ISSN 1023-1072

Pak. J. Agri., Agril. Engg., Vet. Sci., 2014, 30 (1): 43-53

RHIZOBACTERIAL INOCULATION INTEGRATED WITH MINERAL FERTILIZERS PROMOTE MAIZE PRODUCTIVITY IN COMPACTED SALINE-SODIC SOIL

M. Zafar-ul-Hye[1], A. Nasir[1], Z. A. Zahir[2], A. Rehim[1] and M. Ahmad[3]

[1]Department of Soil Science, Faculty of Agricultural Sciences and Technology Bahauddin Zakariya University Multan, Pakistan
[2]Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan
[3]Department of Soil Science, University College of Agriculture and Environmental Sciences, Islamia University, Bahawalpur, Pakistan

ABSTRACT

In the plants ethylene level increases due to stress imposed by soil salinity and sodicity and soil compaction, which exert negative effects on the crops productivity. There are certain rhizobacteria containing an enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase which converts 1-aminocyclopropane-1-carboxylic acid (ACC) (immediate precursor of ethylene biosynthesis in higher plants) into ammonia and α-ketobutyrate instead of ethylene. Reduced ethylene concentration grants resistance to plants against stress conditions. In the present study two rhizobacterial strains were applied in combination with mineral fertilizers which produced significant improvement in maize productivity under multiple stress conditions i.e., saline-sodic and compacted soil. It was observed that rhizobacterial strains increased plant height, cob length, number of cobs per plant, number of grain rows per cob, number of grains per cob, 1000-grain weight, grain yield, stalk yield, nitrogen (N), phosphorus (P) and potassium (K) concentration in grains and N, P and K contents in stalk up to 27.79, 72.79, 1100, 88.88, 827.27, 327.53, 112.93, 29.38, 254, 38.93, 82.5, 635, 108.88 and 107.35%, respectively as compared to the control, when applied with mineral fertilizers. In conclusion, rhizobacteria possessing ACC-deaminase have the potential to improve maize productivity when applied in combination with mineral fertilizers in multiple stress conditions.

Keyword: ACC-deaminase, mineral fertilizers, rhizobacteria, salinity stress.

INTRODUCTION

With lot of diversified industrial uses, maize occupies a central position among world cereal crops. It serves as an important food for a huge world population

Corresponding author: zafarulhyegondal@yahoo.com

especially in Asia and Africa. Several important food items are produced from maize and used around the globe. About 70% of the maize grain consists of starch. Maize is an important oil source which decreases cholesterol level in human blood. Maize may also serve as a big source of carotenoids like beta-carotene, zeaxanthin, lutein and cryptoxanthin which establish beneficial effects on human health especially in maintaining the normal vision and reducing the oxidative stress (Chaudhry *et al.*, 2014). In Pakistan, it is cultivated on an area of 1085 thousand hectares with annual yield of 4631 thousand tons. The average yield remained 4261 kg ha^{-1} (Govt of Pakistan, 2013).

Pakistan is situated in an area where rain fall is very low which may be a big cause of soil salinity and sodicity. It has been reported that, salt-affected soils in Pakistan are extended to an area of 6.67 million hectare (Khan, 1998). An 80% of the saline soils are found to be present in the Punjab. It is documented that 6.14 mha of the salt-affected area has been damaged through salinity and sodicity, out of which 3.9 mha is located in the Punjab, 0.6 mha in the Sindh and 0.2 mha in the Baluchistan (Govt of Pakistan, 2007). Soil salinity reduces the growth and productivity of all arable crops including cereals like maize and rice by impairing several physiological and biochemical processes (Hussain *et al*., 2013; Naz *et al*., 2013).

On the other hand, heavy weight agricultural machinery causes soil compaction during various operations. Load of 4 and 6 tons applied to the silt loam soil surface before sowing reduced 7.5% of maize yield in a two-years experiment (Upadhyaya *et al*., 1991). Compacted soils are noticed to be lowering the yield loss, if they are fertile. However, yield potential of crops cannot be expressed fully even on fertile soils due to compaction (Upadhyaya *et al*., 1991).

