Bio-fertilizer Impact on Production Efficiency and Yield of Corn (*Zea mays*) Cultivars Under Water Deficiency

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Submission: August 12, 2020; Revision: September 11, 2020; September 21, 2020;
Acceptance: September 25, 2020

Abstract

The application of bio-fertilizer (*Glomus mosseae)* and the selection of suitable cultivars are simple solutions to mitigate stress conditions such as water deficiency. This study was conducted in 2016 as a split-plot that was based on randomized complete block design with 3 replications. The effects of irrigation level, bio-fertilizer application and cultivar type on the reproductive efficiency and yield of corn were compared in the field experiment. Irrigation levels (after 70, 110 and 150 mm of pan evaporation) were placed as the main factor in the main plots. Application and non-application of bio-fertilizer (*Glomus mosseae*) as well as cultivar type (cultivars, 640 and 704) were placed in the subplots to study the physiological differences, reproductive efficiency, and yield of corn. This study showed that the cultivars performed differently in their response to water deficiency. The highest grain yield for 704 cultivars was obtained when we applied irrigation after 70 mm evaporation from pan. When subjected to the treatment of irrigation after 110 and 150 mm evaporation from pan, lower grain yield per unit area of 19% and 50.6%, respectively was recorded. The 640 cultivars produced less yield under full irrigation than 704 cultivars. Water deficiency had no beneficial effects on grain yield per unit area ($P > 0.01$). It was also observed that bio-fertilizer treatment increased the corn yield by 25.2 %. Water deficiency, bio-fertilizer and cultivar type affected the grain yield as differences were observed in the main components of kernel row number and 100-kernel weight. Water deficiency had no beneficial effects on 'chlorophyll a' content, but decreased the content of chlorophyll b. Water deficiency and bio-fertilizer application caused an increase in the catalase and peroxidase content. The best plant performance was observed in plants grown under complete bio-fertilizer (704 cultivar) and at after 70 mm irrigation level. Bio-fertilizers can be used in order to improve corn production and also as environmentally friendly fertilizers under deficit irrigation regimes.

Keywords: Bio-fertilizers; corn; water deficiency; cultivar; yield

INTRODUCTION

In recent years, water scarcity has forced many farmers to employ various strategies and conduct many breeding programs to improve drought tolerance. Water deficiency is one of the significant causes of corn's weak performance, and this issue affects the majority of the farming regions around the world. Traditionally, water deficiency is a multi-dimensional stress that affects plants at different levels. Therefore, the physiological response to water deficiency is very complicated and unpredictable. A deficit irrigation system describes exposing crops to a certain level of drought stress by withholding irrigation and/or reducing the amount of irrigation water either during a particular period or throughout the growing season. This system has been successfully employed to maximize water use efficiency and achieve higher yields per unit of irrigation water in different crops (Jahanzad *et al.,* 2013; Afshar *et al.,* 2014). The main impact of water deficiency in corn is

DOI: http://doi.org/10.22146/agritech.58541
ISSN 0216-0455 (Print), ISSN 2527-3825 (Online)

the delay in silk development resulting in an unbalance between pollination and silk development. This resulting unbalance is one of the important factors causing yield reduction (Maazou *et al.,* 2016).

Corn production depends on climate, geographical conditions, irrigation levels, cultivar and application of bio-fertilizers. As a result of the increase in food demand, it is important to determine the effect of bio-fertilizers on quantity and quality of corn. This will help identify the best bio-fertilizers that can be used to increase corn production efficiency. Application of biochemical fertilizers at the beginning of the growing season may result in the conversion of some of its biochemical components to other forms that become unavailable for plants resulting in economic loss. In order to increase the efficiency of nutrients intake, fertilizers must be capable of providing essential nutrients for a long time. Application methods can also have significant effects on plants yield. Applying Mycorrhiza through some fertilizer may have more beneficial effects in comparison to applying it directly to the soil. The encouraging performance of natural and biological fertilizers in crop production and the fact that they have less ecological footprint compared with chemical fertilizers has influenced many studies (Dadrasan *et al.,* 2015). Mycorrhizas are fungi that colonize the roots of crops. This symbiotic elationship is beneficial for both the fungi and the plants. Symbiotic fungi increase the nutrient absorption of host plants and can increase the growth and quality of host plant as well as its resistance to environmental stresses (Oskuie and Cirus, 2015). Studies have reported that bio-fertilizers increase proline, kernel yield, antioxidant activity and the production yield of corn (Chen *et al.,* 2014; Nyaga *et al.,* 2014).

In semi-arid regions, especially regions with limited water availability like Tabriz, in Iran, there is little information about the effect of irrigation, cultivar and fertilization on the growth and yield of corn.The aim of this study was to evaluate the effect of bio-fertilizer application on growth and yield of different cultivars of corn that are grown under different irrigation levels.

