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The effect of plant growth promoting rhizobacteria (PGPR) and zinc fertilizer on forage yield of maize under water deficit stress conditions

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ABSTRACT: Drought is one of the most important abiotic stresses affecting yield of the crops in arid and semiarid regions. Rhizobacterial populations of stressed soils are adapted and tolerant to stress, PGPR strains that can be used as inoculants for crops grown in stressed ecosystems. This study was carried out to study the effects of ACC-deaminase containing PGPR and zinc fertilizer on the yield and some agronomic traits of maize under water deficit stress in the mahvelat region of Iran in 2011. The experiment was conducted as a split- factorial design with 3 replications. The main plots consisted of three water regimes achieved by performed irrigation every 6 (normal condition, I_1), 9 (moderate water deficit stress, I_2) and 12 days (severe water deficit stress, I_3). The sub-plots were six different treatments, including: Grain inoculation with Pseudomonas fluorescens strain169 (T_1), Grain inoculation with P. fluorescens and zinc sulfate fertilizer (T_2), Grain inoculation with Pseudomonas putida strain 108 (T_3), Grain inoculation with P. putida and zinc sulfate fertilizer (T_4), Application of zinc sulfate fertilizer (T_5) and Non-application of grain inoculation or zinc sulfate fertilizer, as a control (T_6). The results showed that water deficit stress decreased leaf, stem and tassel dry weights, cobs weight, grain yield and the harvest index of ear in plant (HIEP). Under water deficit stress, grain inoculation significantly increased stem and tassel dry weights, cob weight and grain yield. The present study also indicated that under water deficit stress using zinc sulfate fertilizer improved tassel dry weights, cob weight and grain yield. Hence, bacterial inoculation of seeds by P. fluorescens strain 169 or P. putida strain108, and using zinc sulphate fertilizer are recommended for maize cultivation in semi-arid and arid regions.

Key words: Maize, water deficit stress, PGPR, zinc fertilizer, grain yield, forage weight.

Abbreviations: ACC- 1-aminocyclopropane-1-carboxylate; HIEP- harvest index of ear in plant; PGPR- plant growth promoting rhizobacterial.

INTRODUCTION

In arid and semi arid regions abiotic stresses such as water stress, salinity, high light, temperature, flooding, toxic metals, wounding and biotic stresses including pests and pathogens can reduce plant growth and yield (Glick et al., 2007b).

There are many free living bacteria in soil that can promot plant growth through direct and in direct ways, these bacteria are called plant growth promoting rhizo bacteria(Glick, 1995) and or effective microbs"EM". In indirect ways, these bacteria reduce or prohibit some of deleterious effects of fungus or other phytopathogenic organism by several different mechanisms. Also the direct mechanisms of PGPR for the plant growh enhancement include of 1- facilitating the uptake of nutrients from the environment via the solubilization of phosphorus, nitrogen fixation, sequestering of iron from the soil by siderophores, 2- production of phytohormones such as gibberellin, auxin, cytokinin and 3- most important than that, the enzymatic lowering of plant ethylene concentrations (Glick, 1995; Mayak et al., 2004). Ethylene is the only gaseous hormone which is produced under stress conditions (abiotic or biotic stress) in plants more than normal and it is called stress ethylene (Glick et al., 2007a). Ethylene prevents roots growth and so plant growth. Plant growth promoting bacteria decrease the plant ethylene levels by enzyme 1- aminocyclopropane-1-carboxylate (ACC) deaminase (Glick et al., 2007a,b). This enzyme break down the ethylene precursor ACC to α-ketobutyrate and ammonium and hence decreasing ethylene concentration will occur in stressed plants (Hontzeas et al., 2004; Glick et al.,2007a,b).

Oktem et al. (2003) has done the irrigation interval in 2, 4, 6 and 8 days interval and set the needed water according to 70, 80, 90 and 100 percent evaporation of class a pan. They got the most and least wet weight of ear in irrigation treatments in 2 and 8 days intervals respectively. Zhang et al. (2004) showed that the water tension causes the sever falling of grain yield in corn.

Seed bacterization of maize with Pseudomonas spp. improved plant biomass, relative water content, leaf water potential (Sandhya et a., 2010). The beneficial effects of coinoculation of Azospirillum brasilense and AM fungus on most of the physiological and biochemical traits of rice (Oriza sativa L.) plants were reported under water deficit stress (Sanchez et al., 2011). Three diffrent strain of Azospirillum lipoferum were used to inoculate wheat (Triticum aestivum L.) seedlings under drought stress. Compared to uninoculated treatments two strains with ACC-deaminase activity increased the wheat yield under drought stress (Arzanesh et al., 2010).

Water stress results in the increased reactive oxygen species (ROS) due to energy accumulation in stressed plants that absorb more light energy than is used during the photosynthetic carbon fixation (Hong and Ji-yun, 2007). Zn plays an important role in lowering the ROS generation and defending cells against ROS attack (Cakmac, 2000). Application of Zn causes reduction in activity of membrane-bound NADPH oxidase producing ROS, while the activities of SOD (superoxide dismutase), POD (peroxidase), and CAT (catalase) enhance (Hong and Ji-yun, 2007). Zinc affects the growth of shoots and roots. Also, Zn acts as a functional component for transcription of About 200 enzymes and factors. This element plays an important role in carbohydrate and protein synthesis and Participates in metabolism regulation of lipids, saccharides and nucleic acid (Sajedi et al., 2009).

