



# Yield and yield components of hybrid corn (*Zea mays* L.) as affected by mycorrhizal symbiosis and zinc sulfate under drought stress

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**Abstract** With respect to the significance of improving hybrid corn performance under stress, this experiment was conducted at the Islamic Azad University, Arak Branch, Iran. A complete randomized block design with three levels of irrigations (at 100%, 75% and 50% crop water requirement), two levels of arbuscular mycorrhizal (AM) fungi (*Glomus intraradisis*) (including control), and three levels of zinc (Zn) sulfate (0, 25 and 45 kg ha<sup>-1</sup>), was performed. Results of the 2-year experiments indicated that irrigation treatment significantly affected corn yield and its components at P=1%. AM fungi and increasing Zn levels also resulted in similar effects on corn growth and production. Although AM fungi did not significantly affect corn growth at the non-stressed irrigation treatment, at moderate drought stress AM fungi significantly enhanced

corn quality and yield relative to the control treatment. The combined effects of AM fungi and Zn sulfate at 45 kg ha<sup>-1</sup> application significantly affected corn growth and production. In addition, the tripartite treatments significantly enhanced corn yield at P=1%. Effects of Zn and AM fungi on plant growth under drought stress is affected by the stress level.

**Keywords** Arbuscular mycorrhizal fungi · Corn (*Zea mays* L.) · Drought stress · Zinc sulfate

## Introduction

Plants are generally subjected to different stresses during their growth period (Bray 1997). Drought is one of the most important abiotic stresses affecting plant growth and yield (Cheong et al. 2003). Drought stress in general has undesirable effects on growth and production of crop plants (Xiong et al. 2002). Drought stress causes damage to cell membrane and photosynthetic system. Moreover, plant root and shoot are damaged by drought stress, decreasing leaf surface area (Hopkins and Huner 2004).

Water is much necessary for plant growth and production because of its important functioning in plant including solubilization and hence uptake of nutrients and their movement in the plant, plant transpiration, and creating the necessary pressure for the growth and development of plant cells. Hence, under drought stress plant activities such as photosynthesis, activities of nitrate reducing and hydrolyzing enzymes (like amylase) decrease, which eventually reduce plant growth and production (Munns 2002).

Plant morphological and biochemical responses to drought stress vary with stress intensity. Corn cellular development decreases under moderate to medium drought

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stress, which eventually reduces plant growth (Laffitte and Edmeades 1995). Lam (2004) showed that corn can be planted using minimum level of irrigation water. Nonetheless, under such conditions corn yield and some of its qualitative characters may be adversely affected.

Many researchers have indicated that arbuscular mycorrhizal (AM) fungi are capable of alleviating the unfavorable effects of drought on plant growth (Auge 2001; Miransari 2010). Symbiotic relationship between AM fungi and a variety of plants produces colonies on the exterior part of root system resulting in the enhanced uptake of water and nutrients by the plant roots. Such characters improve plant performance under drought stress, which is believed to be in part related to the increased absorption of water and some nutrients such as zinc (Zn) and copper (Cu). Improved plant variables may include leaf height, leaf water turgidity, stomatal activities, and root growth and development (Ghazi and Zak 2003). AM fungi perform as the enhancer of plant–water relationship through increasing soil hydraulic conductivity, transpiration ratio and decreasing stomatal resistance by adjusting plant hormonal balance. This chain of improvements enhances plants phosphorous (P) nutrition introduced by AM fungi activities under drought conditions (Elwan 2001).

Higher green leaf surface was observed in mycorrhizal corn subjected to drought stress relative to non-mycorrhizal corn. Such symbiosis resulted in the highest plant height with a 23% increase compared to the control treatment (Auge et al. 2001). One of the most common nutritional obstacles for the production of cultivated and orchard plants is Zn deficient soils (Ramazanzadeh et al. 2007). Zn as a metallic element acts as activator for some enzymes. Zn is attached to some plant enzymes such as dehydrogenase, super oxide dismutase (SOD), carbonic anhydrase and RNA polymerase, and in addition plays a role in the activity of enzymes such as dehydrogenase, aldolase, isomerase, transphosphorylase and DNA polymerase. SOD is an enzyme, found in the cellular chloroplast having Cu and Zn in its structure. This enzyme plays an important role in neutralizing the effect of oxygen free radicals produced during drought stress on plant growth. The protecting role of Zn in maintaining the healthy status of membranes and enhanced plant resistance to diseases has been proved. In addition, Zn regulates auxin (indole acetic acid) level and hence plant growth. Therefore, Zn deficient soils can adversely influence plant growth. Zn deficiency has detrimental effects on male organogenesis and anthesis, and consequently decreases crop yield. Marschner (1993) stated that application of zinc and iron enhanced corn total carbohydrate and protein, resulting in increased grain weight and number, and hence crop yield.