Compaction may be caused by wheel traffic, movement of livestock and/or of roots of trees, etc. Compaction may also cause improper shoot development (Wolfe *et al.,* 1995). It has been documented that in compacted soil poor shoot development may be because of poor water and nutrients provision by the roots. It is thought to be feed forward response (Masle, 1998).

Stress also stimulates synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC), an immediate precursor of ethylene biosynthesis in higher plants (Glick *et al*., 1998). Thus ethylene production increases which produce negative effects on crops productivity. There are certain rhizobacteria which posses ACC-deaminase. This enzyme lowers down the stress ethylene level within plants. It is established that ACC-deaminase converts ACC in ammonia and α-ketobutyrate in place of ethylene. So ethylene level decreases in the plants. In this way plant ethylene concentration increased due to stress like salinity and compaction may be lowered down and its inhibitory growth effects could be minimized (Grichko and Glick*,* 2001). Strategies used to minimize the effects of soil salinity have a lot of importance, as 60 million hectare soils of world are salt-affected (Lewis, 2002). Accumulation of excessive soluble salts in the soil decrease crop yied. Salinity causes physiological drought, compaction of soil (Mahajan and Tuteja, 2005), ionic equilibrium imbalance (Niu *et al.,* 1995), decrease in photosynthesis (Yeo, 1998), nutrients uptake (Grattan and Grieve, 1999), flowering and finally the crop yield (Gill, 1979). Moreover, it has also been observed that balanced chemical fertilization helps rhizobacteria in granting resistance to the plants grown under stress conditions (Naz *et al*., 2013; Zafar-ul-Hye *et al*., 2014).

Keeping in view all these aspects, a field experiment was designed with the hypothesis that ACC-deaminase containing rhizobacterial strains i.e., *Pseudomonas syringae* and *Pseudomonas fluorescens* along with mineral fertilizers may combat the stress effect on plants produced due to saline-sodic and compacted soil conditions and improve the productivity of maize.

MATERIALS AND METHODS

This study was conducted in the research area of Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan. Experimental soil was saline-sodic in nature, characterized as follows; ECe 4.48 dS m^{-1}, pH 9.3, SAR 37 (meq $L^{-1})^{1/2}$ and ESP 18. Maize variety, NK 7002 was taken as test crop. Randomized Complete Block Design (RCBD) was used. The plot size was 3 × 4 m^2. The treatments included, control (without fertilizer and bacterial inoculum), recommended NPK fertilizers dose (200-150-100 kg ha^{-1}), respectively *Pseudomonas syringae*, *Pseudomonas fluorescens*, *Pseudomonas syringae* + half of recommended NPK fertilizers dose, *Pseudomonas fluorescens* + half of the recommended NPK fertilizers dose, *Pseudomonas syringae* + recommended NPK fertilizers dose and *Pseudomonas fluorescens* + recommended NPK fertilizers dose.

Preparation of inocula

The Rhizobacterial strains were obtained from the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad Pakistan. The broths were made in 250 ml flask using minimal salt medium with ACC as the only nitrogen source as described by Dworkin and Foster (1958). The contents of flasks in separate were inoculated with the respective rhizobacterial strains and kept at 28±1°C with continuous shaking @ 100 rpm for a period of 72 hour. A uniform optical density i.e., @ 107-108 colony forming units (CFU) per ml was obtained.

Seed inoculation

The crop seeds were inoculated with slurry, made by adding 12% sugar solution, broth culture and peat and clay mixture. In control, sterilized peat and clay with sterilized broth and sugar solution were applied to the seeds. The coated seeds were dried at room temperature for 24 hours prior to the actual sowing in the field.

Identification of rhizobacterial strains

Each of the rhizobacterial strains to be identified was plated and kept over night on the Biolog Agar plates. The strains were identified with BIOLOG ® Identification Systems (Bochner, 1989). One of the rhizobacterial strains noted to be *Pseudomonas syringae* and the other was *Pseudomonas fluorescens*.

