

Seed inoculation with *Pseudomonas fluorescens* and *Pseudomonas syringae* enhanced maize growth in a compacted saline-sodic soil

La inoculación de semillas con *Pseudomonas fluorescens* y *Pseudomonas syringae* mejoró el crecimiento de maíz en un suelo sódico-salino compactado

Zafar-ul-Hye M¹, A Nasir¹, M Aon¹, S Hussain¹, M Ahmad², I Naz¹

Abstract. Abiotic stresses like salt stress and soil compaction are responsible for increased ethylene production which may adversely affect crop growth. A field experiment was conducted to evaluate the response of seed inoculation with ACC-deaminase containing rhizobacteria (*Pseudomonas fluorescens* and *Pseudomonas syringae*) in the presence of recommended or half of a recommended rate of inorganic fertilizers at different growth stages of fodder maize in a compacted saline-sodic soil. At both fertilizer rates, seed inoculation with *P. fluorescens* and *P. syringae* significantly improved all growth parameters over the control treatment. After 30, 60 and 75 days of sowing, treatment with recommended NPK + *P. fluorescens* produced a statistically maximum increase in root length, shoot length, root fresh weight and shoot fresh weight in comparison to the control and recommended NPK only. As compared to recommended NPK only, seed inoculation with *P. syringae* + recommended NPK increased root dry weight by 4.1, 1.7 and 2.2 folds after 30, 60 and 75 days from sowing, respectively. Similarly, over the recommended NPK only, the recommended NPK + *P. fluorescens* increased shoot dry weight by 1.9, 1.5 and 1.9 folds after 30, 60 and 75 days from sowing, respectively. Conclusively, seed inoculation with *P. fluorescens* and *P. syringae* enhanced maize growth in a compacted saline-sodic soil.

Keywords: ACC-deaminase; Ethylene; Fodder maize; Inoculation; Plant growth promoting rhizobacteria.

Resumen. Los estreses abióticos como el estrés salino y la compactación del suelo son responsables por la mayor producción de etileno, lo cual puede afectar adversamente el crecimiento de las plantas de cosecha. Se condujo un experimento de campo para evaluar la respuesta a la inoculación de semillas con ACC-deaminasa. La inoculación contuvo rizobacterias (bacterias que se asocian a las células de las raíces de las plantas) (*Pseudomonas fluorescens* y *Pseudomonas syringae*) más una tasa recomendada o la mitad de dicha tasa de fertilizantes inorgánicos. Esto se hizo a diferentes estados de crecimiento de maíz forrajero en un suelo salino-sódico compactado. En ambas tasas de fertilización, la inoculación de las semillas con *P. fluorescens* y *P. syringae* mejoró significativamente todos los parámetros de crecimiento estudiados respecto al tratamiento control. Después de 30, 60 y 75 días desde la siembra, el tratamiento con la tasa recomendada de NPK + *P. fluorescens* produjo un incremento máximo significativo en la longitud de la raíz, longitud del tallo, peso fresco de raíces y peso fresco del tallo en comparación al control y a la tasa de NPK solamente. Comparado a esta última tasa, la inoculación de semillas con *P. syringae* + la tasa de NPK recomendada incrementaron el peso seco de la raíz en 4,1; 1,7 y 2,2 veces después de 30, 60 y 75 días desde la siembra, respectivamente. Similarmente, la tasa recomendada de NPK + *P. fluorescens* incrementaron el peso seco del tallo en 1,9; 1,5 y 1,9 veces después de 30, 60 y 75 días desde la siembra, respectivamente. La inoculación de las semillas con *P. fluorescens* y *P. syringae* mejoraron el crecimiento de maíz en un suelo salino-sódico compactado.

Palabras clave: ACC-deaminase; Etileno; Maíz forrajero; Inoculación; Rizobacterias que promueven el crecimiento.

¹ Department of Soil Science, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan-60800, Pakistan.

² University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Bahawalpur-63100, Pakistan.

Address correspondence to: Muhammad Zafar-ul-Hye, e-mail: zafarulhyegondal@yahoo.com

Received 23.VII.2017. Accepted 11.III.2018.

INTRODUCTION

Maize (*Zea Mays* L.) is the most important cereal crop after wheat and rice. It is consumed as staple food in many countries and is also used as a feed for livestock and poultry (Nuss & Tanumihardjo, 2010). Abiotic factors like salinity, soil compaction, extreme temperature, drought, flooding, pollutants and poor or extreme irradiation and other environmental stresses hamper yield potentials of maize cultivars (Akram et al., 2010). Adverse soil and environmental conditions result in poor plant root colonization of plant growth promoting rhizobacteria (PGPR) (Frommel et al., 1993; Dobbelaere et al., 2001).