The interaction effects between P and Zn in plant can adversely affect their uptake. This is especially important in AM symbiosis as one of the most important effects of AM

on plant growth is through the enhanced uptake of nutrients such as P and Zn. Hence, application of Zn may also interfere with the uptake of P by mycorrhizal plants, in addition to the alleviating effects of Zn on plant growth under drought stress through affecting the activities of antioxidant enzymes. Accordingly, the effect of Zn was also tested in this experiment to evaluate how the combined effects of Zn and AM fungi can influence the effects of drought stress on corn growth.

Since there is little data related to the combined effects of AM fungi and Zn application on corn growth and yield under drought stress, these experiments were conducted with the following objectives: 1) to determine the effects of drought stress on corn growth and yield, and 2) to test the hypothesis that the combined effects of AM fungi and Zn can improve the alleviating effects of AM fungi on corn growth and yield under drought stress.

## Materials and methods

A 2-year experiment was performed in 2006 and 2007 at the research farm of Islamic Azad University Arak-Branch, Iran. Before sowing, composite soil samples were collected from the 0–30 and 30–60-cm depths and their physical and chemical properties tested. Soil texture was determined using the hydrometry method (Gee and Bauder 1986). pH of a saturated paste (Rhoades 1982), organic carbon (wet oxidation method, Nelson and Sommers 1982), total nitrogen (Kjeldahl method, Nelson and Sommers 1973), available phosphorus (sodium bicarbonate extraction method, Olsen 1954), and potassium (flame photometer method, emission spectrophotometry, Knudsen et al. 1982), iron and manganese (Diethylenetriaminepentaacetic acid (DTPA) method, Baker and Amachar 1982, using atomic absorption spectrometer, Model Perkin Elmer 3110) were also determined (Table 1).

The experiment was a factorial, on the basis of a complete randomized block design with three replications. Drought stress treatment including control, irrigation at 75% and 50% of corn water requirement was applied during the period of the four-leaf stage to the end of growth period. Crop water requirement was determined using the evaporation basin on a daily basis. The field was irrigated using polyethylene tubes and the amounts of water to plots were controlled by contour. Zn was applied at three levels of 0, 25 and 45 kg ha<sup>-1</sup> using zinc sulfate, applied at sowing as strip, 5 cm underneath and beside the seeds. Seeds were also inoculated with AM species *Glomus intraradices* at 250–300 active propagules at sowing. The seeds of corn cultivar KSC 704 were first sprayed using sugar solution and then inoculated with the powder containing mycorrhizal propagules.

**Table 1** Soil physical and chemical analyses

Years	Depth (cm)	Ec dS/m	pH	OC%	N%	P (ppm)	K (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Sand %	Silt %	Clay %
(2006)	0–30	0.83	7.9	0.8	0.08	4.2	178	0.52	3.8	9.12	1.12	26	42	32
	30–60	1.07	7.9	0.51	0.04	3.5	145	0.31	3.0	6.2	0.79	26	33	41
(2007)	0–30	1.20	7.5	0.82	0.080	5	150	0.8	4.6	10.6	1.14	29	35	36
	30–60	1.70	7.4	0.61	0.061	3.6	120	0.4	4	6.6	0.88	27	29	44

The mycorrhizal inoculum was produced over a 4-month period on sorghum roots under greenhouse conditions using sterilized sand (Miransari et al. 2007, 2008). During the inoculum production, plants were supplied with their nutrient requirements and were irrigated until flowering. After 2 weeks plant shoots were harvested from the soil surface and the mixture of plant roots and sand were used as the inoculum. The inoculation potential of the AM isolate was tested using the Most Probable Number (MPN) method (Feldman and Idczak 1992; Mahaveer et al. 2000; Daei et al. 2009). Using sterilized sand, AM concentrations of 0.1, 0.01, and 0.001 were produced for the 1-month plantation of sorghum plants. The plants were then harvested and the inoculation percentage on the sorghum roots was determined (Mahaveer et al. 2000). Root colonization was determined using the lateral root technique (Read et al. 1976). After preparing the roots, they were put under pressure. Roots were stained with trypan blue. The stained roots with the length of almost 1 cm were used for the determination of root colonization (Giovannetti and Mosse 1980).

The hybrid corn specifications are as follows. It is a late maturity, single cob hybrid, with the American parents of B73 and MO17, which have come to Iran from former Yugoslavia in 1980. According to FAO's classification its growth stage is between 130 to 140 days. This hybrid is amongst the newest ones and is used both for grain and forage production. Its yield ranges from 6000 to 9000 kg ha<sup>-1</sup>. The mature plant has a height of 285 cm with 370 g weight for 1000 seeds. It is relatively tolerant to drought stress as well as fungal diseases. It can be planted at different parts of Iran with the exception of cold areas (Vahedi 1988).

The experimental field was ploughed in fall and disked twice, perpendicularly in mid spring. Each plot included five rows, distanced at 60 cm and with 20 cm spacing between plants in rows. The length of each row was 8 m and two rows were left none cultivated between the adjacent plots. In both years seeds were cultivated on May 18th. According to soil testing the amounts of fertilization were determined including 400 kg ha<sup>-1</sup> urea, 200 kg ha<sup>-1</sup> super phosphate triple and 200 kg ha<sup>-1</sup> potassium sulphate.