Sajedi et al. (2008) declare that different levels of nitrogen, iron and zinc have influence on growth, absorption of nutrition and percentage of field corn (single grass 704). Also Ashoka et al. (2008) studied micro-elements effect and found that using microelements results in increase of corn weight, green fodders and grain yield. Zn application resulted in an improvement in root growth in soybean (Glycine max L.) at all levels of water stress and shoot growth under severe water stress (Gadala, 2000). It is reported that foliar Zn and Mn application can improve the seed yield and seed quality of safflower (Carthamus tinctorius L.) grown under drought stress (Movahhedy-Dehnavy et al., 2009).

Grewal and Williams (2000) showed that the ability of alfalfa plants to cope with water stress during early vegetative growth could be enhanced by providing the plants with an adequate Zn supply. However, Hong and Ji-Yun (2007) reported that the increases of maize growth and Zn uptake due to Zn application were more significant under well-watered conditions than under drying conditions. Also, the other experiment showed that, the increases of plant biomass, stomatal conductance and quantum yield of photosystem 2 due to Zn addition were observed in well-watered maize plants (Wang et al,. 2009).

In this study, the effect of PGPR and zinc fertilizer on some characteristics of maize has been studied under water deficit stress conditions.

MATERIALS AND METHODS

This experiment was conducted in Mahvelat of Khorasan province in Iran in 2011. The experimental design consisted of three randomized complete blocks in a split-factorial arrangement having 18 treatments in every block. Each plot consisted of 5 rows, 5 m long and 60 cm apart (size plot: 15 m^2) and 20 cm distance between plants in the rows. The main plots consisted of three water regimes achieved by performed irrigation every 6 (normal condition, I_1), 9 (moderate water deficit stress, I_2) and 12 days (severe water deficit stress, I_3). The sub-plots included six different treatments, including: Grain inoculation with Pseudomonas fluorescens strain169 (T_1), Grain inoculation with P. fluorescens and zinc sulfate fertilizer (T_2), Grain inoculation with Pseudomonas putida strain 108 (T_3), Grain inoculation with P. putida and zinc sulfate fertilizer (T_4), Application of zinc sulfate fertilizer (T_5) and Non-application of grain inoculation or zinc sulfate fertilizer, as a control (T_6).

The Results of physical and chemical soil analysis is shown in Table 1. Fertilizer requirement for supplemental of phosphorus (P), zinc (Zn) and nitrogen (N) fertilizers were based on the soil test samples. The half of the nitrogen amounts (200kg/ ha) as urea, 200 kg/ha of phosphate as triple super phosphate and 50 kg/ ha of zinc as zinc sulphate (in zinc treatment) at planting were applied on the basis of soil analysis and the remaining nitrogen fertilizer (200 kg/ha) used at 6-8 leaf stages. The field was kept weed-free by weeding. The plants were thinned to 83000 plants ha^{-1}. Water treatments began after maize reached the 8-leaf stage.

Table 1. Result of physical and chemical soil analysis

Fe ppm	Cu Ppm	Zn ppm	Mn ppm	K ppm	P ppm	N%	pH	Electrical conductivity Ds/m	Organic Carbon%	Sand (%)	Silt (%)	Clay (%)
1.23	0.8	0.96	4.82	290	1	0.031	8.24	1.37	0.315	52	32	16

Specific strains of Pseudomonas fluorescens (strain 169) and Pseudomonas putida (strain108) were prepared by the Soil Biology Laboratory of the Water and Soil Research Institute of Khorasan, Razavi, Iran. Some Biochemical characteristics of these strains[1] are shown in Table 2. Maize seeds (SC 704) were inoculated according to Sharma et al. (2003). Seeds were surface-sterilized with 0.02% sodium hypochlorite for 2 min, and rinsed thoroughly in sterile distilled water. For inoculation, seeds (60 g for each plot) were coated with gum Arabic (40%) as an adhesive then bacterial inoculum (0.6 g) mixed with them as uniformly coated and next, placed in the furrow quickly.

Table 2. Some biochemical characteristics of P. fluorescens (strain 169) and P. putida (strain108)

Biochemical characteristics	Strain P. fluorescens169	P. putida 108
ACCdeaminase activity α-ketobutyrate (M mol)/protein (mg)/hour	3.508	5.03
Auxin-like compounds (mg/l)	5.8	8.9
soluble phosphorus (mg/l)	53.5	57.32
Siderophore production	+	+
Ability of auxin production	+	+

At the end of maize growth season grain yield, leaves, stems and tassels dry weight, cobs weight and harvest index of ear per plant (HIEP) were determined.

HIEP calculated by this equation.

$$HIEP = Yep / Ybp \times 100$$

That Yep is ear yield per plant and Ybp is biological yield per plant. Data were analyzed by statistical software MSTAT-C and the means were compared by Duncan Multiple Range Test at 5% level.