The soluble and exchangeable salts in soils reduce maize yield by affecting the physiological and biochemical changes in plants (Hussain et al., 2013; Iqbal et al. 2014; Khan et al. 2015). According to an estimate, salt affected soil causes 50% reduction in crop yield (Zeng & Shannon, 2000). To some extent, the salinity and sodicity problems may be overcome by leaching salts out of the root zone or by adding gypsum into soil either alone and/or in combination with organic materials (Horneck et al., 2007). However, the availability of good quality irrigation water is also low in arid and semiarid areas of Pakistan. Because of this, use of PGPR may be helpful for plant growth (Zahir et al., 2009) along with a balanced fertilization (Hussain et al. 2017). Under stress conditions, higher production of 1-aminocyclopropane-1-carboxylic acid (ACC) and ethylene soon after sowing inhibit root elongation (Alarcón et al., 2009), which lead to significant damage to the plant (Grichko & Glick, 2001; Schallet, 2012). As a result of PGPR containing ACC-deaminase application, ethylene level is reduced which results in growth enhancement (Bhattacharyya & Jha, 2012; Glick, 2014). The PGPR may also improve plant growth and yield, nutrient uptake, adsorption of nitrate ions and phosphate solubilization (Wu et al., 2005; Aon et al., 2015).

The usefulness of inoculation with ACC-deaminase containing PGPR is known under salt, drought and other stresses. Soil compaction causes poor plant growth due to low nutrient and water availability to the plant roots (Nawaz et al., 2013). Such physical impedance also causes an increase in the rate of production of ethylene which ultimately results in root and plant growth reduction (Okamoto et al., 2008). We have also evaluated usefulness of PGPR inoculations for grain yield and yield attributes of maize grown in compacted saline-sodic soil (Zafar-ul-Hye et al., 2014). The plant responses to stress and inoculation at the vegetative and early reproductive stages are included in this manuscript. It was hypothesized that seed inoculation with ACC-deaminase containing PGPR will exert positive effects on maize growth in a compacted, saline-sodic soil.

MATERIALS AND METHODS

This field study was conducted at the research area of the Department of Soil Science, Faculty of Agricultural Sciences

and Technology, Bahauddin Zakariya University, Multan, Pakistan. Detailed methodology is given by Zafar-ul-Hye et al. (2014) and it is summarized here.

After ploughing and planking, tractor was moved fourteen times over the selected area of field to make the soil compacted. A uniform compaction of 2.54 kg/cm² was measured with the help of a Soil Hardness Tester (Table 1). In this salt affected (saline-sodic) and compacted field, the plot size of each experimental unit was 3 × 4 m². Recommended (200 kg N, 65 kg P and 83 kg K/ha) or half of the recommended rates of NPK fertilizers were added to the experimental units as urea, single super phosphate and potassium sulfate. Nitrogen fertilizer was applied to the plots in two splits: first before sowing and second after 30 days of sowing, while full doses of P and K fertilizers were applied before sowing.

Table 1. Pre-sowing physicochemical analyses of the soil.
Tabla 1. Análisis físico-químico del suelo antes de la siembra.

Characteristic	Unit	Value
Sand	%	29.2
Silt	%	57.8
Clay	%	13.0
USDA textural class	---	Silt loam
Soil compaction	kg/cm ²	2.54
Organic matter	%	0.46
Saturation percentage	%	35.0
pH (in saturated paste)	---	9.93
EC (in saturated paste extract)	dS/m	4.48
Organic Matter	%	0.73
Available P	mg/kg	6.3
Extractable K	mg/kg	127
Sodium absorption ratio	(mmol _c /L) ^{-0.5}	17.3

All values are a mean of four replicates.

Inoculum of two strains of PGPR (*Pseudomonas syringae* and *Pseudomonas fluorescens*) was prepared at the laboratory with standard procedures. For seed inoculation, media was prepared and seeds were coated with slurry, prepared with sugar solution (12%), peat, clay and liquid culture (with 1:1:1 ratio). Sterilization of peat and clay, and sugar and broth solution was done for the control treatment. Before sowing, the inoculated seeds were kept 24 hours for drying.

The seeds of fodder maize variety, NK-7002, were sown at a rate of 8 kg/ha on ridges with 20 cm plant to plant distance and 75 cm line to line distance. There were eight treatments in total: full control, recommended NPK, inoculation with *P. syringae*, inoculation with *P. fluorescens*, half of the recommended NPK + *P. syringae*, half of the recommended NPK + *P. fluorescens*, recommended NPK + *Pseudomonas syringae* and

recommended NPK + *P. fluorescens*. The treatments were randomized in three blocks according to the randomized complete block design (RCBD).

After 20 days of sowing, the thinning was done to maintain the plant population at a rate of 7.5 plants/m². The field was irrigated according to the crop requirement. The standard agronomic practices were used to maintain the crop healthy.

After 30, 60 and 75 days of sowing, ten plants were randomly selected from each experimental unit, and root length was measured. One day before each harvesting, all the experimental units were flooded by tube-well water, and at the time of harvesting, roots of selected plants (10 plants per experimental unit) were gently removed from the soil. Likewise, the data of shoot length, root fresh weight, root dry weight, shoot fresh weight and shoot dry weight were recorded.