One third of nitrogen (N) and all of phosphorous (P) fertilizers were applied at planting and the remaining N was

broadcasted twice during the vegetative growth. In both years the final harvest was performed on October the 16th at the physiological maturity stage when a black layer could be seen at the base of the grain. At final harvest 10 plants were harvested from the middle of each plot and their agronomic characters were evaluated as mentioned in the "Results part". Data were subjected to analysis of variance using SAS (SAS Inc. 1988). Means were also compared using Duncan's Multiple Range test at  $P=0.05$  (Steel and Torrie 1980).

## Results

According to the analysis of variance, year and drought and their interaction effects significantly affected corn parameters. Year, AM fungi, the interaction effect of year and AM fungi, irrigation and AM fungi and Zn significantly affected root colonization by AM fungi. Although the effects of AM on corn growth and yield were not significant, however its two way interaction with drought ( $P=0.05$ ) and its three way interaction effect with drought and Zn ( $P=0.01$ ) were significant on corn grain yield (Table 2). Although there were not significant differences between irrigation levels on root colonization, AM inoculation significantly increased root colonization by two fold related to the un-inoculated plants. The highest amount of Zn also significantly increased root colonization by AM species, compared with the control level. Drought stress significantly decreased corn yield and its components. Similar to the AM treatment, application of Zn also numerically increased corn yield and its components (Table 3).

Comparison of the two way interaction effects indicated that although AM did not affect corn grain yield and its components significantly under non-stressed conditions; it had a high impact on corn grain yield at different levels of stress. Zn addition numerically increased corn grain yield and its component at different levels of stress including the control treatment and could partially alleviate the unfavorable effects of drought stress on corn growth and production. AM inoculation at different irrigation levels and the combined effects of Zn and AM fungi significantly enhanced root colonization, however the effects of Zn on root colonization at different levels of irrigation was not

**Table 2** Analysis of variance for different plant parameters as affected by different experimental treatments

S. O. V	df	EL	ED	EKW	KRNE	KNER	KNE	1000KW	RC	GY
year	1	25.501**	0.419**	13294.917**	34.680**	618.725**	303181.238**	165309.381**	1776.49**	74804294.705**
Replication (year)	4	6.338	0.040*	48.386	1.258 <sup>ns</sup>	23.066	2809.898	350.273	55.261	541320.611
Irrigation	2	75.151**	1.302**	14824.483**	3.391 <sup>ns</sup>	994.530**	264354.541**	12844.078**	49.929 <sup>ns</sup>	73409130.375**
Y * I	2	11.343**	0.140*	1256.658**	0.998 <sup>ns</sup>	106.471**	25558.389**	3417.822**	18.387 <sup>ns</sup>	8388927.731**
Mycorrhiza	1	0.013 <sup>ns</sup>	0.002 <sup>ns</sup>	158.583 <sup>ns</sup>	0.018 <sup>ns</sup>	26.305 <sup>ns</sup>	5905.203 <sup>ns</sup>	474.392 <sup>ns</sup>	6252.98**	444097.140 <sup>ns</sup>
Y * M	1	1.541 <sup>ns</sup>	0.016 <sup>ns</sup>	874.098 <sup>ns</sup>	0.045 <sup>ns</sup>	52.780 <sup>ns</sup>	17838.372*	145.116 <sup>ns</sup>	467.00**	7166380.193**
I * M	2	4.663 <sup>ns</sup>	0.091 <sup>ns</sup>	483.625 <sup>ns</sup>	0.016 <sup>ns</sup>	14.382 <sup>ns</sup>	7523.500 <sup>ns</sup>	1101.966*	158.79**	3321973.909*
Y * I * M	2	1.704 <sup>ns</sup>	0.110*	327.242 <sup>ns</sup>	0.487 <sup>ns</sup>	5.143 <sup>ns</sup>	3193.742 <sup>ns</sup>	591.896 <sup>ns</sup>	2.21 <sup>ns</sup>	3617933.274*
Zn	2	0.142 <sup>ns</sup>	0.003 <sup>ns</sup>	358.882 <sup>ns</sup>	0.340 <sup>ns</sup>	4.313 <sup>ns</sup>	1140.335 <sup>ns</sup>	409.995 <sup>ns</sup>	78.94**	2062911.475 <sup>ns</sup>
Y * Zn	34	0.634 <sup>ns</sup>	0.054 <sup>ns</sup>	19.211 <sup>ns</sup>	0.306 <sup>ns</sup>	33.036 <sup>ns</sup>	7241.735 <sup>ns</sup>	268.722 <sup>ns</sup>	50.21 <sup>ns</sup>	49328.979 <sup>ns</sup>
I * Zn	4	1.824 <sup>ns</sup>	0.015 <sup>ns</sup>	156.312 <sup>ns</sup>	0.242 <sup>ns</sup>	9.690 <sup>ns</sup>	1635.463 <sup>ns</sup>	322.363 <sup>ns</sup>	4.80 <sup>ns</sup>	1127658.127 <sup>ns</sup>
M * Zn	2	0.158 <sup>ns</sup>	0.085 <sup>ns</sup>	373.219 <sup>ns</sup>	0.288 <sup>ns</sup>	43.446 <sup>ns</sup>	7591.339 <sup>ns</sup>	482.682 <sup>ns</sup>	52.24 <sup>ns</sup>	1810733.729 <sup>ns</sup>
I * M * Zn	4	5.085*	0.039 <sup>ns</sup>	366.279 <sup>ns</sup>	0.646 <sup>ns</sup>	24.092 <sup>ns</sup>	7502.567 <sup>ns</sup>	525.721 <sup>ns</sup>	34.91 <sup>ns</sup>	4532057.590**
Y * I * M * Zn	10	3.346 <sup>ns</sup>	0.070*	284.833 <sup>ns</sup>	0.468 <sup>ns</sup>	24.940 <sup>ns</sup>	8094.957 <sup>ns</sup>	359.014 <sup>ns</sup>	5.78 <sup>ns</sup>	2877606.505**
Error	68	2.100	0.030	249.199 <sup>ns</sup>	0.733	17.375	4541.475	307.532	18.31	942360.727
C.V	–	8.95	4.01	19.48	6.04	11.81	13.40	9.76	19.01	16.06