RESULTS AND DISCUSSION

Stem dry weights

The result indicated that water deficit stress caused a significant decrease in the dry weights of the stems (Table 3), so that the maximum and minimum stem dry weights can be obtained in I_1 (7156.889 kg/ha) and I_3 (5727.3 kg/ha) respectively (Table 4). Our results were in agreement with those obtained by Hong and Ji-yun (2007) on maize and Thalooth et al. (2006) on mungbean. Drought stress had a negative effect on current photosynthesis. The inhibitory effect of drought on plant growth was suggested to be attributed to an increase in osmotic pressure in the root medium, which tends to decrease synthesis of metabolites, reduce translocation of nutrients from the soil to the plant as well as decrease division and elongation of the cells. Moreover, water stress caused a progressive decline in the net photosynthesis rate which is associated with simultaneous decreases in leaf area and starch content (Thalooth et al., 2006). These factors cause a decrease in dry matter accumulation in stems. Also, water stress increases the amount of remobilized dry matter from stems when grains are growing, and this in turn decreases the stem dry weights in water stress conditions (Beheshti and Behboodi fard, 2010).

The effect of seed inoculation on stem dry weights was significant at the 1% level (Table 3). Mean comparison of treatments indicated that highest stem dry weights were obtained in T3 and T1 with 6787.16 and 6619.35 kg/ha respectively (Table 4). Similar results were reported by Gholami et al. (2009). Also, seed inoculation increased stem dry weights significantly ($P<0.05$) in water deficit stress conditions so that the highest and lowest amount of stems dry weight are related to T1 in normal irrigation with 7171.52 kg/ha and non-inoculated treatment in severe water deficit stress conditions with 5246.7 kg/ha respectively (Table 5).

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[1] . Bacerial density in inoculum:

Strain 108 = 1.01×10^9 cell/gr inoculum

Strain 169 = 1.25×10^9 cell/gr inoculum

Regardless of the irrigation treatment, the application of zinc sulfate fertilizer increased stem dry weights by 3.93% in comparison with the absence of of zinc sulfate fertilizer (Table 3 and Table 4). These results are in agreement with those of obtained by Hong and Ji-yun (2007) on maize and Thalooth et al. (2006) on mungbean. Zinc plays an important role in protein and carbohydrate synthesis and takes part in metabolism regulation of saccharides (Sajedi et al., 2009). Moreover, application zinc sulphate has been reported to increase auxin biosynthesis, chlorophyll concentration, and nitrogen and phosphorous uptake (Malekooti and Tehrani, 1999; Cakmak, 2000). In the present study, using zinc sulfate fertilizer resulted in an increase in stem heights, and leaf areas which can enhance the photosynthetic production and dry matter accumulation.

Leaf dry weights

The result showed that irrigation treatment affected leaf dry weights at the 1% level (Table 3). Severe water deficit stress (I_3) decreased leaf dry weights by 37.5% in comparison to normal irrigation. Highest (6444.9 kg/ha) and lowest (3938.22kg/ha) amount of leaves dry weight were obtained in I_1 and I_3 respectively (Table 4). This result is in agreement with the results of Hong and Ji-yun (2007) and Mahrokh et al. (2011). Water stress caused a reduction in leaf area and chlorophyll concentration, and inhibited both photosynthesis and leaf senescence (Yordanov et al., 2003). These are the protective mechanisms for a plant trying to deal with the effects of drought stress (Hagbabayi et al., 2011). In our study, water deficit stress also resulted in a reduction of ear leaf area.

The effect of seed inoculation on leaf dry weights was significant at the 1% level but the interactive effect between seed inoculation and irrigation was not significant (Table 3). Bacterial inoculation with strains 108 or 169 increased leaf dry weights 10.34% and 7.404% in comparison to non-inoculated plants (Table 4). Similar results were reported by Gholami et al. (2009) and Mahrokh et al. (2011). Enhancement of leaf dry weights presumably reflect the increase in leaf surface area. Arshad et al., (2008) suggested that the role of ACC deaminase-producing bacteria is very important for increasing leaves area. In addition, PGPR synthesize and secrete indole-3-acetic acid (IAA) which can stimulate plant cell proliferation and plant cell elongation (Patten and Glick, 2002; Glick et al., 2007a,b) thereby increasing leaves area. Also, in the present study, bacterial inoculation increased ear leaf areas.

Zinc sulphate fertilizer treatments significantly affected leaf dry weights at the 1% level, but the interaction with irrigation treatments was non-significant (Table 3). Regardless of the irrigation treatment, supplying zinc sulphate (50 kg/ha) resulted in an increase of leaf dry weight of approximately 5% in comparison to the lack of fertilizer treatment (Table 4). These results are in full agreement with those obtained by Hong and Ji-yun (2007) on maize and Thalooth et al. (2006) on mungbean. In the present study, ear leaf area increased as a consequence of Zn treatment. Hence, enhancement of leaf area caused an increase of leaf dry weight.

