in the combination Z22P10. However, there was no nodulation in few combinations. It is concluded that the co-inoculation with rhizobia and PGPR containing ACC-deaminase has improved the growth and nodulation in lentil under axenic conditions and the selected combinations may be evaluated in pot and field trials.

Key words: ACC-deaminase, co-inoculation, lentil, nodulation

The use of plant growth promoting rhizobacteria (PGPR) is a useful technique for sustainable crop production (Glick *et al.,* 2012; Sharafzadeh, 2012). The beneficial effects of the rhizobacteria have been attributed towards their ability to produce various compounds i.e., phytohormones, organic acids and siderophores, fixation of atmospheric nitrogen, phosphate solubilization, and production of antibiotics and chitinase that suppress deleterious effects of pathogens (Arshad and Frankenberger, 1993; Glick, 2012; Tahir and Sarwar, 2013) along with some other unidentified mechanisms. Some PGPR have the ability to reduce the deleterious effects of ethylene on plant growth due to the presence of ACC-deaminase enzyme and thus improve plant growth (Glick *et al.,* 1995; Shaharoona *et al.,* 2006).

Ethylene is required for seed germination by many plant species and rate of ethylene production increases during germination and seedling growth (Abeles *et al.,* 1992). Low level of ethylene appears to enhance root initiation and growth while higher level of ethylene, produced by fast growing roots, can lead to inhibition of root elongation (Ma *et al.,* 1998). Ethylene is the most important factor for inhibiting nodulation in legumes (Madhaiyan *et al.,* 2007). Any factor or stimulus which causes a change in the endogenous level of ethylene in a plant results in modified growth and development (Arshad and Frankenberger, 2002).

The synthesis of ethylene in plants is directly related to the concentration of 1-aminocyclopropane-1-carboxylic acid (ACC) (Yang and Hoffman, 1984; Machacova *et al.,* 1997). It has been well documented that certain microorganisms contain an enzyme ACC-deaminase that hydrolyses ACC into ammonia and α-ketobutyrate (Mayak *et al.,* 1999). Decreased endogenous levels of ACC result in lower levels of ethylene, thus eliminating the potential inhibitory effects of higher ethylene concentration (Glick *et al.,* 1998). Improvement in nodulation in legumes has been reported due to co-inoculation with rhizobia and PGPR (Mishra *et al.,* 2009; Zahir *et al.,* 2011).

Co-inoculation of legumes with PGPR and rhizobia significantly increased the growth and yield of legume crops (Valverde *et al.,* 2006). Rhizosphere bacteria, in combination with symbiotic bacteria may increase nodulation in many ways such as production of phytoalexin, antibiotics against pathogenic organisms and siderophores chelating insoluble cations and colonizing root

*Email: zafarulhyegondal@yahoo.com

surfaces, thereby out-competing pathogens (Parmer and Dadarwal, 1999). The presence of ACC-deaminase positive PGPR on the roots of legumes could suppress endogenous synthesis of ethylene during the rhizobial infection and thus may facilitate nodulation and improve growth and yield. Restoration of nodulation in the presence of Ag^+ (which inhibits ethylene action) and by amino ethoxyvinyl glycine (a chemical inhibitor of endogenous C_2H_4 biosynthesis) strongly supports this premise (Schmidt *et al.,* 1999).

Co-inoculation of legumes with rhizobia and PGPR containing ACC-deaminase could be an effective approach to achieve successful nodulation in legumes. So, the present study has been conducted to assess the potential of co-inoculation with rhizobia and PGPR for improving growth and nodulation in lentil.

Collection of efficient rhizobacterial and rhizobial strains

Ten strains, each of rhizobacteria containing ACC-deaminase and rhizobia (*R. leguminosarum*) were isolated from the rhizosphere soil and nodules of lentil, respectively, and screened for their potential to improve growth and nodulation under axenic conditions (Zafar-ul-Hye *et al*., 2007a, b)

Preparation of inocula

Inocula were prepared in Erlenmeyer flasks by using YEM and DF minimal salt medium containing ACC as substrate, for rhizobia and rhizobacteria strains, respectively. Each flask containing broth was inoculated with respective strains of rhizobia or rhizobacteria and incubated at 28±1 ^oC for 48 hours under shaking (100 rpm) incubator. Optical density was measured and uniform population (OD_{550} = 0.45; 10^7–10^8 cfu mL^{-1}) was achieved by dilution with sterilized water prior to seed inoculation.