\* and \*\* : Significant at 5% and 1% probability ns : Non-significant

EL ear length, ED ear diameter, EKW ear kernel weight, KRNE kernel row / ear, KNER kernel number/row, 1000KW thousand kernel weight, RC Root colonization, GY grain yield

**Table 3** Mean comparisons for different plant parameters averaged at different levels of experimental treatments

Treatment	EL(cm)	ED(cm)	EKW(g)	KRNE	KNER	KNE	1000KW(g)	RC(%)	GY(kg ha <sup>-1</sup> )
Irrigation									
I <sub>100</sub>	17.32 a	4.52 a	99.28 a	14.39 a	39.57 a	571.37 a	198.42 a	23.14 a	7281.55 a
I <sub>75</sub>	16.67 a	4.34 b	84.66 b	14.33 a	36.86 b	530.27 b	180.19 b	21.14 a	6366.16 b
I <sub>50</sub>	14.55 b	4.14 c	59.18 c	13.83 b	29.42 c	406.73 c	160.65 c	21.23 a	4481.00 c
Mycorrhiza									
-M	16.17 a	4.33 a	79.25 a	14.17 a	34.78 a	495.18 a	181.85 a	14.89 b	5978.78 a
+M	16.19 a	4.34 a	72.83 a	14.20 a	35.79 a	510.39 a	187.66 a	30.11 a	6107.03 a
Zinc sulfate									
Zn0	16.12 a	4.33 a	80.52 a	14.09 a	35.16 a	497.91 a	177.63 a	21.33 b	5776.12 a
Zn1	16.15 a	4.34 a	78.17 a	14.28 a	35.01 a	501.50 a	178.09 a	22.01b	6113.66 a
Zn2	16.21 a	4.33 a	84.42 a	14.18 a	35.67 a	508.95 a	183.55 a	24.17 a	6238.93 a

Mean followed by the same letters in each column are not significantly different using Duncan's multiple rang test at P=5%.

EL ear length, ED ear diameter, EKW ear kernel weight, KRNE kernel row / ear, KNER kernel number/row, 1000KW thousand kernel weight, RC Root colonization, GY grain yield

significant. Although at the control level of AM fungi, Zn addition did not influence corn grain yield, the combined effect of AM and the highest level of Zn numerically increased corn grain yield, relative to the control treatment (Table 4).

Both in the absence and in the presence of AM fungi, the highest amount of Zn substantially increased corn growth and production, while the medium level of Zn did not affect such parameters. At the moderate level of drought the combined effects of AM fungi and the highest level of Zn significantly increased corn grain yield. With increasing the stress level, at the highest level of drought and in the absence of AM fungi, the highest level of Zn significantly increased corn grain yield, which has been previously indicated by the significant interaction effects between AM, drought and Zn. The highest level of Zn at the control level of mycorrhization (not-inoculated plants) significantly increased root colonization. This was also the case for Zn application at the medium and highest level of water stress for inoculated plants. The medium level of Zn, in the presence of AM fungi substantially increased corn grain yield at the highest level of drought (Tables 2 and 5).

## Discussion

According to the analysis of variance, the effects of AM fungi on corn growth and production were not significant. However, the two and the three way significant interaction effects indicate that AM fungi can be more effective in alleviating the drought stress on corn growth when combined with Zn, which is also evident from the root colonization results. It can also be mentioned that AM effectiveness is dependent on the level of drought stress

(Miransari 2010) and Zn concentration. Accordingly, the results indicate that AM fungi significantly increased corn grain yield at the moderate level of drought stress, relative to the control and the highest level of stress.