The results of the present study revealed that the interaction effect between bacterial inoculation and zinc sulphate was significant at the 5% level (Table 3). Means comparisons showed that maximum and minimum amount of leaf dry weights obtained in T4 with 5100.42kg/ha and T6 with 4298.93kg/ha respectively (Table 7). It seems that, elimination of the ethylene inhibiting effect by the ACC deaminase-containing PGPR (Glick et al., 2007a, b) plus the role of Zn in plant nutrition (Cakmak, 2000; Sajedi et al., 2009; Hong and Ji-yun, 2007) increased both leaf surface area and dry weight.

Table3. Mean square of the effect of Water deficit stress and application of Zinc sulphate on the traits of maize

Source of Variation	df	Stem dry weights (kg/ha)	Leaf dry weights (kg/ha)	Tassel dry weights (kg/ha)	Cob weights (kg/ha)	Grain yield (kg/ha)	HIEP
Replication	2	412561.073^{*}	811125.807^{*}	614.462^{*}	225035.66^{**}	1484006.78^{*}	1.38^{ns}
Irrigation	2	10278308.469^{*}	36441395.013^{**}	30601.613^{**}	4427279.6^{**}	254352627.49^{**}	874.04^{**}
Error a	4	1037993.173	406447.308	69.756	10014.599	155444.759	2.802
Bacterial inoculation	2	899634.692^{**}	1052415.789^{**}	6883.675^{**}	860233.711^{**}	8525393.376^{**}	35.135^{**}
I*B	4	386663.713^{*}	64863.179^{*}	258.883^{**}	21652.856^{*}	664655.652^{**}	0.729^{ns}
Zinc sulfat	1	944523.818^{**}	742809.33^{**}	4398.153^{**}	542015.879^{**}	11234629.574^{**}	12.77^{**}
I*Zn	2	237062.251^{*}	132217.004^{ns}	361.828^{**}	46949.213^{**}	1370926.9^{**}	0.035^{ns}
B*Zn	2	284507.343^{*}	215201.752^{*}	354.893^{**}	5292.121^{ns}	526254.402^{*}	2.714^{**}
I*B*Zn	4	75200.86^{*}	88732.118^{*}	79.031^{*}	1157.316^{ns}	186247.064^{ns}	0.457^{ns}
Erorr b	30	102663.647	53698.121	26.167	5845.986	153465.047	0.3
CV%		14.87	14.82	12.92	15.96	14.17	11.14

(* significant at 5%; ** significant at 1%;^{ns} non significant)

Tassel dry weights

Water deficit stress caused a significant decrease in tassel dry weights at the 1% level (Table 3). Also, soil moisture appears to be very important for tassel growth so that tassel dry weights decreased in I_2 and I_3 to 17.98% and 38.28%, respectively, in comparison to normal irrigation treatment (Table 4). Similar results were obtained by Monneveux et al. (2006) and Nbati and Rezvani (2010). Under drought stress conditions, flowering is the most sensitive stage of maize plant growth (Rabani and Emam, 2011). Under drought conditions, the increasing ethylene concentration and decreasing photosynthetic production results in inhibition of tassel initiation and growth both of which lead to a decrease in tassel dry weights.

Seed bacterization of maize with Pseudomonas spp. strains improved tassel dry weights significantly at the 1% level (Table 3). Mean comparison of treatments indicated that bacterial inoculation by strain 108 or 169 increased tassel dry weights by 16.09% and 25.1% respectively compared to control levels (Table 4). Interaction effects of bacterial inoculation and water deficit stress were significant at the 1% level (Table 3). The highest (239 kg/ha) and lowest (118.3 kg/ha) tassel dry weights were obtained following bacterial inoculation (strain 169) under normal condition (I_1) and control treatment in water deficit stress conditions (I_3), respectively (Table 5). Increased plant ethylene concentrations cause to a decrease in root length (Mayak et al., 2004) and a increase in leaf senescence (Taiz and Zeiger, 2002) under water stress condition. These events severely affect photosynthesis as reflected by declines in photosynthetic production at the flowering stage. Moreover, water stress leads to a shortened flowering stage and to decreasing inflorescence growth. Elimination of ethylene inhibition by Pseudomonas strains containing ACC deaminase can obviate water deficit stress (Glick et al., 2007a) and increase tassels growth.

The effect of zinc sulphate on tassel dry weights was significant at the 1% level (Table 3). Application of 50 kg/ha zinc sulfate increased tassel dry weights by 10.88% compared to controls without zinc sulfate (Table 4). Also, the interaction effect of this fertilizer and water deficit stress was significant at the 1% level. Highest (228.51 kg/ha) and lowest (128.54 kg/ha) tassel dry weights were related to supplying zinc sulphate fertilizer treatments in normal irrigation, and the absence of this fertilizer in severe water deficit stress, respectively (Table 6). The present study indicated that, using zinc sulphate fertilizer increased leaf surface area in both normal and water deficit stress conditions. Also, higher Zn concentrations caused an increase in auxin concentration which effects rooting induction (Zand et al., 2010). Thus, enhancement of water and nutrient uptake due to root development plus increasing leaf area can result in improvement of photosynthetic production for tassel growth at the flowering stage.

The interaction effect of bacterial inoculation and zinc sulphate on tassel dry weight was significant at the 1% level (Table 3). Moreover, co-application of PGPR strains and zinc sulphate enhanced tassel dry weights (Table 7). It seems that, tassels growth increased due to enzymatic lowering of plant ethylene concentrations by PGPR associated with supplying zinc to plants (Sajedi et al., 2009; Talooth et al., 2006), ultimately yielding higher photosynthetic productivity in plants.