Data computations of all the three harvests and standard error of means were calculated on Microsoft Excel 2013[®] software (Microsoft Corporation, Redmond, WA-USA) and to test the significance of treatments, statistical software, Statistix 8.1[®] (Analytical Software, Tallahassee-USA) was used. Significantly different treatment means were separated by Tukey-HSD test at $P \leq 0.05$ (Steel et al., 1997).

RESULTS

Crop response at 30 days after sowing. Table 2 and Figure 1 are presenting the plant responses at 30 days from sowing. Seed inoculation with the rhizobacterial strains, with or without NPK fertilizer, significantly ($P \leq 0.05$) improved length and fresh weight of shoots, and length, fresh weight and dry weight of roots, over the control treatment (Table 2; Fig. 1).

As compared to the recommended NPK only, recommended NPK + *Pseudomonas fluorescens* and recommended NPK + *Pseudomonas syringae* significantly ($P \leq 0.05$) increased root length by ≥ 2 folds. Comparing the treatments with recommended NPK fertilizer only, seed inoculation with *Pseudomonas fluorescens* and *Pseudomonas syringae* along with recommended NPK significantly ($P \leq 0.05$) improved shoot length by 84 and 68%, respectively.

Both treatments having seed inoculation increased root fresh weight by more than 130% over the recommended NPK without seed inoculation (Table 2). Treatment having recommended NPK + *P. fluorescens* significantly ($P \leq 0.05$) increased shoot fresh weight by 96% over recommended NPK only. At the same time, other treatments (i.e., half of NPK fertilizer + *P. fluorescens* and recommended NPK fertilizer + *P. syringae*) showed statistically comparable increase in shoot fresh weight, over the treatment with recommended NPK fertilizer only. As compared to the sole application of recommended NPK, recommended NPK + *P. syringae* and recommended NPK + *P. fluorescens* significantly ($P \leq 0.05$) increased root dry weight by 4.1 and 4.2 folds, respectively, and shoot dry weight by 1.3 and 1.7 folds, respectively (Fig. 1).

Crop response at 60 days after sowing. Seed inoculation also similarly improved plant growth and fodder yield 60 days after sowing (Table 3 and Fig. 2). Seed inoculation with rhizobacterial strains *P. fluorescens* or *P. syringae*, with half and recommended NPK fertilizers, significantly ($P \leq 0.05$) improved length, fresh weight and dry weight of root, and length and fresh weight of shoot over the control and recommended NPK fertilizer treatments (Table 3, Fig. 2). *Pseudomonas fluo-*

Table 2. Effect of seed inoculation with *Pseudomonas syringae* and *Pseudomonas fluorescens* on length and fresh weight of roots and shoots of maize after 30 days from sowing in a compacted saline-sodic soil supplied with the recommended or half the recommended rates of NPK fertilizers.

Tabla 2. Efecto de la inoculación de la semilla con *Pseudomonas syringae* y *Pseudomonas fluorescens* sobre la longitud y el peso fresco de raíces y tallos de maíz luego de 30 días desde la siembra en un suelo salino-sódico compactado con las tasas recomendadas o la mitad de las tasas recomendadas de los fertilizantes NPK.

Treatment	Root length (cm)	Shoot length (cm)	Root fresh weight (Mg/ha)	Shoot fresh weight (Mg/ha)
Control	11.3 ± 0.32 f	10.0 ± 0.23 f	0.038 ± 0.002 f	0.56 ± 0.002 e
Recommended NPK fertilizer	12.2 ± 0.42 f	17.5 ± 0.20 e	0.049 ± 0.003 ef	0.67 ± 0.009 de
<i>Pseudomonas syringae</i>	15.5 ± 0.19 e	21.2 ± 0.80 d	0.053 ± 0.001 e	0.69 ± 0.017 d
<i>Pseudomonas fluorescens</i>	17.1 ± 0.32 d	18.3 ± 0.42 e	0.067 ± 0.002 d	0.75 ± 0.015 d
Half of recommended NPK fertilizer + <i>Pseudomonas Syringae</i>	18.2 ± 0.15 d	23.1 ± 0.32 d	0.105 ± 0.003 b	0.99 ± 0.035 c
Half of recommended NPK fertilizer + <i>Pseudomonas fluorescens</i>	20.2 ± 0.16 c	26.1 ± 0.40 c	0.082 ± 0.003 c	1.14 ± 0.028 b
Recommended NPK fertilizer + <i>Pseudomonas syringae</i>	24.5 ± 0.32 b	29.4 ± 0.28 b	0.114 ± 0.003 ab	1.06 ± 0.031 bc
Recommended NPK fertilizer + <i>Pseudomonas fluorescens</i>	28.1 ± 0.26 a	32.3 ± 0.63 a	0.116 ± 0.002 a	1.31 ± 0.030 a

Means ± standard error of means for three replicates. Treatments sharing similar letter(s) for each parameter do not differ significantly based on Tukey-HSD test at $P \leq 0.05$.