In addition, the combined effects of AM fungi and the highest amount of Zn resulted in significant increase in corn yield. Hence, it can be stated that although the single effects of the experimental treatments can affect corn grain yield under stress, however there are some favorable interactions, which can intensify such enhancing effects. Recognition and hence application of such combinations can improve plant growth and production under drought stress.

The positive effects of AM fungi on plant growth and production under different conditions are through extending plant host root system and hence increased water and nutrients uptake.

AM fungi develop an extensive network of hypha when in symbiosis with the host plant. This can significantly enhance the absorbing capacity of the host plant roots. It has also been indicated that AM fungi may behave more effectively with increased stress level (Miransari et al. 2007, 2008), which is somehow in accordance with the results of this experiment. AM fungi can substantially enhance the uptake of different nutrients under different conditions, because of their extensive network of hypha and production of different enzymes such as phosphatase, enhancing the solubility of nutrients including P and the less mobile micronutrients (Marschner and Dell 1994; Miransari et al. 2009a, b). The alleviating effects of AM fungi on plant growth under drought in this research work are in agreement with the results of other researchers.

There is some sort of interaction between P and Zn in the plant. Extra application of either nutrient inhibits the uptake of the other one. This can be of significance as the results of

**Table 4** Mean comparisons for different plant parameters, averaged for different two-way interaction effects

Treatment		EL(cm)	ED(cm)	EKW(g)	KRNE	KNER	KNE	1000KW(g)	RC (%)	GY(kg ha <sup>-1</sup> )
Irrigation	Mycorrhiza									
I <sub>100</sub>	+M	17.72 a	4.56 a	104.05 a	14.40 a	40.78 a	593.77 a	203.65 a	32.96 a	7507.24 a
	-M	16.92 ab	4.48 ab	94.50 ab	14.38 a	38.36 ab	548.97 ab	193.20 ab	17.61 c	7055.86 ab
I <sub>75</sub>	+M	16.50 b	4.29 c	82.11 c	14.31 a	37.11 b	526.50 b	175.90 cd	28.71 b	6559.88 c
	-M	16.83 ab	4.40 bc	87.21 bc	14.36 a	38.61 b	534.05 b	184.48 bc	13.57 d	6072.45 bc
I <sub>50</sub>	+M	14.28 c	4.11 d	60.59 d	13.81 a	29.44 c	400.28 c	166.01 de	28.61b	4733.97 d
	-M	14.83 c	4.11 d	57.76 d	13.86 a	29.40 c	413.17 c	155.30 e	13.50 d	4228.03 d
Irrigation	Zinc sulfate									
I <sub>100</sub>	Zn0	17.82 a	4.54 a	101.12 ab	14.20 a	39.48 ab	557.70 ab	198.10 ab	22.23 ab	6965.09 abcd
	Zn1	16.99 a	4.52 ab	93.42 abc	14.53 a	38.56 ab	564.72 ab	194.98 abc	22.17 ab	7306.90 ab
	Zn2	17.14 a	4.49 ab	103.29 a	14.44 a	40.68 a	591.69 a	202.20 a	25.08 a	7572.67 a
I <sub>75</sub>	Zn0	16.62 a	4.34 c	80.51 c	14.29 a	37.30 ab	525.05 ab	170.51 cde	22.31 b	5957.32 c
	Zn1	16.53 a	4.38 bc	85.42 c	14.53 a	36.26 b	528.28 b	174.14 de	21.33 ab	6517.60 bc
	Zn2	16.83 a	4.31 cd	88.06 bc	14.18 a	37.01 ab	537.48 b	181.93 cd	19.80 b	6623.58 bc
I <sub>50</sub>	Zn0	14.21 b	4.11 e	54.93 d	13.77 a	28.72. c	401.00 c	153.27 f	22.47 ab	4150.71 d
	Zn1	14.80 b	4.13 e	60.68 d	13.79 a	30.31c	411.50 c	162.16 ef	22.58 ab	4405.96 d
	Zn2	14.65 b	4.17 de	61.93 d	13.94 a	29.33 c	407.68 c	166.53 ef	21.62 a	4886.33 d
Mycorrhiza	Zinc sulfate									
-M	Zn0	16.21 a	4.31 a	81.37 ab	14.15 a	34.63 ab	494.21 a	182.95 a	14.95 cd	6101.78 ab
	Zn1	16.16 a	4.35 a	82.77 ab	14.29 a	35.66 a	515.34 a	181.18 a	13.22 d	6092.65 ab
	Zn2	16.13 a	4.30 a	82.61 ab	14.07 a	36.04 ab	511.01 a	181.42 a	16.51 c	6126.66 ab
+M	Zn0	16.22 a	4.35 a	79.66 ab	14.02 a	35.70 ab	501.62 a	174.30 a	27.72 b	6125.53 ab
	Zn1	16.05 a	4.28 a	73.58 b	14.27 a	33.36 b	477.67 a	173.01 a	30.79 a	5459.60 b
	Zn2	16.30 a	4.40 a	86.24 a	14.30 a	36.31 ab	526.88 a	185.68 a	31.83 a	6351.21 a

Means followed by the same letters in each column are not significantly different using Duncan's multiple rang test at P=5%.