Also, co-application of bacterial inoculants and zinc sulphate fertilizer significantly increased tassel dry weights in water deficit stress (Table 3). Maximum and minimum amount of tassel dry weights were obtained in the bacterial inoculation and supplying 50 kg/ha zinc sulphate under normal irrigation conditions (I_1) with 256.407 kg/ha, and non-application of PGPR and zinc sulphate in severe water deficit stress conditions (I_3) with 114.58 kg/ha, respectively (Table 8).

Cob weights

Water deficit stress caused a significant decrease in cob weights (Table 3). The cob weights decreased 25.76% and56.18% in I_2 and I_3, respectively compared to control (Table 4). The results of this research parallel the results of Rafiai et al. (2009) and Mostafavi et al. (2011). Reduced cob weights are attributed to a disturbance in photosynthesis, increasing ethylene concentration, and decreasing cell division and cell elongation under the impact of the water stress conditions (Glick et al., 2007a,b; Yordanov et al., 2003).

Application of bacterial inoculants increased cob weights significantly at the 1% level (Table 3). Seed inoculation by strain108 or 169 enhanced cob weights, by 30.05% and 40.61%, respectively, compared to the control (Table 4) in agreement with the results of Shahroona et al. (2006) and Naveed et al. (2008). In this investigation, cob weights were affected by PGPR due to developing leaf surface area that can enhance transferred photosynthetic production to ear and ear length. Also, the interaction effect of bacterial inoculation and different irrigation regimes was significant at the 1% level (Table 3). Highest (1963.15 kg/ha) and lowest (589.56 kg/ha) cob weights were obtained in the bacterial inoculation treatment by strain 169 under normal conditions, and the lack of inoculation in severe water deficit stress conditions, respectively (Table 5). Similar results were reported by Ghoorchiyani et al. (2011). This result may reflect the enzymatic lowering of plant ethylene concentrations and higher auxin production which can result in longer roots and shoots in inoculated plants (Glick et al., 2007a; Mayak et al., 2004).

Zinc sulphate fertilizer significantly affected the cob weights at the 1% level (Table 3). Using 50 kg/ha zinc sulphate increased cob weights 16.95% in comparison to the control (Table 4). The result of this research is consistent with the results of Harris et al. (2007) and Ghazvineh and Yousefi (2012). Also, the interaction effect of zinc sulphate and different irrigation regimes was significant at the 1% level (Table 3). Maximum (1920.31 kg/ha) and minimum (715.259 kg/ha) amounts of cob weights were related to using zinc sulphate treatment in normal irrigation and non-application of zinc sulphate treatment under severe water deficit stress conditions (I_3), respectively (Table 6). Hong et al. (2007) reported that, Zn affects rooting, plant water status and oxygen metabolism, hence it can alleviate some of the impacts of water deficit stress. Also, in this study, using zinc sulphate fertilizer (in I_3) improved the leaf area of ears, which can result in the enhancement of photosynthetic production and dry matter accumulation in cobs.

Grain yield

Water deficit stress caused a significant decrease in grain yield (Table 3). The maximum (13110.848 kg/ha) and minimum (5594.851 kg/ha) grain yield was obtained in I_1 and I_3 respectively (Table 4). Our results confirmed by the finding of Habibi et al. (2010), Farajzadeh et al. (2011) and Sajedi et al. (2009) who also showed decreasing grain yield because of water stress. Drought stress led to decreased cell growth and division, photo-oxidation of chlorophyll, impaired plant metabolism, and changes in cellular osmotic potential, decreased enzyme activity and stomatal closure (Yordanov et al., 2003); and a higher ethylene concentration resulted in reduced shoot and root growth and leaf senescence (Glick et al., 2007a; Mayak et al., 2004; Taiz and Zeiger. 2002). Thus, all of these factors can reduce photosynthetic production and grain yield. In this experiment, economic yield of the maize plant was affected because of decreased leaf water potential, ear leaf area and yield components under water deficit stress.

The effect of bacterial inoculation and the interaction effect of bacterial inoculation and irrigation on grain yield were significant at the 1% level (Table 3). Regardless of the irrigation treatments, the results shown in Table 4 reveal that seed inoculation by P. fluorescents strain 169 and P. putida strain 108 increased grain yield by 14.72% and 12.73% respectively in comparison to the control. These results are in agreement with those obtained by Gholami et al. (2009), Rosas et al. (2009), Shahroona et al. (2006), Seyed Sharifi et al. (2011); Naveed et al. (2008) on maize, and Shahroona and Navid (2009) on wheat. Also, means comparison of the interaction effect of irrigation and bacterial inoculation showed that application of PGPR containing ACC deaminase improved grain yield in water deficit stress. The highest (13930.333 kg/ha) and lowest (5087.632 kg/ha) amount of grain yield were obtained respectively following bacterial inoculation treatment by strain 169 in normal condition (I_1) and non-inoculants treatment under severe water deficit stress(I_3) conditions (Table 5). Similar results were obtained by Aghaalikhani and Ehteshami (2008), Ghoorchiyani et al. (2012) on maize; Arzanesh et al. (2010) and Rafat et al. (2011) on wheat and Arshad et al. (2008) on pea. PGPR that decrease ethylene levels increase the lifetime of the plant, and increased plant IAA concentration results in increased rooting and plant ability for nutrient and water uptake (Glick et al., 2007a,b), siderophore production supplies needed iron to the plant, provide essential elements such as phosphorus (Hayat et al., 2010) and nitrogen (Shahroona et al., 2006; Hontzeas et al., 2006), increased photosynthetic surface via developed leaf area and preventing leaf senescence, and finally more transferred photosynthetic production into ears result in improved grain yield in water stress conditions. In addition, both bacterial strains applyed in this study provided a positive change on yield components such as the number of kernels per ear, 1000 grain weight and increased grain yield.