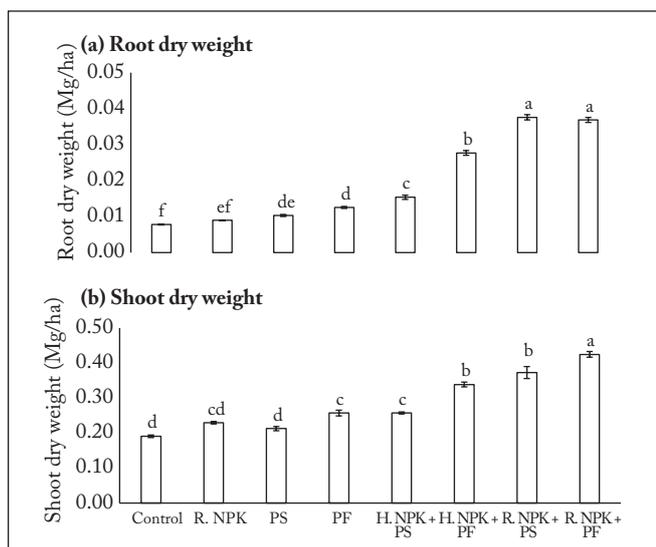


Fig. 1. Effect of seed inoculation with *Pseudomonas syringae* (PS) and *Pseudomonas fluorescens* (PF) on root (a) and shoot (b) dry weight of maize after 30 days from sowing in a compacted saline-sodic soil supplied with recommended (R. NPK) or half the recommended (H. NPK) rate of NPK fertilizers. Bars are means and error bars are standard error of means for three replicates. Treatments sharing similar letter(s) for each parameter do not differ significantly based on Tukey-HSD test at $P \leq 0.05$.

Fig. 1. Efecto de la inoculación de semillas con *Pseudomonas syringae* (PS) y *Pseudomonas fluorescens* (PF) en el peso seco de raíces (a), y tallos (b) de maíz después de 30 días desde la siembra en un suelo salino-sódico compactado fertilizado con la tasa recomendada (R. NPK) o la mitad de la recomendada (H. NPK) de fertilizantes NPK. Los histogramas son promedios de $n=3$. Las barras verticales el error estándar de la media. Los tratamientos que comparten letras similares para cada parámetro no difieren significativamente basado en el test de Tukey-HSD test a $P \leq 0.05$.

Table 3. Effect of seed inoculation with *Pseudomonas syringae* and *Pseudomonas fluorescens* on length and fresh weight of roots and shoots of maize after 60 days from sowing in a compacted, saline-sodic soil supplied with the recommended or half the recommended rates of NPK fertilizers.

Tabla 3. Efecto de la inoculación de la semilla con *Pseudomonas syringae* y *Pseudomonas fluorescens* sobre la longitud y el peso fresco de raíces y tallos de maíz luego de 60 días desde la siembra en un suelo salino-sódico compactado con las tasas recomendadas o la mitad de las tasas recomendadas de los fertilizantes NPK.

Treatment	Root length (cm)	Shoot length (cm)	Root fresh weight (Mg/ha)	Shoot fresh weight (Mg/ha)
Control	28.1 ± 0.92 e	41.2 ± 1.40 e	1.35 ± 0.056 e	2.97 ± 0.08 e
Recommended NPK fertilizer	54.4 ± 1.62 d	48.5 ± 0.97 d	3.03 ± 0.115 c	4.51 ± 0.14 cd
<i>Pseudomonas syringae</i>	48.1 ± 1.54 d	52.1 ± 1.11 d	2.63 ± 0.072 c	3.86 ± 0.11 d
<i>Pseudomonas fluorescens</i>	32.1 ± 0.98 e	58.8 ± 0.76 c	2.10 ± 0.047 d	4.88 ± 0.08 c
Half of recommended NPK fertilizer + <i>Pseudomonas Syringae</i>	62.8 ± 0.95 c	62.1 ± 0.58 bc	3.83 ± 0.133 b	5.78 ± 0.11 b
Half of recommended NPK fertilizer + <i>Pseudomonas fluorescens</i>	67.1 ± 1.54 bc	67.7 ± 1.68 b	3.54 ± 0.069 b	6.23 ± 0.11ab
Recommended NPK fertilizer + <i>Pseudomonas syringae</i>	71.5 ± 1.56 b	82.6 ± 2.19 a	4.48 ± 0.094 a	5.86 ± 0.12 b
Recommended NPK fertilizer + <i>Pseudomonas fluorescens</i>	77.5 ± 1.24 a	79.3 ± 2.84 a	4.28 ± 0.081 a	6.76 ± 0.42 a

Means ± standard error of means for three replicates. Treatments sharing similar letter(s) for each parameter do not differ significantly based on Tukey-HSD test at $P \leq 0.05$.

rescens in combination with recommended NPK significantly ($P \leq 0.05$) improved root length by 42% in comparison to recommended NPK only. Recommended NPK + *P. syringae* and recommended NPK + *P. fluorescens* produced statistically comparable increases in shoot length that was $\geq 64\%$ more than with recommended NPK only.