EL ear length, ED ear diameter, EKW ear kernel weight, KRNE kernel row / ear, KNER kernel number/row, 1000KW thousand kernel weight, RC Root colonization, GY grain yield

**Table 5** Mean comparisons for different plant parameters, averaged for different three-way interaction effects

Treatment			EL(cm)	ED(cm)	EKW(g)	KRNE	KNER	KNE	1000KW(g)	RC (%)	GY(kg ha <sup>-1</sup> )
Irrigation	Mycorrhiza	Zn									
I <sub>100</sub>	-M	Zn0	18.88 a	4.62 a	109.44 a	14.20 ab	40.10 ab	574.93 ab	198.66 abcd	17.83 de	7520.46 abc
	-M	Zn1	17.23 abc	4.57 ab	100.28 abc	14.60 ab	40.53 ab	598.66 ab	200.55 abc	14.22 ef	7517.95 abc
	-M	Zn2	17.05 abc	4.48 ab	102.41 ab	14.41 ab	41.73 a	607.71 a	213.73 a	20.78 d	8106.31 a
	+M	Zn0	16.75 bcd	4.46 abc	92.79 abcd	14.21 ab	38.86 ab	540.46 abc	182.54 cdef	26.62 c	7039.03 abcd
	+M	Zn1	16.77 bcd	4.47 abc	86.56 bcd	14.46 ab	36.60 abc	530.78 abc	189.42 bcde	30.00 bc	6412.23 bcde
	+M	Zn2	17.23 abc	4.51 ab	104.16 ab	14.46 ab	39.63 ab	575.66 ab	207.66 ab	29.38 bc	7716.33 ab
I <sub>75</sub>	-M	Zn0	16.20 bcde	4.25 cdef	79.71 cde	14.26 ab	36.10 abc	516.30 abc	179.61cdef	11.52 f	5897.63 de
	-M	Zn1	16.93 bc	4.42 abcd	86.94 bcd	14.76 a	39.33 ab	581.50 ab	173.25defgh	14.22 ef	6438.78 bcde
	-M	Zn2	16.39 bcd	4.20 def	79.68 cde	13.90 ab	35.90 bc	511.70 bc	174.85defg	14.98 ef	5903.23 de
	+M	Zn0	17.04 abc	4.43 abcd	91.30 abcd	14.31 ab	38.50 abc	553.81 abc	189.42 bcde	27.10 c	7137.56 abcd
	+M	Zn1	16.12 bcde	4.35 bcde	73.90 def	14.30 ab	33.20 cd	475.06 cd	175.03 gh	28.32 bc	5475.86 efg
	+M	Zn2	17.31 ab	4.41 abcd	96.44 abc	14.46 ab	38.13 abc	543.26 abc	189.02 efg	30.62 abc	7343.93 abc
I <sub>50</sub>	-M	Zn0	13.55 f	4.06 f	54.96 f	14.00 ab	27.70 d	391.40 d	155.61efgh	15.52 ef	4301.41 gh
	-M	Zn1	14.32 ef	4.22 def	61.09 ef	13.51 b	30.13 d	395.83 d	169.75 h	11.22 f	4321.21 gh
	-M	Zn2	14.97 def	4.20 def	65.74 ef	13.91 ab	30.50 d	413.63 d	172.69 gh	13.76 ef	5579.30 ef
	+M	Zn0	14.86 def	4.16 ef	54.89 f	13.55 b	29.75 d	410.60 d	150.94 f	29.43 bc	4000.01 h
	+M	Zn1	15.27 cdef	4.04 f	60.28 ef	14.06 ab	30.30 d	427.18 d	154.58 g	33.95ab	4490.71 fgh
	+M	Zn2	14.34 ef	4.14 ef	58.12 f	13.96 ab	28.16 d	401.73 d	160.38 h	35.48 a	4193.36 h

Mean followed by the same letters in each column are not significantly (Duncan's multiple rang test 5%)

*EL* ear length, *ED* ear diameter, *EKW* ear kernel weight, *KRNE* kernel row / ear, *KNER* kernel number/row, *1000KW* thousand kernel weight, *RC* Root colonization, *GY* grain yield

this experiment also test the combined effects of these nutrients on plant growth under stress. AM fungi are able to enhance plant P uptake, which can contribute to the production of energy in the plant. In addition P can also substantially increase root growth, which can improve plant growth under stress by enhancing water and nutrient uptake (Miransari et al. 2007, 2008, 2009a, b). When conditions are unfavorable to plant growth, for example under high level of drought stress, the host plant may not be able to develop a symbiosis with AM fungi, as the plant prefer to spend its energy to alleviate the stress rather than developing a symbiosis with AM fungi (Miransari et al. 2007, 2008).