The application of zinc sulfate fertilizer affects the seed yield significantly at the 1% level (Table 3). Zinc sulfate fertilizer increased grain yield 10.19% in comparison with the control (Table 4). The results of this research is in agreement with the results of Harris et al. (2007), Ghazvine and Yousefi (2012), El-Badawy and Mehasen (2011), Farajzadeh et al. (2011), Gamali et al. (2012), Sheykhbeygloo et al. (2009) on maize; Talooth et al. (2006) on mungbean; Khan et al. (2003) on pea; and Dehghanian and Madandoost (2008) on wheat. Moreover, some researchers have suggested that, Zn plays an important role in IAA production (which can enhance cell growth and proliferation), protein and carbohydrate synthesis and takes part in the regulation of saccharides (Zand et al., 2010; Sajedi et al., 2009). In the present study, using zinc sulfate improved ear leaf area (as a photosynthetic surface) which can increase the transferred photosynthetic production into seed, and also increased yield components such as the number of seeds per ear and 1000 grain weight, and grain yield.

The interaction effect of irrigation and zinc sulphate was significant at the 1% level (Table 3). The highest (13848.54 kg/ha) and lowest (5408.74 kg/ha) grain yield were related to using zinc sulphate treatment in condition I_1 and non-application of zinc sulphate in condition I_3 (Table 6). Similar results have been reported by Dehghaniyan and Madandoost (2008) on wheat; Baniabbas et al. (2012) on sunflower; and Talooth et al. (2006) on Mungbean, Movahhedy et al. (2009) on safflower. In this experiment, supplying zinc sulphate facilitated the development of ear leaf area as a photosynthetic surface, increase of seed number per ear and higher grain yield. Some researchers have also reported that Zn application lead to higher photosynthesis in the plant (Thalooth et al., 2006). Moreover, interaction of soil potassium and Zn results in an increase of potassium uptake and enrichment of potassium in plant shoots (Malakuoti, 2004). Talooth et al. (2006) also commented that potassium plays an important role in chlorophyll formation and regulating photosynthesis, and thereby enrichment of photosynthetic material, transmiting and supply carbohydrate in drought stress. Also potassium is an essential factor in maintenance of osmotic potential and water uptake and has a positive effect on stomatal closure which increases plant tolerance to water stress. Potassium application on mungbean resulted in a higher grain yield in water stress conditions (Talooth et al., 2006).

Nevertheless, Zn treatment was only significant on grain yield in I_1 and I_2 and it could not improve the economic yield of maize in I_3 compared to a lack of Zn treatment (Table6). These results agree with those obtained by Farajzadeh et al. (2011), Wang et al. (2009) and Hong and Jj-yun (2007) who also reported that Zn application could not improve grain yield in severe water stress.

Regardless irrigation treatments, co-application of bacterial inoculants and zinc sulphate fertilizer significantly increase of maize grain yield (Table 3). Maximum (10185.89 kg/ha) and minimum (7998.163 kg/ha) grain yield were obtained in T4 and T6 (control) respectively (Table7). It seems that, elimination of ethylene effects associated with suppling needed Zn to the plant enhances the seed yield.

Harvest index of ear in plant (HIEP)

Irrigation treatments effected on HIEP significantly at 1% level (Table 3). The impact of water deficit stress caused HIEP to decrease by 4.02% and 24.51% in I_2 and I_3 treatments compared to I_1, respectively (Table 4). The result of this research is consistent with the results of Hagbabayi et al. (2011). In drought conditions, the flowering phase is the most sensitive stage of maize plant growth and water stress has the most negative effects on ear growth and yield (Rabani and Emam, 2011). Hence, reduced ear yield leads to a decline in HIEP.

The effect of PGPR on HIEP was significant at the 1% level (Table 3). Application of strain 169 and 108 increased HIEP by 5.87% and 4.06% respectively compared to the non-inoculated control (Table 4). Most probably, application of a bacterial inoculant lead to prevention of leaf senescence, stability of leaf area in the flowering stage and extending the duration of the reproductive stage by lowering plant ethylene concentrations. These factors can result in enhancement of the photosynthetic material in ear, ear yield and finally HIEP.

