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CO-INOCULATION WITH *RHIZOBIUM* AND PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) FOR INDUCING SALINITY TOLERANCE IN MUNG BEAN UNDER FIELD CONDITION OF SEMI-ARID CLIMATE

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ABSTRACT

Salinity stress severely affects the growth, nodulation and yield of mung bean (*Vigna radiata* L.). However, inoculation/co-inoculation with rhizobia and plant growth promoting rhizobacteria (PGPR) containing 1-Aminocyclopropane-1-carboxylic acid (ACC) deaminase improve the plant growth by reducing the stress induced ethylene production through ACC-deaminase activity. A study was conducted under salt affected field condition where pre-isolated strains of *Rhizobium* and PGPR were used alone as well as in combination for mitigating the salinity stress on growth, nodulation and yield of mung bean by following the randomized complete block design (RCBD). Results revealed that single and combine inoculation enhanced the 1000 grain weight and grain yield up to 14 and 30%, respectively, compared to un-inoculated control. Moreover, nodulation, relative water content (RWC) and total dry matter (TDM) were improved in case of inoculated/co-inoculated plant. Similarly, improved protein content (48%) and K/Na ratio (95%) in grain were observed by single and combine inoculation. Thus, inoculation/co-inoculation with *rhizobium* and PGPR could be a sustainable approach to improve plant growth under salinity stress.

Keywords: ACC-deaminase, salt stress, rhizobia, legume, *Vigna radiata* L.

INTRODUCTION

In Pakistan, mung bean has been cultivated on an area of 141 thousand hectares with a total production of about 93 thousand tonnes (Anonymous, 2012). This average production is much lower compared to many areas of the world. Salinity is one of the most important abiotic constraints that adversely affect plant growth and development throughout the world. In Pakistan, out of the total land area of 80 million hectares, about 10 million hectares is under salinity stress (FAO, 2008). Moreover, about one-third of the irrigated land is affected by salinity where major causes are high temperature, low annual rainfall, high evapotranspiration, lack of fresh water and/or salt rich poor quality irrigation water and poor irrigation management (Plaut et al., 2013). Ethylene is known as gaseous plant hormone and synthesized endogenously in all type of plants. Various biotic and abiotic stresses also produce ethylene in plant that consequently induces remarkable physiological changes in plants at molecular level. Besides regulating the plant growth, ethylene also involves in different stress responses (Saleem et al., 2007). Under stress condition, plants produce higher level of ethylene which is received by different receptors that ultimately trigger cellular responses (Jouyban, 2012). It has also been reported that in various plant species ethylene acts as negative regulator of nodulation (Schaller, 2012). Therefore, it is assumed that plant growth promoting rhizobacteria (PGPR) containing ACC-deaminase present in close vicinity of legume roots could reduce the endogenous ethylene concentration during bacterial infection and thereby may improve nodulation and plant growth. So, combined inoculation of competitive rhizobia and PGPR-containing ACC-deaminase could be an attractive and sustainable approach to enhance nodulation in legumes (Shaharoona et al., 2006). Compared to traditional breeding and plant genetic modifications, the application of PGPR may prove beneficial and cost effective strategy to promote plant growth under salinity stress (Mayak et al., 2004). Plants showed more resistance to the deleterious effects of ethylene that is produced during different stresses such as drought (Zahir et al., 2008), flooding

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(Grichko and Glick, 2001), salinity (Nadeem et al., 2007), heavy metals stress (Gupta et al., 2002; Khan et al., 2013) and pathogenicity stress (Wang et al., 2000) when treated with PGPR. Rhizobia are well perceived for symbiotic association with legumes that reside in legume nodules where they convert atmospheric nitrogen into plant available form (Sessitsch et al., 2002). Free living diazotrophs promote the rhizobial efficiency by altering root architecture providing more niches for nodulation and thus enhance the N_2-fixing ability of legumes (Qureshi et al., 2009). Plant growth and nodulation improved by rhizobia and certain rhizobacteria (Ladwal et al., 2012). Keeping this in view, present study was planned to evaluate the pre-isolated *Rhizobium* and PGPR strains for their ability to promote mung bean growth, nodulation and yield under salt affected fields.