Zn is a necessary micronutrient for plant growth as it is involved in the structure of many plant enzymes including the antioxidant enzymes, which can help the plant to alleviate different stresses (for example by maintaining chloroplast and mitochondria membranes) through degrading different unfavorable oxidative radicals, produced under stress. Under drought the production of radical oxygen increases resulting in unfavorable effects on plant growth and production. The radicals of oxygen result in the peroxidation of cell membrane lipid. In addition, properties including the production of osmolytes and membrane stability can determine plant species resistance to drought stress (Moussa and Abdel Aziz 2008). It may be speculated that the alleviating effects of Zn on drought through activating the antioxidant activities have resulted in a situation more ideal for root colonization by AM species. However, the stress by itself is also able to intensify the alleviating effects of AM species on the un-favorable effects of stress on plant growth and crop production (Miransari et al. 2007, 2008).

Zn is necessary for the production and activity of some antioxidants including malondialdehyde and superoxidase dismutase, necessary for the alleviation of drought adverse effects on plant growth (Wang and Jin 2007). Hence, Zn has some impotent roles in plant systematic acquired resistance and can alleviate the effects of different stresses including pathogens on plant growth. This is somehow similar to some of the effects of AM fungi on plant growth under stress as AM fungi with its great abilities are able to enhance plant growth under stress and in the presence of pathogens (Song 2005).

Different researchers have indicated that addition of micronutrients especially Zn can enhance corn grain yield (Brown et al. 1993; Marschner 1993; Yilmaz et al. 1997; Cakmak 2000; Wang and Jin 2007; Wang et al. 2009). Similar to AM fungi, Zn can also influence plant growth through influencing the balance of plant hormones including indole acetic acid (IAA) (Brown et al. 1993; Elwan 2001). Under drought stress, AM fungi are able to decrease abscisic acid concentration and increase IAA and gibber-

ellins concentrations (Liu et al. 2000) and hence alleviate the stress. Zn can also adjust cellular osmotic pressure under severe stress conditions. The synergistic effects of AM fungi and Zn can be attributed to the enhanced uptake of water and nutrients through AM hypha development.

## Conclusion

It can be stated that with respect to the alleviating effects of AM fungi under moderate drought stress on corn grain yield, these fungi can enhance corn water efficiency under drought conditions and hence increase corn yield. In addition, the results indicate the important effects of Zn on corn growth and production under drought. The combined effects of AM fungi and Zn can intensify the alleviating effects of AM fungi on corn growth and production under drought stress.

## References

- Auge RM (2001) Water relations, drought and vesicular-arbuscular mycorrhiza symbiosis. *Mycorrhiza* 11:3–42
- Auge RM, Stodola AJW, Tims JE (2001) Moisture retention properties of a mycorrhizal soil. *Plant Soil* 230:87–97
- Baker DE, Amachar MC (1982) Nickel, copper, zinc and cadmium. In: Page AL, Miller RH, Keeney DR (eds) *Methods of soil analysis, part 2*. American Society of Agronomy, Madison, pp 323–338
- Bray DE (1997) Plant responses to water deficit. *Trends Plant Sci* 2:48–54
- Brown PH, Cakmak I, Zhang Q (1993) Form and function of zinc in plants. In: Robson AD (ed) *Zinc in soil and plants*. pp 93–1.6. Kluwar Academic Publishers
- Cakmak I (2000) Possible roles of Zinc in protecting plant cells from damage by reactive oxygen species. *New Phytol* 2:85–205
- Cheong YH, Kim KN, Pandey GK, Gupta R, Grant JJ, Luan S (2003) CLB1, a calcium sensor that differentially regulates salt, drought, and cold responses in *Arabidopsis*. *Plant Cell* 15:1833–1845
- Daei G, Ardakani MR, Rejali F, Teimuri S, Miransari M (2009) Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. *J Plant Physiol* 166:617–625
- Elwan LM (2001) Effect of soil water regimes and inoculation with mycorrhizae on growth and nutrients content of maize plants. *J Agric Research* 28:163–172
- Feldman F, Idczak E (1992) Inoculum production of vesicular-arbuscular mycorrhizal fungi for use in tropical nurseries. In: Norris JR, Read DJ, Varma AK (eds) *Methods in microbiology, techniques for the study of mycorrhiza*. Academic, New York
- Gee GW, Bauder JW (1986) Particle-size analysis. In: Klute A (ed) *Methods of soil Analysis, Part 1*. 2<sup>nd</sup> ed., Agronomy Monographs, vol. 9. ASA and SSSA, Madison, pp 383–411
- Ghazi AK, Zak BM (2003) Field response of wheat to arbuscular mycorrhizal fungi and drought stress. *Mycorrhiza* 14:263–269
- Giovannetti M, Mosse B (1980) Estimating the percentage of root length colonized (Gridline-Intersect Method). *New Phytol* 84:489–500
- Hopkins WG, Huner NP (2004) *Introduction to plant physiology* (3rd ed). John Wiley & Sons. Inc, New york, 560 p