Growth pouch experiment

Screening of effective (PGPR × *Rhizobium*) combinations was carried out under axenic conditions in the growth room. For co-inoculation, broth cultures of PGPR strains and rhizobia were used in the 1:1 ratio. Surface-sterilized lentil seeds were dipped in this combined broth for ten minutes. Three seeds were sown in autoclaved growth pouches. In the case of control, sterilized broths were used for seed dipping. Nitrogen free sterilized Hoagland's solution was used for nutrients supply (Fahraeus, 1957). There were three replications for each treatment. The temperature in the growth room was adjusted to 28±1 ^oC with 10 hours of light (275 µmol $m^{-2}s^{-1}$) and 14 hours dark period. Data regarding seedlings growth were recorded. The experiments were conducted in the growth room and all operations were carried out aseptically in a laminar flow-hood. After 50 days, data regarding root, shoot growth and nodulation were recorded.

The standard error of means was calculated as described by Steel *et al*. (1997). The significance of the high growth promoting combinations was further analyzed by General Linear Model using Minitab™ version 15 software (Mead *et al.,* 2003).

Characterization of PGPR strains

The rhizobacterial strains were characterized for ACC-deaminase activity, Root colonization ability and i*n vitro* auxin production by following the standard protocols, and the results have already been published as Zahir *et al.* (2011).

The results showed that co-inoculation of *Rhizobium* and PGPR strains containing ACC-deaminase activity significantly increased the growth and nodulation of lentil seedlings.

It was revealed from the data that co-inoculation with *Rhizobium* and PGPR strains improved the shoot length of lentil seedlings. The maximum increase in shoot length (1.87 fold) compared to the un-inoculated control was recorded with the combination P10Z22 (Table 1). Most of the combinations significantly increased the shoot length of lentil seedlings and the increase ranged from 1.67 – 1.87 fold over the un-inoculated control. However, four combinations showed negative effect and decreased the shoot length up to 1.25-fold, as compared to the control.

The root length of lentil seedlings was improved due to co-inoculation with *Rhizobium* and PGPR strains. The increase in root length due to co-inoculation ranged from 1.11 to 1.97-fold as compared to the un-inoculated control (Table 2). Four combinations (P25Z4, P1Z7, P11Z7 and P1Z45) showed negative effect on lentil growth.

The data (Table 3) showed that co-inoculation with rhizobia and PGPR strains improved the total biomass of lentil seedlings. The increase in total biomass due to co-inoculation ranged from 1.04 to 1.98-fold except four combinations (P25Z4, P1Z7, P11Z7 and P1Z45) which decreased the total biomass up to 1.14-fold, when compared with the control. The combination P10Z22 was the most effective one which increased total biomass up to 1.98-fold than the un-inoculated control.

Co-inoculation also increased the nodulation in lentil seedlings grown in growth pouches under axenic conditions. The maximum number of nodules was observed in the combination P10Z22 which produced 24.3 nodules $plant^{-1}$, while there was no nodulation in the un-inoculated control and a few co-inoculated treatments (Table 4).

Table 1: Synergistic effect of rhizobacteria and rhizobia on shoot length (cm) of lentil seedlings under axenic conditions