Also, the effect of zinc sulphate fertilizer on HIEP was significant at the 1% level but the interaction effect between zinc sulphate fertilizer and irrigation was not significant (Table 3). Supplying 50 kg/ha zinc sulphate increased HIEP 2.04% compared to the control (Table 4). Khalili and Roshdi (2009) reported similar results and suggested that HIEP is generally a quantitative trait which can effect the quality of silage corn. They also suggested that the accumulated protein and carbohydrate in grain are greater than in the stem in maize. Hence, all factors that can increase the protein (as a heavy organic component) and carbohydrate concentration have a significant effect on HIEP. Whereas, zinc plays an important role in protein and carbohydrate synthesis (Sajedi et al., 2009), higher zn concentrations in plants can cause an enhancement of these organic component and their accumulation in grain.

Co-application of zinc sulphate fertilizer and bacterial inoculation affected HIEP significantly at 1% the level but the interaction effect between them and water deficit stress was not significant (Table 3). Maximum (49.97%) and minimum (46.353%) HIEP are related to T2 and T6, respectively (Table 7).

CONCLUSION

According to the results, the yield of maize (forage and grain) declined in both water deficit stress conditions. Application of P. fluorescens strain 169 or P. putida strain108 (both containing ACC deaminase) for seed inoculation caused an enhancement of forage and grain yield under normal and water deficit stress conditions. Supplying 50 kg/ha zinc sulphate fertilizer increased the yield of maize in both normal and water deficit stress conditions; as well, co-application of a bacterial inoculation and 50 kg/ha zinc sulphate caused higher grain yield in normal irrigation conditions. Hence, bacterial inoculation of seeds by P. fluorescens strain 169 or P. putida strain108, and using zinc sulphate fertilizer are recommended for maize cultivation in semi-arid and arid regions where water deficit stress is one the most important inhibiting factors in plant growth.

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Table 4. Mean comparison of the maize traits under water deficit stress application of bacterial inoculation and Zinc sulfate

Traits Treatment	Stems dry weight (kg/ha)	Leaves dry weight (kg/ha)	Tassels dry weight (kg/ha)	Grain yield (kg/ha)	Cobs weight (kg/ha)	HIEP (%)
I1	7156.889a	6444.933a	a 215.269	13110.848 a	1763.536 a	52.997a
I2	6866.673a	3938.224 b	176.558 b	9509.175 b	1309.073 b	50.866b
I3	5727.3 b	4025.013 b	132.855 c	5594.851 c	772.775 c	40.004 c
B0	6344.343 b	4534.378 b	153.776 c	8616.485 b	1037.416 c	46.417 c
B1	6787.160 a	5003.683 a	178.530 b	9713.371 a	1349.211 b	48.304b
B2	6619.359 a	4870.109 a	192.377 a	9885.018 a	1458.757 a	49.146a
Zn0	6451.366 b	4685.439 b	165.869 b	8948.834 b	1181.608 b	47.469b
Zn1	6715.875 a	4920.008 a	183.919 a	9861.081 a	1381.981 a	48.442a

Mean followed by the same letters in each column are not significant (Duncan's multiple rang test 5%):I1= Normal irrigation (control), I2= Changing irrigation interval from 6 to every 9 days (moderate water deficit stress), I3= changing irrigation interval from 6 to every 12 days (sever water deficit stress); B0 = non-inoculation, B1 = Seed inoculation by Pseudomonas putida strain108, B2 = Seed inoculation by Pseudomonas fluorescens strain169; Zn0= Non-application of zinc sulfate fertilizer, Zn1= Application of zinc sulfate 50 kg/ha.

Table 5. Mean comparison for ineteraction effect of water deficit stress and bacterial inoculant on maize traits.

Traits Treatment		Stems dry weight (kg/ha)	Leaves dry weight (kg/ha)	Tassels dry weight (kg/ha)	Grain yield (kg/ha)	Cobs weight (kg/ha)	HIEP (%)
I1	B0	6846.585 ab	6195.362 a	187.2 cd	11923. 237 b	1523.55 c	51.42 c
	B1	7455. 557 a	6717.363 a	219.7 b	13478.975 a	1803.9 b	53.14 ab
	B2	7171.525 a	6422.073 a	239.0 a	13930.333 a	1963.158 a	54.43 a
I2	B0	6942.742 ab	3745. 880 b	155.9 e	8838. 586 d	999.138 d	49.01 d
	B1	6854.138 ab	4045.252 b	179.7 d	9789.985 c	1410.570c	51.49 c
	B2	6804.138 ab	4023.540 b	194.2 c	9898.953 c	1517.510c	52.10 bc
I3	B0	5246.702 c	3661.892 b	118.3 g	5087.632 f	589.560f	38.82 f
	B1	6053.785 bc	4248.435 b	136.3 f	5871. 153e	833.162e	40.28 e
	B2	5883.413 c	4164.713 b	144.0f	5825.767e	895.603de	40.91 e

Mean followed by the same letters in each column are not significant (Duncan's multiple rang test 5%):I1= Normal irrigation (control), I2= Changing irrigation interval from 6 to every 9 days (moderate water deficit stress), I3= changing irrigation interval from 6 to every 12 days (sever water deficit stress); B0 = non-inoculation, B1 = Seed inoculation by Pseudomonas putida strain108, B2 = Seed inoculation by Pseudomonas fluorescens strain169.