- Knudsen D, Peterson GA, Pratt PF (1982) Lithium, sodium and potassium. In: Page AL et al (ed) *Methods of soil analysis: part 2. Chemical and microbiological properties*. ASA monograph number 9, p 225–246
- Laffitte HR, Edmeades GO (1995) Stress tolerance in tropical maize is linked to constitutive change in ear growth characteristics. *Crop Science* 3:820–826
- Lam F (2004) Corn production as related to sprinkler irrigation capacity. 16th annual central plains irrigation conference, Kearney, Nebraska, Feb 17–18
- Liu R, Li M, Meng X (2000) Effects of AM fungi on endogenous hormones in corn and cotton plants. *Mycosystem* 19:91–96
- Mahaveer PS, Singh R, Adholeya A (2000) *Laboratory manual for basic techniques in arbuscular mycorrhizal research*. Center for Mycorrhizal Research, Tata Energy Research Institute, New Delhi
- Marschner H (1993) Nutrient dynamics at the soil–root interface (Rhizosphere). In: Read DJ, Lewis DH, Fitter AH, Alexander IJ (eds) *Mycorrhizae in ecosystems*. CAB International Publisher
- Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. *Plant Soil* 159:89–102
- Miransari M, Bahrami HA, Rejali F, Malakuti MJ, Torabi H (2007) Using arbuscular mycorrhiza to reduce the stressful effects of soil compaction on corn (*Zea mays* L.) growth. *Soil Biol Biochem* 39:2014–2026
- Miransari M, Bahrami HA, Rejali F, Malakuti MJ (2008) Using arbuscular mycorrhiza to reduce the stressful effects of soil compaction on wheat (*Triticum aestivum* L.) growth. *Soil Biol Biochem* 40:1197–1206
- Miransari M, Rejali F, Bahrami HA, Malakouti MJ (2009a) Effects of soil compaction and arbuscular mycorrhiza on corn (*Zea mays* L.) nutrient uptake. *Soil Tillage Res* 103:282–290
- Miransari M, Rejali F, Bahrami HA, Malakouti MJ (2009b) Effects of arbuscular mycorrhiza, soil sterilization, and soil compaction on wheat (*Triticum aestivum* L.) nutrients uptake. *Soil Tillage Res* 104:48–55
- Miransari M (2010) Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stresses. Review article. *Plant Biol* 12:563–569
- Moussa HR, Abdel Aziz M (2008) Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. *Aust J Crop Sci* 1:31–36
- Munns R (2002) Comparative physiology of salt and water stress. *Plant Cell Environ* 25:239–250
- Nelson DW, Sommers LE (1973) Determination of total nitrogen in plant material. *Agron J* 65:109–112
- Nelson DW, Sommers LE (1982) Total carbon, organic carbon, and organic matter. In: Page AL, Miller RH, Keeney DR (eds) *Methods of soil analysis, part 2, 2nd edn*. American Society of Agronomy, Madison, pp 539–573
- Olsen RS (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Department of Agriculture. No. 939
- Ramazan-zadeh H, Avestan SH, Reihanitabar A (2007) Effect of some factors on zinc absorption in soil calcareous. In 10th Soil Science Congress. Karaj. Iran
- Read DJ, Koucheki HK, Hodgson J (1976) Vesicular-arbuscular mycorrhiza in natural vegetation systems. *New Phytol* 77:641–653
- Rhoades DJ (1982) Soluble salts. In: Page AL, Miller RH, Keeney DR (eds) *Methods of soil analysis, Part 2, 2nd edn*. American Society of Agronomy, Madison, pp 167–178
- Sas Institute Inc. (1988) SAS/STAT user's guide. Version 6. Fourth edition. Statistical Analysis Institute Inc., Cary North Carolina. 1988
- Song H (2005) Effects of VAM on host plant in condition of drought stress and its mechanisms. *Electron J Biology* 3:44–48
- Steel RGD, Torrie JH (1980) *Principles and procedures of statistics: a biometrical approach*, second edition. McGraw-Hill Book Company
- Vahedi M (1988) Corn plantation, growth and harvest. In Persian, published by the Bureau of Tarvij Keshavarzi
- Wang H, Jin JY (2007) Effects of zinc deficiency and drought on plant growth and metabolism of reactive oxygen species in maize (*Zea mays* L.). *Agric Sci China* 6:988–995
- Wang H, Liu RL, Jin JY (2009) Effects of zinc and soil moisture on photosynthetic rate and chlorophyll fluorescence parameters of maize. *Biol Plant* 53:191–194
- Xiong L, Schumaker K, Zhu JK (2002) Cell signaling during cold, drought, and salt stress. *Plant Cell* 14:165–183
- Yilmaz A, Ekiz H, Torun B, Gultekin I, Karanlik S, Bagei SA, Cakmak I (1997) Effect of different zinc application methods on grain and zinc concentration in wheat cultivares grown on zinc deficient calcareous soils. *J Plant Nutr* 20:461–471