Combination	Mean ± SE	Combination	Mean ± SE	Combination	Mean ± SE
Control	17.72±1.27	Z7P10	19.25±2.39	Z22P15	24.30±1.67
Z1P1	26.93±1.36	Z7P11	19.75±1.98	Z22P24	30.67±1.32
Z1P3	25.86±1.75	Z7P12	22.33±1.11	Z22P25	26.98±1.03
Z1P5	27.73±1.37	Z7P13	19.97±0.85	Z23P1	28.82±2.42
Z1P10	24.72±1.36	Z7P15	15.85±1.41	Z23P3	19.08±0.85
Z1P11	22.13±1.72	Z7P24	19.75±1.66	Z23P5	27.48±1.34
Z1P12	23.47±1.73	Z7P25	26.00±2.37	Z23P10	27.45±2.42
Z1P13	21.32±1.95	Z9P1	26.72±1.08	Z23P11	28.46±1.40
Z1P15	23.60±1.39	Z9P3	23.83±1.06	Z23P12	18.81±1.04
Z1P24	25.02±1.81	Z9P5	27.75±0.71	Z23P13	29.48±1.83
Z1P25	26.78±1.68	Z9P10	23.25±1.43	Z23P15	27.12±1.99
Z3P1	24.40±1.60	Z9P11	15.83±1.83	Z23P24	28.99±1.62
Z3P3	26.58±1.91	Z9P12	25.92±1.12	Z23P25	27.33±1.21
***Z3P5**	**29.82±1.45**	Z9P13	24.52±0.32	Z38P1	26.82±1.15
Z3P10	28.40±1.84	Z9P15	21.80±1.50	Z38P3	21.45±1.09
Z3P11	25.00±0.72	Z9P24	25.07±0.59	Z38P5	26.17±1.63
Z3P12	26.83±1.26	Z9P25	27.25±2.05	Z38P10	26.94±1.89
Z3P13	25.17±1.42	Z14P1	27.98±1.33	Z38P11	27.33±2.22
Z3P15	27.25±1.31	Z14P3	29.05±1.61	Z38P12	23.42±0.59
Z3P24	30.75±1.75	Z14P5	30.07±1.10	Z38P13	27.35±1.08
Z3P25	26.05±1.55	Z14P10	29.17±1.74	Z38P15	26.00±1.58
Z4P1	24.95±1.32	Z14P11	25.17±1.91	Z38P24	27.68±1.71
Z4P3	24.68±2.07	Z14P12	28.37±0.52	Z38P25	25.30±0.74
Z4P5	27.35±1.83	Z14P13	24.73±1.37	Z45P1	19.53±1.31
Z4P10	27.13±1.45	Z14P15	24.93±0.61	Z45P3	30.95±0.78
Z4P11	23.50±1.52	Z14P24	25.92±0.65	Z45P5	29.61±1.56
Z4P12	25.37±0.52	Z14P25	30.43±1.04	Z45P10	28.52±1.98
Z4P13	22.29±0.33	Z22P1	30.83±0.83	Z45P11	27.99±1.47
Z4P15	25.17±2.12	Z22P3	32.33±1.27	Z45P12	26.46±1.03
Z4P24	27.17±0.94	Z22P5	31.63±0.57	Z45P13	28.28±0.88
Z4P25	14.22±0.56	***Z22P10**	**33.10±1.40**	Z45P15	28.36±1.41
Z7P1	16.36±0.64	Z22P11	29.38±1.15	***Z45P24**	**31.95±2.15**
Z7P3	20.02±1.13	Z22P12	27.88±1.20	Z45P25	28.49±1.11
Z7P5	19.30±1.48	Z22P13	31.67±0.83		

SED (Standard error of difference between two mean) = 2.19; P value at 5% = 0.015, n = 3 *These were more promising than the rest of the combinations according to General Linear Model using the minitab™ version 15 software

Z: *Rhizobium* strains, P: PGPR strains

Characterization of PGPR strains of the selected combination

The biochemical characterization of the PGPR strains from the selected combinations was performed (Zahir *et al.,* 2011). All the three PGPR strains from the selected combinations were positive for phosphate solubilization. The maximum ACC deaminase activity (537 nmol (g biomass)$^{-1}$ h^{-1}) was observed in P10. The results of the root colonization assay showed that all the strains colonized the roots, but the maximum root colonization ability (7.64 × 10^5 cfu g^{-1}) was observed by strain P10. Similarly, all the rhizobacterial strains produced auxins measured in terms of IAA equivalents in the presence and absence of L-TRP but they varied in their ability to produce auxin. The maximum auxins were produced by the strain P10 which gave significantly different results compared with other strains.

Ten strains, each of rhizobacteria containing ACC-deaminase and rhizobial strains were isolated from the rhizosphere soil and nodules of lentil, and screened for their potential to improve growth and nodulation under axenic conditions (Zafar-ul-Hye *et al.,* 2007a, b). These strains were screened for their efficiency as co-inoculants in

Table 2: Synergistic effect of rhizobacteria and rhizobia on root length (cm) of lentil seedlings under axenic conditions