As compared to the treatment with recommended NPK only, root fresh weight significantly ($P \leq 0.05$) increased by 41 and 48% with recommended NPK + *P. fluorescens* and recommended NPK + *P. syringae*, respectively. *Pseudomonas fluorescens* along with recommended NPK significantly ($P \leq 0.05$) improved shoot fresh weight by 51% in comparison to recommended NPK only (Table 3). As compared to recommended NPK only, root dry weight significantly ($P \leq 0.05$) increased by 69 and 63% with recommended NPK + *Pseudomonas syringae* and recommended NPK + *Pseudomonas fluorescens*, respectively. Shoot dry weight was maximum with recommended NPK + *Pseudomonas fluorescens*, and it did not differ with recommended NPK + *Pseudomonas syringae* (Fig. 2).

Crop response at 75 days after sowing. Crop response at 75 days after sowing also confirmed the importance of seed inoculation for improving plant growth and fodder yield of maize (Table 4; Fig. 3). *Pseudomonas fluorescens* or *Pseudomonas syringae* seed inoculation, along with half and recommended NPK fertilizer significantly ($P \leq 0.05$) improved all the studied parameters over the control treatment (Table 4; Fig. 3). As compared to recommended NPK only, *P. fluorescens* and *P. syringae* along with recommended NPK significantly ($P \leq 0.05$) increased root length by 87 and 74%, respectively. There was a 2.4 fold increase in shoot length with recommended NPK + *P. fluorescens* in comparison to recommended NPK only.

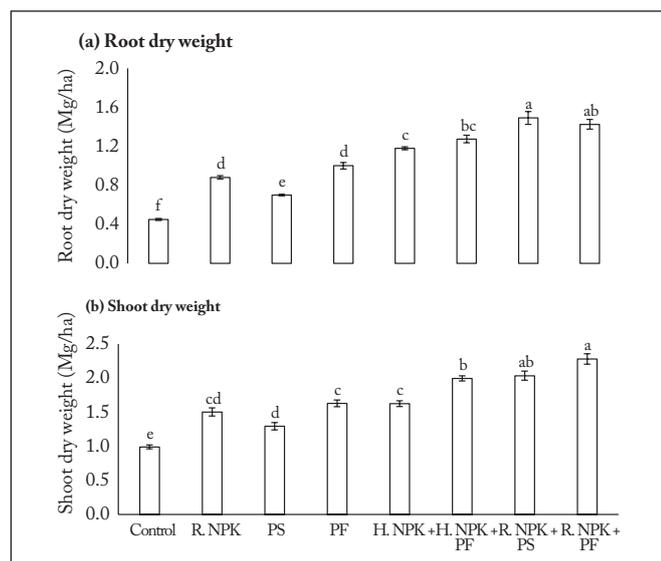


Fig. 2. Effect of seed inoculation with *Pseudomonas syringae* (PS) and *Pseudomonas fluorescens* (PF) on root (a) and shoot (b) dry weight of maize after 60 days from sowing in a compacted saline-sodic soil supplied with recommended (R. NPK) or half the recommended (H. NPK) rate of NPK fertilizers. Bars are means and error bars are standard error of means for three replicates. Treatments sharing similar letter(s) for each parameter do not differ significantly based on Tukey-HSD test at $P \leq 0.05$.

Fig. 2. Efecto de la inoculación de semillas con *Pseudomonas syringae* (PS) y *Pseudomonas fluorescens* (PF) en el peso seco de raíces (a), y tallos (b) de maíz después de 60 días desde la siembra en un suelo salino-sódico compactado fertilizado con la tasa recomendada (R. NPK) o la mitad de la recomendada (H. NPK) de fertilizantes NPK. Los histogramas son promedios de $n=3$. Las barras verticales el error estándar de la media. Los tratamientos que comparten letras similares para cada parámetro no difieren significativamente basado en el test de Tukey-HSD test a $P \leq 0,05$.

Table 4. Effect of seed inoculation with *Pseudomonas syringae* and *Pseudomonas fluorescens* on length and fresh weight of roots and shoots of maize after 75 days from sowing in a compacted saline-sodic soil supplied with recommended or half the recommended rates of NPK fertilizers.

Tabla 4. Efecto de la inoculación de la semilla con *Pseudomonas syringae* y *Pseudomonas fluorescens* sobre la longitud y el peso fresco de raíces y tallos de maíz luego de 75 días desde la siembra en un suelo salino-sódico compactado con las tasas recomendadas o la mitad de las tasas recomendadas de los fertilizantes NPK.