MATERIALS AND METHODS

A field experiment was conducted for evaluating the effect of inoculation/co-inoculation with *Rhizobium* and PGPR strains for inducing salinity tolerance in mung bean in a salt affected field (EC; 5.59 dS m^{-1}) having textural class sandy clay loam under semi-arid climatic conditions. Inoculum was prepared in flasks by using glucose peptone broth medium (Khan et al., 2013) and yeast extract mannitol broth medium (Singh et al., 2011) for strains of PGPR and *Rhizobium*, respectively. The inoculum of each isolate was injected into sterile peat (100 ml kg^{-1}) and was incubated for 24 hrs at 28±1°C before using it for seed coating. For mung bean seed inoculation, seed dressing was carried out with inoculated peat mixed with clay and 10% sugar solution. But in case of un-inoculated control, seeds were coated with same but autoclaved broth and sugar solution. In case of co-inoculation equal quantity of broth culture of respective strain was mixed and used for seed coating. Seeds were sown in lines where Row × Row distance of 60 cm and Plant × Plant distance of 10 cm was maintained after 15 days of germination. Recommended doses of NPK fertilizers were applied at the rate of 20-60-60 kg ha^{-1} in the form of urea, DAP and sulfate of potash, respectively. One strain of *Rhizobium* (Mg6) and two strains of PGPR (A1 and A2) were tested separately and in possible combinations as co-inoculation. Control treatment was kept un-inoculated neither with *Rhizobium* nor with PGPR. There were three replications of each treatment following RCBD. Canal water was used for irrigation. The data regarding nodulation were collected at flowering while that of growth and yield parameters were collected at harvesting. Plant samples were collected and analyzed for nitrogen (N), phosphorus (P), potassium (K) and sodium (Na) contents by following the method described by Ryan et al. (2001). Relative water contents (RWC) was determined by following the method used by Barrs and Weatherly (1962). The data recorded were subjected to statistical analysis by using Statistix-9 computer software (Copyright 2005, Analytical software, USA).

RESULTS

Results obtained from this study revealed that inoculation/co-inoculation improved the plant growth and yield as compared to un-inoculated control under salt affected field conditions. It was observed that plant height and number of pods were increased in inoculated and/or co-inoculated plants, but statistically increase was non-significant compared to un-inoculated control plants (Table 1). The maximum increase in pod weight (27%) was recorded by combined inoculation (A2 × Mg6) while these results were statistically non-significant from all other treatments except control that did not receive any inoculation. Moreover, data (Table 1) revealed that sole inoculation enhanced the 1000 grain weight and grain yield up to 13 and 23 %, respectively, over the control plant. But, combined inoculation of A2 × Mg6 play effective role in minimizing the deleterious effect of salinity stress caused considerable increase in the 1000 grain weight and grain yield, 14 and 30% , respectively, than un-inoculated plants. It was also found that single (A1) as well as combined inoculation (A2 × Mg6) improved the relative water content (RWC) up to 11% and 14%, respectively, as compared to control. Co-inoculation with A2 × Mg6 isolates caused substantial increase (50%) in total dry matter (TDM) that was statistically at par with inoculation (A2). Inoculation with *Rhizobium* and PGPR alone or in combination improved the number of nodules significantly compared with un-inoculated plants but among the treatments difference was non-significant. Same trend was observed for nodule fresh and dry weight (Table 2). Furthermore, data regarding nutrient contents in grain, revealed that dual inoculation improved the P concentration (32%) which was considerably superior compared to alone inoculation and un-inoculated control plant while single (A1) and combined application (A1 × Mg6) showed better results regarding the K concentration and improved K uptake by 27 and 24%, respectively (Table 3). The results indicated that A1 × Mg6 increased N concentration in grain which was comparable to the non-inoculated plants. Highest protein content (48%) was obtained when inoculated with A1 × Mg6. It was also revealed from the table (3) that co-inoculation improved the K/Na ratio (95%) by decreasing the Na content compared to the control. Study revealed that co-inoculation could be better approach for inducing salt tolerance and enhancing growth, nodulation and yield of mung bean grown on salt-affected fields.

Table 1 Effect of inoculation/co-inoculation *Rhizobium* and PGPR on growth parameters on mung bean

Treatments	Plant height (cm)	Number of pods	Pod fresh weight(g)	1000 grain weight (g)	Grain Yield (Mg ha^{-1})	RWC[a] (%)	TDM[b] (Mg ha^{-1})
Control	50.8 a	21.13 a	16.7 b	52.13 d	1.2 d	68.7 c	1.72 d
A1	55.0 a	24.33 a	19.9 ab	56.57 abc	1.4 abc	76.6 ab	2.53 b
A2	58.3 a	25.07 a	18.1 ab	59.17 ab	1.3 cd	73.1 bc	2.38 a
Mg6	54.3 a	25.13 a	20.5 ab	53.97 cd	1.5 ab	76.0 ab	1.85 cd
A1 X Mg6	58.2 a	23.27 a	19.2 ab	56.23 bc	1.4 bcd	76.2 ab	2.10 c
A2 X Mg6	58.7 a	25.47 a	21.2 a	59.77 a	1.6 a	78.9 a	2.62 a