Combination	Mean ± SE	Combination	Mean ± SE	Combination	Mean ± SE
Control	11.03±0.80	Z7P10	21.75±1.41	Z22P15	15.42±0.85
Z1P1	15.53±0.45	Z7P11	11.0±1.23	Z22P24	18.88±1.69
Z1P3	17.11±0.64	Z7P12	16.58±0.67	Z22P25	14.58±1.81
Z1P5	16.33±0.65	Z7P13	13.42±0.58	Z23P1	16.02±0.83
Z1P10	18.52±0.88	Z7P15	15.98±0.55	Z23P3	16.42±0.45
Z1P11	16.80±0.61	Z7P24	17.00±0.43	Z23P5	15.00±0.66
Z1P12	14.22±0.73	Z7P25	17.67±0.84	Z23P10	15.57±0.61
Z1P13	16.35±0.19	Z9P1	14.45±0.79	Z23P11	16.92±0.77
Z1P15	16.05±1.14	Z9P3	19.25±0.51	Z23P12	16.65±1.57
Z1P24	13.88±0.60	Z9P5	18.83±0.96	Z23P13	15.22±0.80
Z1P25	15.22±0.94	Z9P10	17.96±1.67	Z23P15	14.85±1.03
Z3P1	15.75±0.88	Z9P11	19.33±0.45	Z23P24	16.75±0.72
Z3P3	17.53±0.61	Z9P12	18.75±0.70	Z23P25	15.67±0.56
***Z3P5**	**19.82±0.94**	Z9P13	18.05±0.39	Z38P1	18.17±1.07
Z3P10	18.96±1.11	Z9P15	17.37±0.77	Z38P3	17.83±0.20
Z3P11	17.00±0.71	Z9P24	16.42±0.83	Z38P5	17.83±0.90
Z3P12	16.42±0.30	Z9P25	15.92±0.65	Z38P10	15.00±0.71
Z3P13	14.50±0.83	Z14P1	15.60±0.50	Z38P11	17.35±0.80
Z3P15	16.25±0.47	Z14P3	18.52±0.70	Z38P12	15.38±0.79
Z3P24	17.95±0.57	Z14P5	17.43±0.44	Z38P13	16.93±0.48
Z3P25	13.17±0.84	Z14P10	18.08±0.67	Z38P15	16.95±0.44
Z4P1	17.08±0.30	Z14P11	16.42±0.67	Z38P24	17.68±0.37
Z4P3	17.82±0.61	Z14P12	15.88±0.64	Z38P25	16.95±0.44
Z4P5	19.23±1.09	Z14P13	15.18±0.78	Z45P1	10.92±0.61
Z4P10	18.17±0.69	Z14P15	17.47±0.12	Z45P3	16.28±0.59
Z4P11	18.73±0.66	Z14P24	16.58±0.38	Z45P5	15.00±0.35
Z4P12	17.92±0.65	Z14P25	15.22±0.91	Z45P10	18.80±0.71
Z4P13	16.71±0.71	Z22P1	17.25±0.66	Z45P11	16.08±0.38
Z4P15	16.67±0.58	Z22P3	18.27±0.46	Z45P12	15.75±0.51
Z4P24	12.28±0.32	Z22P5	18.20±0.37	Z45P13	16.08±0.30
Z4P25	10.58±0.76	***Z22P10**	**21.78±0.55**	Z45P15	15.98±0.55
Z7P1	10.55±0.47	Z22P11	16.12±0.64	***Z45P24**	**19.76±1.33**
Z7P3	16.23±0.78	Z22P12	17.33±0.45	Z45P25	16.09±0.46
Z7P5	21.03±1.36	Z22P13	15.00±0.83		

SED (Standard error of difference between two mean) = 2.19; P value at 5% = 0.015, n = 3 *These were more promising than the rest of the combinations according to General Linear Model using the minitab™ version 15 software
Z: *Rhizobium* strains, P: PGPR strains

different combinations to improve lentil growth and nodulation. Co-inoculation of rhizobia with selected strains of PGPR containing ACC deaminase activity was more effective in improving growth and nodulation of lentil than the others. It is highly likely that these rhizobacteria might have improved the plant growth by lowering the endogenous levels of ethylene (Arshad *et al.,* 2008). It is highly likely that these rhizobacteria might also have stimulated nodulation indirectly through increased root growth that provides rhizobia with more infection sites for nodulations. Improvement in nodule occupancy of *Bradyrhizobium* in soybean has been reported due to co-inoculation with *P. fluorescens* (Mishra *et al.,* 2009). Co-inoculation improves plant growth by reducing ethylene level (Ahmad *et al.,* 2011), directly stimulating rhizobial growth and survival in the soil, enlarging the root system by hormone production for enhanced nutrient uptake and increased number of potential colonization sites for *Rhizobium*, solubilizing phosphate, and suppressing pathogens as a result of production of antibiotics (Naveed *et al.,* 2008). There are many evidences to show that ACC-deaminase as one of the main mechanisms by which PGPR