Table 6. Mean comparison for ineteraction effect of water deficit stress and zinc sulfate on maize traits.

Traits Treatment		Stems dry weight (kg/ha)	Tassels dry weight (kg/ha)	Grain yield (kg/ha)	Cobs weight (kg/ha)
I1	Zn0	6918.719 a	202.028 b	12373.157 b	1606.762 b
	Zn1	7395.059 a	228.51 a	13848.540 a	1920.310 a
I2	Zn0	6718. 409 a	167.04 d	9064.599 d	1222.803 d
	Zn1	7014.937 a	186.077 c	9953.751 c	1395.342 c
I3	Zn0	5716.971 b	128.540 e	5408. 748 e	715.259 e
	Zn1	5737.629 b	137.170 e	5780.953 e	830.291 e

Mean followed by the same letters in each column are not significant (Duncan’s multiple rang test 5%):I1= Normal irrigation (control), I2= Changing irrigation interval from 6 to every 9 days (moderate water deficit stress), I3= changing irrigation interval from 6 to every 12 days (sever water deficit stress); Zn0 = Non-application of zinc sulfate fertilizer, Zn1= Application of zinc sulfate 50 kg/ha.

Table 7. Mean comparison for ineteraction effect of bacterial inoculants and zinc sulfate on maize traits.

Traits Treatment		Stems dry weight (kg/ha)	Leaves dry weight (kg/ha)	Tassels dry weight (kg/ha)	Grain yield (kg/ha)	Cobs weight (kg/ha)	HIEP (%)
B0	Zn0	6088.260b	4298.936 c	142.224 e	7998.163 c	956.999 f	46.35 e
	Zn1	6600.426 a	4769.82 b	165.327 d	9346.807 b	1117.833 e	46.48 e
B1	Zn0	6651.201 a	4906.944 ab	174.632 c	9240.852 b	1240.082 d	47.74 d
	Zn1	6923.119 a	5100.422 a	182.428 b	10185.89 a	1458.339 b	48.87 b
B2	Zn0	6614.638 a	4850.436 b	180.751 b	9607.488 b	1347.643 c	48.32 c
	Zn1	6624.08 a	4889.782 ab	204.002 a	10162.548 a	1569.771 a	49.97a

Mean followed by the same letters in each column are not significant (Duncan’s multiple rang test 5%): B0 = non-inoculation, B1 = Seed inoculation by Pseudomonas putida strain108, B2 = Seed inoculation by Pseudomonas fluorescens strain169; Zn0= Non-application of zinc sulfate fertilizer, Zn1= Application of zinc sulfate 50 kg/ha.

Table 8. Mean comparison for interaction effect of water deficit stress, bacterial inoculation and zinc sulphate on maize traits.

Traits Treatment			Stems dry weight (kg/ha)	Leaves dry weight (kg/ha)	Tassels dry weight (kg/ha)	Grain yield (kg/ha)
I1	B0	Zn0	6344.707abcd	5726.45 b	168.7 e	10878.017d
		Zn1	7346.463 ab	6664.27 a	205.7 c	12968.45bc
	B1	Zn0	7254.067 ab	6584.78ab	215.853bc	12650.826 c
		Zn1	7655.047 a	6850.94a	223.5 b	14307.123 a
	B2	Zn0	7157. 383 ab	6436.77 ab	221.573 b	13590.62 b
		Zn1	7184.667 ab	6407. 37 ab	256.407 a	14270.04 a
I2	B0	Zn0	6742.18 abc	3576.18 c	143.437 g	8365.936 f
		Zn1	7141.303 ab	3916. 58 c	168.270 e	9311. 236 e
	B1	Zn0	6646.96 abcd	3896. 58 c	174.44de	9321. 85e
		Zn1	7061.31 ab	4195 c	184.88 d	10258. 12 d
	B2	Zn0	6766.08 abc	3952.97c	183.243 d	9506.01 e
		Zn1	6842.19 ab	4094.14 c	205.1c	10291. 897 d
I3	B0	Zn0	5177.813 d	3594.173 c	114.58 i	4750. 537 h
		Zn1	5313.514 cd	3729.61 c	122.1 hi	5425.727gh
	B1	Zn0	6052.57 bcd	4241.467 c	133.6 gh	5749. 88 g
		Zn1	6053 bcd	4255.403 c	138.93 g	5992.427g
	B2	Zn0	5920.45 bcd	4161.593 c	137.437 g	5725.827 g
		Zn1	5846.37 bcd	4167.833 c	150.523 f	5926.707g

Mean followed by the same letters in each column are not significant (Duncan’s multiple rang test 5%):I1= Normal irrigation (control), I2= Changing irrigation interval from 6 to every 9 days (moderate water deficit stress), I3= changing irrigation interval from 6 to every 12 days (sever water deficit stress); B0 = non-inoculation, B1 = Seed inoculation by *Pseudomonas putida* strain108, B2 = Seed inoculation by *Pseudomonas fluorescens* strain169; Zn0= Non-application of zinc sulfate fertilizer, Zn1= Application of zinc sulfate 50 kg/ha.