Crop husbandry

An irrigation of 10 cm was applied to the selected field to achieve adequate moisture level. After plowing and planking, a tractor was moved 14 times on the field to make the soil compacted. A compaction of 2.5 kg cm^{-2} was measured with the help of "soil hardness tester".The seed rate used remained 8 kg ha^{-1}, while 20 cm plant to plant distance and 75 cm row to row distance were kept. A plant population @ 75000 plants $ha^{-1}$was acquired by thinning at the third leaf stage. The 100% of recommended NPK fertilizers dose @ 200-150-100 kg ha^{-1}, respectively and 50% of the recommended NPK fertilizers dose @ 100-75-50, respectively were applied through urea, single super phosphate (SSP) and muriate of potash (MOP) in accordance with the requirement. The irrigations were applied as per requirements.

Data collected

From every plot, ten palnt were taken after attaining maturity and plant height was noted in centimeters with the help of measuring tape and average height was found out. From every plot, ten plants were taken and average no. of cobs $plant^{-1}$ was computed. From every plot, ten cobs were taken and cob length was measured using scale, average was found out and no. of grain rows cob^{-1} was noted too. Average no. of grains cob^{-1} was obtained from the same samples. The 1000-grain weight was noted using balance from produce of every plot. The grains produced in each plot were weighed in grams and then converted to kg per hactare by unitary method. The stalk yield for every sub-plot was noted and found out as kg ha^{-1}.

Plant analysis

After drying and grinding, the grain and stalk samples were digested in H_2SO_4 and H_2O_2 (Wolf, 1982). With the addition of distilled water, 50 ml volume was made followed by filtration. The K was determined through flame photometer (Jenway PFP-7). The phosphorus content was obtained using spectrophotometer (Richards, 1954; Ashraf *et al.* 1992). The N contents were determined through Kjeldhal method (Jackson, 1962).

Statistical analysis

The data, noted and determined were statistically analyzed.The means of four replications were compared applying Duncan's Multiple Range test at 5% level of probability using Mstate (Steel *et al*., 1997).

RESULTS AND DISCUSSION

The data reveal that on saline sodic soil subjected to compactness, rhizobacterial inoculations with recommended NPK fertilizers dose significantly improved maize growth and productivity as compared to the situations, when either only rhizobacterial inoculations were used or merely recommended dose of NPK fertilizers were applied (Tables 1-3).

In case of plant height, cob length, no. of cobs plant^{-1} and no of grain rows cob^{-1}, bacterial inoculations caused significant improvement over control and when applied along with recommended NPK fertilizers dose, further improvement was noticed in the parameters mentioned above (Table 1). The recommended NPK fertilizers dose improved plant height, cob length, no. of cobs plant^{-1} and no of grain rows cob^{-1} up to 5.74, 14.41, 300 and 22.22%, respectively as compared to the control and only bacterial inoculations caused up to 12.12, 26.12, 400 and 33.33% increase over control, respectively in these parameters. On the other hand improvement in plant height, cob length, no. of cobs plant^{-1} and no of grain rows cob^{-1} was noted up to 27.79, 72.79, 1100 and 88.88%, respectively as compared to the control upon the application of rhizobacterial strains and mineral fertilizers together.

Both the *Pseudomonas* species along with recommended NPK fertilizers dose caused similar improvement in plant height with respect to each other and significantly improved results were obtained over all other treatments. *Pseudomonas fluorescens* with recommended NPK fertilizers dose produced maximum and significant promotion in cob length, no. of cobs plant^{-1} and no. of grain rows cob^{-1} comparing with all other treatments. It is evident that the rhizobacterial strains significantly increased plant height, no. of cobs plant^{-1}, cob length and no. of grain rows cob^{-1} as compared to the control. This improvement might be due to decrease in stress ethylene level with ACC-deaminase containing bacteria which might have changed ACC into ammonia (NH_3) and α-ketobutyrate in place of ethylene. The same was described by others (Shaharoona *et al*., 2003).