Table 3: Synergistic effect of rhizobacteria and rhizobia on total biomass (g) of lentil seedlings under axenic conditions

Combination	Mean ± SE	Combination	Mean ± SE	Combination	Mean ± SE
Control	1.89±0.04	Z7P10	2.50±0.13	Z22P15	2.58±0.08
Z1P1	2.66±0.15	Z7P11	1.86±0.14	Z22P24	2.85±0.21
Z1P3	2.82±0.17	Z7P12	2.18±0.11	Z22P25	2.49±0.08
Z1P5	2.90±0.13	Z7P13	2.28±0.08	Z23P1	2.48±0.34
Z1P10	2.92±0.13	Z7P15	2.05±0.10	Z23P3	2.54±0.16
Z1P11	2.68±0.20	Z7P24	2.17±0.8	Z23P5	2.52±0.22
Z1P12	2.49±0.10	Z7P25	2.17±0.12	Z23P10	2.57±0.09
Z1P13	2.71±0.04	Z9P1	2.20±0.24	Z23P11	2.45±0.22
Z1P15	2.64±0.15	Z9P3	2.24±0.11	Z23P12	2.26±0.11
Z1P24	2.55±0.17	Z9P5	2.70±0.25	Z23P13	2.03±0.07
Z1P25	2.62±0.12	Z9P10	2.64±0.11	Z23P15	2.13±0.10
Z3P1	2.48±0.15	Z9P11	2.52±0.14	Z23P24	2.54±0.12
Z3P3	3.11±0.14	Z9P12	2.50±0.08	Z23P25	2.31±0.07
***Z3P5**	**3.35±0.08**	Z9P13	2.46±0.05	Z38P1	2.57±0.06
Z3P10	3.14±0.29	Z9P15	2.43±0.10	Z38P3	2.09±0.07
Z3P11	2.53±0.15	Z9P24	2.08±0.19	Z38P5	2.32±0.12
Z3P12	2.72±0.27	Z9P25	1.97±0.08	Z38P10	2.36±0.05
Z3P13	2.83±0.09	Z14P1	2.39±0.38	Z38P11	2.48±0.07
Z3P15	2.76±0.19	Z14P3	2.93±0.03	Z38P12	2.38±0.05
Z3P24	3.13±0.46	Z14P5	3.11±0.22	Z38P13	2.27±0.10
Z3P25	2.69±0.28	Z14P10	2.89±0.24	Z38P15	2.31±0.04
Z4P1	2.91±0.35	Z14P11	2.38±0.26	Z38P24	2.56±0.01
Z4P3	2.82±0.24	Z14P12	2.12±0.07	Z38P25	2.40±0.09
Z4P5	2.92±0.21	Z14P13	2.04±0.10	Z45P1	1.86±0.10
Z4P10	2.74±0.25	Z14P15	2.33±0.09	Z45P3	2.33±0.15
Z4P11	2.26±0.14	Z14P24	2.37±0.06	Z45P5	2.72±0.16
Z4P12	2.25±0.14	Z14P25	2.24±0.06	Z45P10	2.96±0.02
Z4P13	2.39±0.21	Z22P1	2.49±0.17	Z45P11	2.72±0.03
Z4P15	2.26±0.13	Z22P3	3.25±0.14	Z45P12	2.33±0.13
Z4P24	2.05±0.17	Z22P5	3.33±0.16	Z45P13	2.48±0.04
Z4P25	1.66±0.12	***Z22P10**	**3.74±0.11**	Z45P15	2.14±0.06
Z7P1	1.74±0.15	Z22P11	2.78±0.24	***Z45P24**	**3.39±0.12**
Z7P3	2.01±0.04	Z22P12	2.61±0.43	Z45P25	2.32±0.28
Z7P5	2.45±0.20	Z22P13	2.55±0.22		

SED (Standard error of difference between two mean) = 2.19; P value at 5% = 0.015, n = 3 *These were more promising than the rest of the combinations according to General Linear Model using the minitab™ version 15 software
Z: *Rhizobium* strains, P: PGPR strains

have improved the growth of plants, especially root elongation (Madhaiyan *et al.,* 2006).

The strains varied in their ability to improve plant growth, and the maximum response was observed with the combination P10Z22. It is very likely that PGPR strains varied in their ACC deaminase ability along with some other characters that contributed differently for growth promotion. Similarly, it was reported in previous findings that strains differ in their ability to promote plant growth due to differences in ACC deaminase activity (Nadeem *et al.,* 2009; Ahmad *et al.,* 2011, 2012).