Materials and Method

This experiment was conducted in 2016 at the Agriculture station of Islamic Azad University, Malekan-Iran in north-west Iran (37° 24′ N, 46° 17′ E; 1360 m).The split-plot design was used in this experiment, and it was based on completely randomized block design with 3 replications. The first factor, irrigation levels (after 70, 110 and 150 mm of evaporation from evaporation pan), was placed in the main plots. The second factor, bio-fertilizer treatments (no-application and application of bio-fertilizer), and the third factor, cultivars (640 and 704) were placed in the subplots. In early April 2016, when the soil was ready for sowing, superficial ploughing was done to control weeds after the soil was sampled. Land preparation and construction of ridge and furrow were done as well in accordance to land cultivation practices. First irrigation was performed on 29th April 2016. Corn seeds were gotten from Pakan Institute, Isfahan-Iran. On 4th May, 2016, the seeds were sown at depth of 4 cm as dry farming within a row distance of 15 cm on the ridges' water trail, and the distance between rows was 60 cm. To ensure germination, two seeds were planted in each location. The first irrigation was carried out 2 days after sowing and further irrigations were applied subsequently at 5 days intervals. After planting and establishing of plants, thinning and weeding were carried out in the 2-4 leaf stage, weeding was continued until the end of vegetative growth.

For soil analysis, 6-point field samples were taken from depths of 0-30 cm and sent to the laboratory at pre-sowing stage. After analysis, the physical and chemical properties of soil were determined and are shown in Table 1.

Nitrogen

Sampling was conducted at the kernel filling stage, and 0.5 m of each plot was eliminated as marginal effect. At the flowering stage, the upper leaf of a competing plant was selected and separated from the mother plant before being transferred to the laboratory. First, a solution was prepared by mixing liquid glue and white alcohol. A brush is then used to apply a thin layer of the solution to the leaf's underside, and the prepared sample was then dried and transferred onto glass slides using adhesive tape to hold it on. While on each slide, a lens with a magnification of 40 was used to count 6 microscopic field of view by and the average was calculated (Abdallah *et al.,* 2013; Šantrůček *et al.,* 2014).

Table 1. Some of the physicochemical characteristics of the field soil

EC (ds/m)	pH	Saturation (%)	TNV	OC (%)	TN (%)	Absorbable P (%)	Absorbable K (%)	Zn (Mg/kg)	Sand (%)	Silt (%)	Clay (%)	Soil texture
1.42	7.56	47	10.8	1.29	0.12	51.85	20.85	0.84	37	50	13	Silt loam

EC: Electrical conductivity; TNV: Total Neutralizing Value; OC: Organic Carbon; TN: Total Nitrogen.

The final harvest was carried out from an area equivalent to one square meter from the second row of each plot. The kernels were then separated from the corn plant and the kernel weight of the kernels harvested from one square meter was determined. To measure chlorophyll content of leaves at the end of flowering, samples were taken from terminal leaves of competing plants and transferred to the laboratory,1.0 g of plant tissue was pulverized in a porcelain mortar with liquid nitrogen, and 10 mL of 80% acetone was added, it was then centrifuged for 10 minutes at a speed of 6,000 rpm and a high solution absorbance at the wavelengths of 663, 645 and 470 nm, respectively, was set on the spectrophotometer for the measurement of chlorophyll a, chlorophyll b and carotenoid content. Subsequently, chlorophyll a, chlorophyll b, and sample carotenoid content were obtained using the Equation 1 and 2.

$$\text{Chlorophyll a} = \frac{(19.3\times A_{663} - 0.86\times A_{645})/\text{V}}{100\,W} \quad (1)$$

$$\text{Chlorophyll b} = \frac{(19.3\times A_{645} - 3.6\times A_{663})/\text{V}}{100\,W} \quad (2)$$

V is the volume of the centrifuged solution, A is light absorption at mentioned wavelengths, and W is the weight of the fresh sample in grams (Khalil and El-Noemani, 2015).

Measurement of Malondialdehyde

The gas chromatographic HPLC technique was employed to determine the amount of malondialdehyde (MDA). The extract used for the measurement of H-dG0-8 was transferred to an octadecyl silica-gel column based on thiobarbituric acid method with 12 moles of MDA chloroacetic acid. After reaching equilibrium, the column was washed with the mobile phase consisting of a methanol-phosphate buffer. A visible spectrophotometer at a wavelength of 532 was used to detect the MDA peak, and the measurement was based on the area under the curve peak. As a standard, pure MDA with different ratios was drawn in wash buffer and used to prepare the standard curve (Mirzaei *et al.,* 2011).