Similarly, pronounced results were obtained with the bacterial inoculations as far as no. of grains cob^{-1}, 1000 grain weight and yield of grain and stalk are concerned and in all these parameters further promotion is evident from the data, with the integrated use of bacterial strains and NPK fertilizers (Table 2). Comparing with control, an increase up to 88.88, 66.60, 18.59 and 2.14% respectively was noted in no. of grains cob^{-1}, 1000 grain weight and yield of grain and stalk with merely recommended NPK fertilizers dose and the improvement in these parameters remained up to 263.63, 211.59, 56.33 and 11.43%, respectively over the control with simple bacterial inoculations. The results flashed that combined application of NPK fertilizers and bacterial inoculations improved no. of grains cob^{-1}, 1000grain weight and yield of grain and stalk up to 827.27, 327.53, 112.93 and 29.38%, respectively comparing with the control.

Table 1. Effect of bacterial inoculations integrated with mineral fertilizers on plant height and yield related traits of maize in saline-sodic soil subjected to compactness.

Treatments	Plant height (cm)	Cob length (cm)	Number of cobs plant^{-1}	Number of grain rows cob^{-1}
Control (No fertilizers and no rhizobacterial strain)	127.00 e	11.10 e	0.25 d	9.00 f
NPK fertilizers @ 200-150-100 kg ha^{-1}	134.30 de	12.70 d	1.00 c	11.00 e
Pseudomonas syringae	138.40 cde	13.50 d	0.75 cd	12.00 de
Pseudomonas fluorescens	142.40 bcd	14.00 d	1.25 bc	12.00 de
Pseudomonas syringae + NPK fertilizers @ 100-75-50 kg ha^{-1}	144.50 bcd	15.90 c	1.25 bc	13.00 cd
Pseudomonas fluorescens + NPK fertilizers @100-75-50 kg ha^{-1}	148.30 bc	16.20 bc	1.75 b	14.00 bc
Pseudomonas syringae + NPK fertilizers @ 200-150-100 kg ha^{-1}	155.50 ab	17.50 b	1.75 b	15.00 b
Pseudomonas fluorescens + NPK fertilizers @ 200-150-100 kg ha^{-1}	162.30 a	19.20 a	3.00 a	17.00 a
LSD Value	13.49	1.44	0.66	1.56

Means sharing the same alphabets in a column are similar at 5% probability level

It is evident that *Pseudomonas fluorescens* in combination with recommended NPK fertilizers dose produced maximum and significant increase in no. of grains cob^{-1} and 1000 grain weight. Although both the species remained at par with each other in case of grain and stalk yield when applied along with recommended NPK fertilizers dose, while both the species in conjunction with recommended NPK fertilizers dose caused maximum and significant improvement in grain and stalk yield as compared to all other treatments.

This might be again due to the decrease in ethylene with ACC-deaminase bacteria which changed ACC into ammonia (NH_3) and α-ketobutyrate in palce of ethylene. Reduced ethylene level would have produced resistance in plants against stress and improved root growth and consequently shoot and plant growth and yield. This may supported with work of others (Glick *et al*., 1998; Ahmed *et al*., 2004; Shaharoona *et al.*, 2006; Zahir *et al*., 2009).

The data opened up that the rhizobacterial strains proved to be very effective to improve NPK content of maize on saline sodic soil subjected to compactness and more promising results were noted through integrated application of NPK fertilizers with bacterial inoculations (Table 3). An improvement in NPK concentration in maize grains and stalk were found up to 80, 12.97, 225, 150, 73.33 and 68.38%, respectively, over control when only NPK fertilizers were applied and remained up to 120, 32.82, 458.33, 190, 35.55 and 27.21%, respectively, over control upon the use of only bacterial inoculation. On the other hand, due to the combined application of NPK fertilizers and bacterial inoculation, improvement in NPK contents of maize grains and stalk reached up to 254, 38.93, 82.5, 635, 108.88 and 107.35%, respectively comparing with the control. Considering N content in grain and stalk and P concentration in stalk, both the bacterial strains along with recommended NPK fertilizers dose although found to be similar with each other, but caused maximum and significant increase over all rest of the treatments. As far as grains P and K concentration in grains and stalk are concerned, *Pseudomonas fluorescens* caused maximum and significant improvement when applied in combination with recommended NPK fertilizers dose.

Table 2. Effect of bacterial inoculations integrated with mineral fertilizers on yield related traits and that of maize in saline-sodic soil subjected to compactness.