The variation in growth promotion by different combinations in this study might also be due to the differences in their efficiency to colonize the germinating roots. The combinations with the rhizobacterial strain having greater efficiency of deaminating endogenous ACC might have caused more root growth promotion by eliminating the inhibitory effects of higher ethylene concentrations (Zahir *et al.,* 2011). Similar results have been reported by other researchers (Shaharoona *et al.,* 2006; Ahmad *et al.,* 2011).

On the other hand, a few combinations could not promote growth parameters as compared to the control.

Table 4: Synergistic effect of rhizobacteria and rhizobia on number of nodules per plant of lentil under axenic conditions

Combination	Mean ± SE	Combination	Mean ± SE	Combination	Mean ± SE
Control	0	Z7P10	0	Z22P15	5.7±0.7
Z1P1	0	Z7P11	3.3±1.1	Z22P24	6.7±0.7
Z1P3	2.0±0.5	Z7P12	2.3±0.3	Z22P25	5.7±0.7
Z1P5	3.7±0.7	Z7P13	2.3±0.7	Z23P1	6.0±0.9
Z1P10	1.7±0.3	Z7P15	2.0±0.5	Z23P3	5.3±0.7
Z1P11	4.3±0.7	Z7P24	2.3±0.3	Z23P5	13.0±0.9
Z1P12	3.3±0.7	Z7P25	2.0±0.5	Z23P10	11.3±1.5
Z1P13	2.0±0.5	Z9P1	2.3±0.7	Z23P11	7.7±0.7
Z1P15	0	Z9P3	3.0±0.5	Z23P12	0
Z1P24	2.7±0.3	Z9P5	4.7±0.7	Z23P13	0
Z1P25	2.3±0.3	Z9P10	11.7±1.2	Z23P15	7.3±0.9
Z3P1	2.3±0.7	Z9P11	8.7±0.7	Z23P24	7.0±0.5
*Z3P3	4.0±0.5	Z9P12	4.0±0.5	Z23P25	5.7±1.2
***Z3P5**	**15.0±0.9**	Z9P13	2.0±0.5	Z38P1	5.7±1.4
Z3P10	10.3±1.2	Z9P15	3.3±0.7	Z38P3	9.0±1.2
Z3P11	2.7±1.19	Z9P24	3.7±0.9	Z38P5	12.7±1.2
Z3P12	3.0±0.5	Z9P25	3.3±0.7	Z38P10	9.0±0.5
Z3P13	3.0±0.5	Z14P1	3.0±0.5	Z38P11	10.3±1.4
Z3P15	2.7±0.7	Z14P3	0	Z38P12	11.0±0.9
Z3P24	9.0±0.5	Z14P5	10.0±0.9	Z38P13	0
Z3P25	2.3±0.3	Z14P10	12.3±1.4	Z38P15	5.3±0.7
Z4P1	2.7±0.7	Z14P11	13.7±1.2	Z38P24	5.7±1.2
Z4P3	2.3±0.3	Z14P12	4.3±0.9	Z38P25	0
Z4P5	3.3±1.1	Z14P13	0	Z45P1	0
Z4P10	3.7±0.7	Z14P15	3.0±0.5	Z45P3	5.0±0.9
Z4P11	4.3±0.7	Z14P24	3.3±0.7	Z45P5	6.7±1.1
Z4P12	3.0±0.5	Z14P25	3.0±0.5	Z45P10	5.0±0.5
Z4P13	2.0±0.5	Z22P1	4.7±0.7	Z45P11	0
Z4P15	3.0±0.5	Z22P3	13.3±1.4	Z45P12	0
Z4P24	1.7±0.3	Z22P5	13.0±1.2	Z45P13	0
Z4P25	0	***Z22P10**	**24.3±1.3**	Z45P15	0
Z7P1	0	Z22P11	7.0±0.5	***Z45P24**	**14.3±1.2**
Z7P3	0	Z22P12	0	Z45P25	0
Z7P5	3.0±0.47	Z22P13	7.7±1.19		

SED (Standard error of difference between two mean) = 2.19; P value at 5% = 0.015, n = 3 *These were more promising than the rest of the combinations according to General Linear Model using the minitab™ version 15 software
Z: *Rhizobium* strains, P: PGPR strains

These PGPR might have produced certain compounds (i.e. toxic for seedlings) and thus showed negative effect on lentil seedlings. Negative effects of co-inoculation of PGPR with *Rhizobium* might have been attributed to the production of antibiotics and competition for attachment sites on the root surfaces (Mirza *et al.,* 2007).