Treatment	Root length (cm)	Shoot length (cm)	Root fresh weight (Mg/ha)	Shoot fresh weight (Mg/ha)
Control	35.8 ± 1.25 e	041 ± 1.33 g	1.88 ± 0.07 g	2.92 ± 0.09 f
Recommended NPK fertilizer	51.4 ± 1.21 d	055 ± 1.62 f	2.41 ± 0.08 f	4.93 ± 0.08 d
<i>Pseudomonas syringae</i>	58.0 ± 0.95 d	075 ± 1.49 e	3.61 ± 0.12 de	3.91 ± 0.14 e
<i>Pseudomonas fluorescens</i>	69.2 ± 1.69 c	081 ± 1.21 de	3.42 ± 0.08 e	5.35 ± 0.11 d
Half of recommended NPK fertilizer + <i>Pseudomonas Syringae</i>	73.3 ± 1.71 c	090 ± 1.73 cd	3.94 ± 0.10 cd	6.53 ± 0.10 c
Half of recommended NPK fertilizer + <i>Pseudomonas fluorescens</i>	87.1 ± 1.70 b	095 ± 1.76 c	4.18 ± 0.07 bc	8.85 ± 0.19 a
Recommended NPK fertilizer + <i>Pseudomonas syringae</i>	89.5 ± 1.82 ab	117 ± 3.18 b	4.56 ± 0.11 ab	7.16 ± 0.12 b
Recommended NPK fertilizer + <i>Pseudomonas fluorescens</i>	96.7 ± 1.45 a	130 ± 3.38 a	4.89 ± 0.09 a	9.45 ± 0.13 a

Means ± standard error of means for three replicates. Treatments sharing similar letter(s) for each parameter do not differ significantly based on Tukey-HSD test at $P \leq 0.05$.

Root fresh weight was significantly ($P \leq 0.05$) improved by 2.1 and 1.8 folds, and root dry weight by 2.0 and 2.2 folds, respectively, with recommended NPK + *Pseudomonas fluorescens* and recommended NPK + *Pseudomonas syringae*, respectively, in comparison to the recommended NPK only (Table 4 and Figure 3). Recommended and half of recommended NPK combined with seed inoculation with *Pseudomonas fluorescens* significantly ($P \leq 0.05$) increased shoot fresh weight by 92 and 80%, respectively, in comparison to the recommended NPK only. Maximum shoot dry weight was observed with recommended NPK + *Pseudomonas fluorescens* that was statistically similar to that observed with recommended NPK + *Pseudomonas syringae*.

DISCUSSION

Abiotic stresses, like salt and compaction, suppress shoot growth. Plants grown in compacted soils frequently exhibit impaired root growth and crop yield (Lipiec et al., 2003). In response to mechanical impedance, it is known that roots exhibit increased production of ACC and ethylene, which is considered a major reason for root growth inhibition (Okamoto et al., 2008).

Growth and yield of various crop species were significantly enhanced with PGPR under stressed soil conditions (Farzana et al., 2009). At each studied growth stage, it was observed that root length and biomass production were significantly ($P \leq 0.05$) higher with inoculation than using the recommended NPK fertilizer only (Tables 2 to 4, Fig. 1 to 3). In stressed environments, root growth and seed germination are negatively affected with high levels of ethylene production (Beaudoin et al., 2000). The ACC-deaminase activity of both *Pseudomonas* strains suppress-

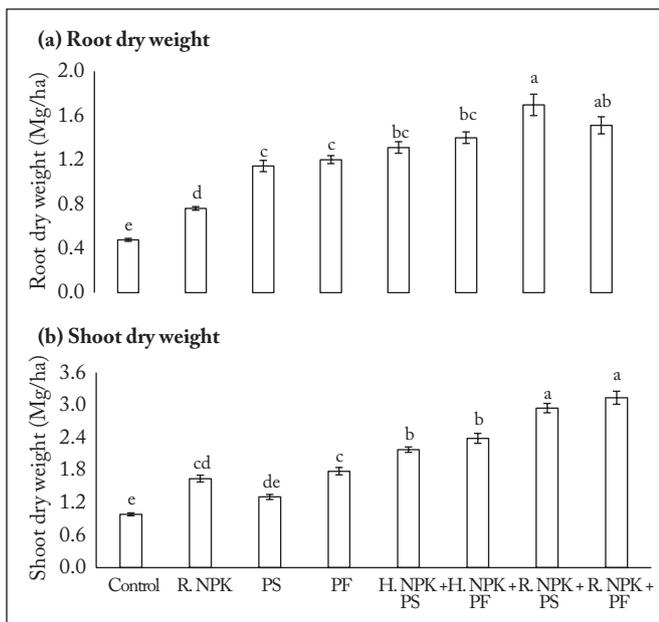


Fig. 3. Effect of seed inoculation with *Pseudomonas syringae* (PS) and *Pseudomonas fluorescens* (PF) on root (a) and shoot (b) dry weight of maize after 75 days from sowing in a compacted saline-sodic soil supplied with recommended (R. NPK) or half the recommended (H. NPK) rate of NPK fertilizers. Bars are means and error bars are standard error of means for three replicates. Treatments sharing similar letter(s) for each parameter do not differ significantly based on Tukey-HSD test at $P \leq 0.05$.