Means sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test (*p<0.05*) [a] Relative Water Content [b] Total Dry Matter

Table 2 Effect of inoculation/co-inoculation of Rhizobium and PGPR on nodule number, nodule fresh and dry weight on mung bean

Treatments	Number of nodules	Nodule fresh weight (g)	Nodule dry weight (g)
Control	4.67 b	0.156 b	0.014 b
A1	16.00 a	0.537 a	0.045 a
A2	12.67 a	0.427 a	0.037 a
Mg6	16.00 a	0.537 a	0.047 a
A1 X Mg6	14.67 a	0.493 a	0.040 a
A2 X Mg6	13.33 a	0.449 a	0.039 a

Means sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test (*p<0.05*)

Table 3 Effect of inoculation/co-inoculation of *Rhizobium* and PGPR on nutrient concentration on mung bean

Treatments	Grain					
	N (%)	**P (%)**	**K (%)**	**Na (%)**	**K/ Na ratio**	**Protein (%)**
Control	1.5 e	0.22	1.55 c	0.81 a	1.81c	9.63 e
A1	1.8 cd	0.24 ab	1.98 a	0.59 b	3.00 b	11.32 cd
A2	1.9 bc	0.23 b	1.82 ab	0.57 bc	2.94 b	12.18 bc
Mg6	1.7 de	0.23 b	1.88 ab	0.54 cd	2.97 b	10.56 de
A1 X Mg6	2.3 a	0.26 a	1.92 ab	0.47e	3.55 a	14.34 a
A2 X Mg6	2.1 b	0.26 a	1.74 bc	0.53 d	2.83 b	12.90 b

Means sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test *($p<0.05$)*

DISCUSSION

The results of present study revealed that inoculation/co-inoculation with *Rhizobium* and PGPR had potential to improve plant growth by reducing the inhibitory effect of salinity under salt affected field condition. Single and combined inoculation have shown positive response to the measured growth parameters that might be attributed to changes in endogenous ethylene level by presence of plant growth promoting rhizobacteria (PGPR) containing ACC-deaminase on the roots of legumes (Shahroona et al., 2006; Nadeem et al., 2009; Ahmad et al., 2011). It is also likely to be better colonization ability of rhizobacteria, increased nitrogen fixation, phosphorous solubilization, production of phytohormones, chitanase activity and production of biological active substances (Remans et al., 2008). Similar trends were observed by Guinazu et al. (2010) and Panjebashi et al. (2012).

It has also been observed that relative water content and dry matter production was improved in inoculated and co-inoculated plant that might be due better and longer roots which ultimately resulted in increased water uptake from the deeper soil (Ahmed et al., 2011; Egamberdiyeva, 2007). Roopa et al. (2012) found that plant dry matter significantly improved when co-inoculated with PGPR strain PUK-17171 and *Rhizobium*. Higher level of ethylene which is applied either directly or indirectly had significant inhibitory effect on nodulation (Guinel and Sloetjes, 2000). Compared to non-inoculated plant, nodule number, nodule fresh and dry weight was considerably improved by sole and dual inoculation. Similar, results were also found by Shahzad et al. (2010). Similarly, Zahir et al. (2011) found that PGPR containing ACC-deaminase in combination with rhizobia enhanced the nodule formation in lentil by regulating the ethylene synthesis which subsequently effected nodulation.

In our study, results regarding nutrient concentration in grain showed that N, P and K concentration was more pronounced in inoculated and co-inoculated plants. The reason behind this might be better root growth, increased nitrogen fixation and greater phosphorus solubilization (Ahmed et al., 2011). Yao (2004) demonstrated that effective P concentration could be increased by inoculation with rhizobacteria. Similarly, Qureshi et al. (2011) observed that nutrient concentration in grain and different parts of plant increased by co-inoculation of *Bacillus* and *Rhizobium* in a pot experiment. Compared to un-inoculated plant, inoculation/co-inoculation significantly increased protein content of grain that might be attributed to more availability of N which is an important constituent of protein. Aslam et al. (2013) found that grain protein content improved in chickpea by *rhizobium* inoculum. Moreover, Na concentration in grain decreased and K/Na ratio improved under salinity stress by single and combine inoculation. This might be due to increase in K concentration which subsequently decreased Na^+ concentration.

Similar response was also observed by Munns et al. (2006).

CONCLUSION

Inoculation/co-inoculation with *Rhizobium* and PGPR could be an effective approach to induce salinity tolerance and improve nodulation, growth and yield of mung bean under salt affected conditions.

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