Some combinations showed similar results to the un-inoculated control in case of number of nodules plant^{-1}. This might be due to the lack of compatibility of combinations or/and due to production of toxic substances for plant health. Similar results were obtained by Okon *et al*. (1998) and Paul and Verma (1999).

Conclusion

It is concluded from our findings that co-inoculation with PGPR containing ACC-deaminase activity and rhizobia may be an effective approach for better root elongation, nodulation and consequently improved growth of lentil. Further evaluation of the selected combinations should be carried out under pot and field conditions.

Acknowledgement

We are thankful to the Higher Education Commission, Pakistan and ALP/PARC for providing the financial support for this research project.

References

Abeles, F.D., P.W. Morgan and M.E. Saltveit, Jr. 1992. Ethylene in Plant Biology. 2nd Ed. Academic Press, Inc., San Diego, CA.

Ahmad, M., Z.A. Zahir, H.N. Asghar and M. Arshad. 2012. The combined application of rhizobial strains and plant growth promoting rhizobacteria improves growth and productivity of mung bean (*Vigna radiata* L.) under salt-stressed conditions. *Annals of Microbiology* 62(3): 1321-1330.

Ahmad, M., Z.A. Zahir, H.N. Asghar and M. Asghar. 2011. Inducing salt tolerance in mung bean through co-inoculation with *Rhizobium* and PGPR containing ACC-deaminase. *Canadian Journal of Microbiology* 57(7): 578-589.

Arshad, M. and W.T. Frakenberger, Jr. 2002. Ethylene: Agricultural Sources and Applications. Kluwer Academic Publishers, New York, USA. p. 342.

Arshad, M. and W.T. Frankenberger, Jr. 1993. Microbial production of plant growth regulators. p. 307-347. In: Soil Microbial Ecology. F.B. Metting Jr. (ed.). Marcel Dekker, Inc., New York, USA.

Fahraeus, G. 1957. The infection of white clover root hair by nodule bacteria studied by a simple slide technique. *Journal of General Microbiology* 16: 374-381.

Glick, B.R. 2012. Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica* 2012: 1-15. http://dx.doi.org/10.6064/2012/963401 [Accessed 24/04/2013].

Glick, B.R., D.M. Karaturovic and P.C. Newell. 1995. A novel procedure for rapid isolation of plant growth promoting *Pseudomonads*. *Canadian Journal of Microbiology* 41: 533-536.

Glick, B.R., D.M. Penrose and J. Li. 1998. A model for lowering plant ethylene concentration by plant growth promoting rhizobacteria. *Journal of Theoretical Biology* 190: 63-68.

Ma, J.H., L. Yao, D. Chen and B. Morris. 1998. Ethylene inhibitors enhance vitro formation from apple shoot culture. *Plant Cell Reporter* 17: 211-214.

Machacova, I., N. Chavaux, W. Dewitte and H.V. Onckelen. 1997. Diurnal fluctuations in ethylene formation in *Chenopodium rubrum*. *Plant Physiology* 113: 981-985.

Madhaiyan, M., Poonguzhali, S., and Sa, T.M. 2007. Characterization of 1-aminocyclopropane-1-carboxylate deaminase (ACC) deaminase containing *Methylobacterium oryzae* and interactions with auxins and ACC regulation of ethylene in canola (*Brassica campestris*). *Planta* 226(4): 867–876.

Madhaiyan, M., S. Poonguzhali, J. Ryu and T. Sa. 2006. Regulation of ethylene levels in canola (*Brassica campestris*) by 1-aminocyclopropane-1-carboxylate deaminase-containing *Methylobacterium fujisawaense*. *Planta* 224: 268-278.

Mayak, S., T. Tirosh and B.R. Glick. 1999. Effect of wild type and mutant plant growth promoting rhizobacteria on the rooting of mung bean cuttings. *Journal of Plant Growth Regulators* 18: 49-53.

Mead, R., R.N. Curnow and A.M. Hasted. 2003. Statistical Methods in Agriculture and Experimental Biology. 3rd Ed. Chapman and Hall, CRC Press. 488p.

Mirza, B.S., M.S. Mirza, A. Bano and K.A. Malik. 2007. Co-inoculation of chickpea with *Rhizobium* isolates from roots and nodules and phytohormone-producing *Enterobacter* strains. *Australian Journal of Experimental Agriculture* 47: 1008-1015.