Fig. 3. Efecto de la inoculación de semillas con *Pseudomonas syringae* (PS) y *Pseudomonas fluorescens* (PF) en el peso seco de raíces (a), y tallos (b) de maíz después de 75 días desde la siembra en un suelo salino-sódico compactado fertilizado con la tasa recomendada (R. NPK) o la mitad de la recomendada (H. NPK) de fertilizantes NPK. Los histogramas son promedios de $n=3$. Las barras verticales el error estándar de la media. Los tratamientos que comparten letras similares para cada parámetro no difieren significativamente basado en el test de Tukey-HSD test a $P \leq 0,05$.

es the stress-induced ethylene levels (Nadeem et al., 2009; Ahmad et al., 2011). The immediate precursor of ethylene, ACC, was possibly converted to ammonia and α -ketobutyrate. Therefore, the limited ability of a crop to convert ACC to ethylene would result in the maintenance of plant growth.

The improved root growth by rhizobacteria containing ACC-deaminase also caused better nutrient uptake by plants (Biari et al., 2008). Therefore, it was also another possible reason of better plant growth (Tables 2 to 4, Fig. 1 to 3) as a result of seed inoculation with *Pseudomonas fluorescens* and *Pseudomonas syringae*. Inoculation with bacteria also increased the efficiency of the fertilizers applied at the appropriate rates (Zahir et al., 2004); higher rates of which can be dangerous for the environment (Aziz et al., 2015).

The inoculation of *Pseudomonas* strains increased the root and shoot lengths, which was the major reason for the improved fresh (Tables 2 to 4) and dry weights (Fig. 1 to 3) of maize fodder. The ACC-deaminase containing rhizobacteria decreased the ethyl-

ene level and increased plant growth. Although ethylene concentrations and fluxes have yet to be measured, it appears likely that ACC may have a role in the seed inoculation with various rhizobacterial strains. ACC-deaminase activity improved plant growth of various plant species when they were exposed to stress (Zahir et al. 2004; Kausar & Shehzad 2006; Nadeem et al. 2009; Mayak et al. 2014). Due to the same possibility, seed inoculation with *Pseudomonas fluorescens* and *Pseudomonas syringae* improved maize growth and yield under field conditions.

CONCLUSION

It may be concluded that under stress condition, the use of ACC-deaminase containing rhizobacterial strains is an effective technique for improving plant growth. Seed inoculation with *Pseudomonas fluorescens* and *Pseudomonas syringae* improved plant growth in the presence of recommended or half of the recommended inorganic fertilizer doses, possibly by decreasing the ethylene levels. High levels of ethylene have inhibitory effects on plant growth. Seed inoculation with recommended inorganic fertilizers is an effective strategy to improve yield of fodder maize in salt-affected, compacted soils.

REFERENCES

- Ahmad, M., Z.A. Zahir, H.N. Asghar & M. Asghar (2011). Inducing salt tolerance in mung bean through coinoculation with rhizobia and plant-growth promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate-deaminase. *Canadian Journal of Microbiology* 57: 578-589.
- Akram, M., M.Y. Ashraf, E.A. Waraich, M. Hussain, N. Hussain & A.R. Mallahi (2010). Performance of autumn planted maize (*Zea mays* L.) hybrids at various nitrogen levels under salt affected soils. *Soil and Environment* 29: 23-32.
- Alarcón, M.V., A. Lloret-Salamanca, P.G. Lloret, D.J. Iglesias, T. Talón & J. Salguero (2009). Effects of antagonists and inhibitors of ethylene biosynthesis on maize root elongation. *Plant Signal Behavior* 4: 1154-1156.
- Aon, M., M. Khalid, S. Hussain, M. Naveed & M.J. Akhtar (2015). Diazotrophic inoculation supplemented nitrogen demand of flooded rice under field conditions. *Pakistan Journal of Agricultural Sciences* 52: 145-150.
- Aziz, T., M.A. Maqsood, S. Kanwal, S. Hussain, H.R. Ahmad and M. Sabir. 2015. Fertilizer and environment: Issues and challenges. In: K.R. Hakeem (ed.), pp. 575-598. Crop Production and Global Environmental Issues. Springer, Switzerland.
- Beaudoin, N., C. Serizet, F. Gosti & J. Giraudat (2000). Interactions between abscisic acid and ethylene signaling cascades. *Plant Cell* 12: 1103-1115.
- Bhattacharyya, P.N. & D.K. Jha (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology* 28: 1327-1350.
- Biari, A., A. Gholami & H.A. Rahmani (2008). Growth promotion and enhanced nutrient uptake of maize (*Zea mays* L.) by application of plant growth promoting rhizobacteria in arid region of Iran. *Journal of Biological Science* 8: 1015-1020.