Treatments	No. of grains cob^{-1}	1000 grain weight (g)	Grain yield (kg ha^{-1})	Stalk yield (kg ha^{-1})
Control (No fertilizers and no rhizobacterial strain)	99 g	69 h	371 f	13912 e
NPK fertilizers@200-150-100 kg ha^{-1}	187 f	115 g	440 e	14211 de
Pseudomonas syringae	324 e	175 f	540 d	14988 cda
Pseudomonas fluorescens	360 e	215 d	580 cd	15511 bcd
Pseudomonas syringae + NPK fertilizers@100-75-50 kg ha^{-1}	546 d	199 e	611 c	16777 ab
Pseudomonas fluorescens + NPK fertilizers@100-75-50 kg ha^{-1}	611 c	250 c	675 b	16000 bc
Pseudomonas Syringae + NPK fertilizers@200-150-100 kg ha^{-1}	720 b	275 b	750 a	17500 a
Pseudomonas fluorescens + NPK fertilizers @200-150-100 kg ha^{-1}	918 a	295 a	790 a	18000 a
LSD Value	47	13	53	15

Means sharing the same alphabets in a column are similar at 5% probability level

It is obvious that the bacterial inoculationss increased the NPK concentrations in grains and straw comparing with the control. The *Pseudomonas fluorescen* gave best performance. The obacteria strains improved plants nutrients uptake as decreased ethylene concentration would have resulted in better root development even in the compacted and saline sodic soil. Our results are similar with the findings of many other research workers (Zahir *et al*., 1996; Shaharoona *et al*., 2003; Ahmed *et al.*, 2004; Zahir *et al.*, 2009). The rhizobacterial strains along with mineral fertilizer exerted pronounced effect on maize growth as compared to the rhizobacterial inoculation without mineral fertilizers. Combined application of the bacterial strains and mineral fertilizers would have resulted in improved solubilization and availability of certain nutrients to the maize plants and consequently in excellent synthesis of plant growth regulators (Zahir *et al.,* 2004). Similar conclusions were reached by Pal *et al.* (2000).

Table 3. Effect of bacterial inoculation integrated with mineral fertilizers on NPK contents of maize in saline-sodic soil subjected to compactness.

Treatments	Grain N contents (%)	Stalk N contents (%)	Grain P contents (%)	Stalk P contents (%)	Grain K contents (%)	Stalk K contents (%)
Control (No fertilizers and no rhizobacterial strain)	0.50 f	1.31 e	0.12 f	0.20 d	0.45 f	1.36 h
NPK fertilizers @ 200-150-100 kg ha^{-1}	0.90 e	1.48 d	0.39 e	0.50 c	0.78 cd	2.29 c
Pseudomonas syringae	0.98 de	1.71 ab	0.40 e	0.48 c	0.56 e	1.61 g
Pseudomonas fluorescens	1.10 cd	1.74 ab	0.67 d	0.58 c	0.61 e	1.73 f
Pseudomonas syringae + NPK fertilizers@100-75-50 kg ha^{-1}	1.18 c	1.52 cd	0.71 cd	0.78 b	0.75 d	2.00 e
Pseudomonas fluorescens + NPK fertilizers@100-75-50 kg ha^{-1}	1.45 b	1.62 bc	0.75 c	0.80 b	0.81 c	2.15 d
Pseudomonas Syringae + NPK fertilizers@200-150-100 kg ha^{-1}	1.75 a	1.81 a	0.95 b	1.38 a	0.88 b	2.67 b
Pseudomonas fluorescens + NPK fertilizers @200-150-100 kg ha^{-1}	1.77 a	1.82 a	1.11 a	1.47 a	0.94 a	2.82 a
LSD Value	0.13	0.13	0.07	0.07	0.05	0.13

Means sharing the same alphabets in a column are similar at 5% probability level

CONCLUSION

We may summarize our findings that using biotechnology of ACC-deaminase along with mineral fertilizers is more effective and useful in crop growth under multiple stress conditions.

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(Received April 15, 2014; Accepted June 12, 2014)