Mishra, P.K., S. Mishra, G. Selvakumar, S. Kundu and H.S. Gupta. 2009. Enhanced soybean (*Glycine max* L.) plant growth and nodulation by *Bradyrhizobium japonicum*-SB1 in presence of *Bacillus thuringiensis*. *Acta Agriculturae Scandinavica, Section B* 59: 189-196.

Nadeem, S.M., Zahir, Z.A., Naveed, M., and Arshad, M. 2009. Rhizobacteria containing ACC deaminase confer salt tolerance in maize grown on salt affected soils. *Canadian Journal of Microbiology* 55(11): 1302-1309.

Naveed, M., Z.A. Zahir, M. Khalid, H.N Asghar, M.J. Akhatr and M. Arshad. 2008. Rhizobacteria containing ACC-deaminase for improving growth and yield of wheat under fertilized conditions. *Pakistan Journal of Botany* 40(3): 1231-1241.

Okon, Y., G.V. Bloemberge and J.J. Lugtenberg. 1998. Biotechnology of biofertilization and phytostimulation. p. 327-349. In: Agriculture Biotechnology. A. Altman, (ed.). Marcel Dekker, New York.

Parmar, N. and K.R. Dadarwal. 1999. Stimulation of nitrogen fixation and induction of flavonoid-like compounds by rhizobacteria. *Journal of Applied Microbiology* 86: 36-44.

Paul, S. and O.P. Verma. 1999. Influence of combined inoculation of *Azotobacter* and *Rhizobium* on the yield of chickpea (*Cicer arietinum* L.). *Indian Journal of Microbiology* 39: 249-251.

Schmidt, J.S., J.E. Harper, T.K. Hoffman and A.F. Bent. 1999. Regulation of soybean nodulation independent of ethylene signalling. *Plant Physiology* 119: 951-959.

Shaharoona, B., M. Arshad, Z.A. Zahir and A. Khalid. 2006. Performance of *Pseudomonas* spp. containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous

fertilizers. *Soil Biology and Biochemistry* 38: 2971-2975.

Sharafzadeh, Sh. 2012. Effect of PGPR on growth and nutrients uptake of tomato. *International Journal of Advances in Engineering & Technology* 2: 27-31.

Steel, R.G.D., J.H. Torrie and D.A. Dicky. 1997. Principles and Procedures of Statistics- A Biometrical Approach. 3rd Ed. McGraw-Hill Book International Co., Singapore. p. 204-227.

Tahir, M. and M.A. Sarwar. 2013. Plant growth promoting rhizobacteria (PGPR): A budding complement of synthetic fertilizers for improving crop production. *Pakistan Journal of Life and Social Sciences* 11: 1-7.

Valverde, A., A. Burgos, T. Fiscella, R. Rivas, E. Velazquez, C. Rodriguez-Barrueco, E. Cervantes, M. Chamber and J-M. Igual. 2006. Differential effects of coinoculation with *Pseudomonas jessenii* PS06 (a phosphate-solubilizing bacterium) and *Mesorhizobium ciceri* C-2/2 strains on the growth and seed yield of chickpea under greenhouse and field conditions. *Plant and Soil* 287: 43-50.

Yang, S.F. and N.E. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. *Annual Review in Plant Physiology* 35: 155-189.

Zafar-ul-Hye, M., Z.A. Zahir, S.M. Shahzad, M. Naveed, M. Arshad and M. Khalid. 2007a. Preliminary screening of rhizobacteria containing ACC-deaminase for promoting growth of lentil seedlings under axenic condition. *Pakistan Journal of Botany* 39(5): 1725-1738.

Zafar-ul-Hye, M., Z.A. Zahir, S.M. Shahzad, U. Irshad and M. Arshad. 2007b. Isolation and screening of rhizobia for improving growth and nodulation of lentil (*Lens culinaris* Medic) seedlings under axenic conditions. *Soil and Environment* 26(1): 81-91.

Zahir, Z. A., M. Zafar-ul-Hye**,** S. Sajjad and M. Naveed. 2011. Comparative effectiveness of *Pseudomonas* and *Serratia sp*. containing ACC-deaminase for co-inoculation with *Rhizobium leguminosarum* to improve growth, nodulation and yield of lentil. *Biology and Fertility of Soils* 47: 457-465.