- Dobbelaere, S., A. Croonenborghs, A. Thys, D. Ptacek, J. Vanderleyden, P. Dutto, C. Labandera-Gonzalez, J. Caballero-Mellado, J.F. Aguirre & Y. Kapulnik (2001). Responses of agronomically important crops to inoculation with *Azospirillum*. *Functional Plant Biology* 28: 871-879.
- Farzana, Y., R.O.S. Saad & S. Kamaruzaman (2009). Growth and storage root development of sweet potato inoculated with rhizobacteria under glass house conditions. *Australian Journal of Basic and Applied Science* 3: 1461-1466.
- Frommel, M.I., J. Nowak & G. Lazarovits (1993). Treatment of potato growth responses and bacterium distribution in the rhizosphere. *Plant and Soil* 150: 51-60.
- Glick, B.R. (2014). Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiological Research* 169: 30-39.
- Grichko, V.P. & B.R. Glick (2001). Amelioration of flooding stress by ACC deaminase containing plant growth promoting bacteria. *Plant Physiology and Biochemistry* 39: 11-17.
- Horneck, D.A., J.W. Ellsworth, B.G. Hopkins, D.M. Sullivan & R.G. Slevens (2007). Managing salt-affected soils for crop production. A Pacific Northwest Extension publication, pp. 1-21.
- Hussain, A., C.R. Black, I.B. Taylor, & J.A. Roberts (1999). Soil compaction: A role for ethylene in regulating leaf expansion and shoot growth in tomato? *Plant Physiology* 121: 1227-1237.
- Hussain, M., H.W. Park, M. Farooq, K. Jabran & D.J. Lee (2013). Morphological and physiological basis of salt resistance in different rice genotypes. *International Journal of Agriculture and Biology* 15: 113-118.
- Hussain, S., M.B. Hussain, A. Gulzar, M. Zafar-ul-Hye, M. Aon, M. Qaswar & R. Yaseen (2017). Right time of phosphorus and zinc application to maize depends on nutrient-nutrient and nutrient-inoculum interactions. *Soil Science and Plant Nutrition* 63: 351-356.
- Iqbal, J., S. Kanwal, S. Hussain, T. Aziz & M.A. Maqsood (2014). Zinc application improves maize performance through ionic homeostasis and ameliorating devastating effects of brackish water. *International Journal of Agriculture and Biology* 16: 383-388.
- Kausar, R. & S.M. Shahzad (2006). Effect of ACC-deaminase containing rhizobacteria on growth promotion of maize under salinity stress. *Journal of Agricultural Society for Science* 2: 216-218.
- Khan, M.K., M.A. Maqsood, M.A. Naeem, S. Hussain, T. Aziz & J. Schoenau (2015). High sodium in irrigation water caused B toxicity at low soil solution and shoot B concentration in maize (*Zea mays* L.). *Journal of Plant Nutrition* 38: 728-741.
- Lipiec, J., V.V. Medvedew, M. Birkas, E. Dumitru, T.E. Lyndina, S. Rousseva & E. Fulajtar (2003). Effect of soil compaction on root growth and crop yield in Central and Eastern Europe. *International Agrophysics* 17: 61-69.
- Mayak, S., T. Tirosh & B.R. Glick (2004). Plant growth promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiology and Biochemistry* 42: 565-572.
- Nadeem, S.M., Z.A. Zahir, M. Naveed & M. Arshad (2009). Rhizobacteria containing ACC-deaminase confers salt tolerance in maize grown on salt affected fields. *Canadian Journal of Microbiology* 55: 1302-1309.
- Nawaz, M.F., G. Bourrie & F. Trolard (2013). Soil compaction impact and modelling: A review. *Agronomy for Sustainable Development* 33: 291-309.
- Nuss, E.T. & S.A. Tanumihardjo (2010). Maize: a paramount staple crop in the context of global nutrition. *Comprehensive Reviews in Food Science Food Safety* 9: 417-436.
- Okamoto, T., S. Tsurumi, K. Shibasaki, Y. Obana, H. Takaji, Y. Oono & A. Rahman (2008). Genetic Dissection of Hormonal Responses in the Roots of *Arabidopsis* Grown under Continuous Mechanical Impedance. *Plant Physiology* 146: 1651-1662.
- Schallet, G.E. (2012). Ethylene and the regulation of plant development. *BMC Biology*. 10: 1-3.
- Steel, R.G.D., J.H. Torrie & D.A. Deekey (1997). Principles and procedures of statistics: A biometrical approach, 3rd ed. McGraw Hill Book. Int. Co., New York.
- Wu, S.C., Z.H. Cao, Z.G. Li, K.C. Cheung, & M.H. Wong (2005). Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: A greenhouse trial. *Geoderma* 125: 155-166.
- Zafar-ul-Hye, M., A. Nasir, Z.A. Zahir, A. Rehim & M. Ahmad (2014). Rhizobacterial inoculation integrated with mineral fertilizers promote maize productivity in compacted saline-sodic soil. *Pakistan Journal of Agriculture, Agricultural Engineering and Veterinary Sciences* 30: 43-53.
- Zahir, Z.A., M. Arshad, W.T. Frankenberger & Jr (2004). Plant growth promoting rhizobacteria: Applications and perspectives in agriculture. *Advances in Agronomy* 81: 97-168.
- Zahir, Z.A., U. Ghani, M. Naveed, S.M. Nadeem & H.N. Asghar (2009). Comparative effectiveness of *Pseudomonas* and *Serratia* sp. containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.) under salt-stressed conditions. *Archives of Microbiology* 191: 445-424.
- Zeng, L. & M.C. Shannon (2000). Salinity effects on seedling growth and yield components of rice. *Crop Science* 40: 996-1003.