Measuring Proline Content

Leaf samples were taken at the flowering stage and were crushed using an electric mill to obtain the green extract. The transparent and clear part of the extract was transferred into a test tube. This operation was repeated multiple times. The extract taken from all treatments were centrifuged at 3500 rpm for 10 min at 10 °C. The top centrifuged pure green extract was separated and kept in test tubes with stoppers at 4 °C to determine the free proline content. 1 mL of the separated extract was mixed with 10 mL of doubled-distilled water and then stirred using a shaker. 5 mL of ninhydrin reagent was added to the sample. To prepare the ninhydrin reagent, 0.125 g of ninhydrin, 2 mL of 6 M phosphoric acid and 3 mL of glacial acetic acid were mixed. In order to fully dissolve ninhydrin in phosphoric acid and acetic acid, the mixture was stirred for 16 hours by a magnetic shaker. After adding ninhydrin reagents to each sample, 5 mL of glacial acetic acid was added again. The mixture was thoroughly stirred and put into the boiling water bath for 45 minutes at 100 °C. After cooling, benzene was added to each sample and the samples were shaken vigorously, and then they were allowed to settle for 30 minutes to prevent proline from entering benzene phase. The top phase that contains benzene and proline is separated and its absorption intensity was measured using a spectrophotometer at 515 nm wavelength (Chorfi and Taibi, 2011).

Measuring Carbohydrate Content

To measure the dissolved carbohydrate, an extraction similar to that of proline was performed. After extraction, 0.1 mL of alcoholic extract was mixed with 3 mL of freshly prepared anthrone (150 mg anthrone + 100 mL of 72 % sulfuric acid). The solution was then placed in a boiling water bath for ten minutes to react and become colored. The absorption rate was then measured using a spectrophotometer at 625 nm wavelength, and the sugar content was calculated (Ghobadi *et al.,* 2011).

Plant Extraction for Catalase Activity Assay

0.5 g of fresh leaf tissue, 3 mL of extraction buffer, 50 mM Tris-HCl (pH = 7), containing 3 mM $MgCl_2$, and 1 mM EDTA were pulverized in a cooled mortar. The resulting homogenate was centrifuged at 5,000 rpm for 20 min at 4 °C, using a refrigerated centrifuge of model 'Vision VS-15000 CFN'. The supernatant was then used to measure the activities of catalase and peroxidase (Wu, 2011).

Guaiacol Peroxidase Enzyme Activity Assay (GPX, EC 1.11.1.7)

To measure Guaiacol peroxidase (GPX) activity, the reaction mixture is 2.5 mL of 50 mM phosphate buffer (pH=7) containing 1 mL of 1 % guaiacol, 1 mL of 1 % H_2O_2 and 0.1 mL of the extract. GPX activity based on the rate of absorption increase per minute was calculated using a spectrophotometer (model UV / VIS Lambda25) set at 420 nm wavelength. To assay the activity, the Equation 3 and the extinction coefficient (26/6 mM^{-1} cm^{-1}) were used.

$$\text{Units (Mm/min)} = \frac{\frac{\text{doD}}{\text{min (slop)}} * \text{Vol. of assay} * (0.0001)}{\text{Extinction cofficient} * (26.6)} \quad (3)$$

Where, extinction coefficient is 26.6 mM^{-1} cm^{-1}, doD is the difference between the highest and lowest numbers, and vol. of the assay is the absorption time during the test.

Catalase enzyme activity assay (CAT, EC 1.11.1.6)

The reaction mixture is 2.5 mL of 50 mM phosphate buffer (pH=7) containing 0.2 mL of 1% H_2O_2 and 0.3 mL of the extract. Catalase (CAT) activity based on the rate of absorption decrease per minute was calculated using a spectrophotometer (model UV / VIS Lambda25) set at 240 nm wavelength. To assay, the activity, the following formula and the extinction coefficient (0.0436 mM^{-1} cm^{-1}) were used.

$$\text{Units (Mm/min)} = \frac{\frac{\text{doD}}{\text{min (slop)}} * Vol.\ of\ assay\ (0.0003)}{\text{Extinction cofficient (0.0436)}} \quad (4)$$

Before starting the statistical analysis, the data was tested for normality; then, the statistical analysis was performed using SAS. LSD and Duncan's tests at a 5% probability level were used to compare means. The required figures were created using Microsoft Excel software.

Results and Discussion

The results showed that the effects of the different dehydration levels, cultivar types and application of * Mycorrhiza *on proline of corn were significant at 5% level. Significant differences were observed between the effects of dehydration and cultivar type on stem dry weight and chlorophyll b content at a 1% statistical level. Similarly, they were also significant for catalase content and grain yield at a 5 % level. The two effects of Mycorrhiza application and cultivar type on the number of stomata below the leaf area were significant at 1 %. The combined impact of Mycorrhiza application and dehydration on the number of stomata at the leaf surface was also significant at a 5% probability level (Table 2).

Shoot Dry Weight

In this study, the highest shoot dry weight (139.5 g) was obtained for cultivar 640 when irrigation after 70 mm evaporation from pan was applied. The lowest weight (88.1 g) was obtained from using irrigation after 150 mm evaporation from pan for cultivar 704 (Table 3). In general, susceptibility to water deficiency varied among cultivars, evident in their shoot dry weight. Cultivar 640 was more susceptible to water deficiency in terms of shoot dry weight than cultivar 704 (Table 4). Madani *et al.* (2010) reported that limited availability of resources like water would result in resource restrictions such as reducing the current photosynthesis rate; therefore, the dry matter accumulation in various plant parts decreases. Anjum et al (2011) stated that multiple mechanisms of root growth could be enhanced by the application of mycorrhiza. Studies show that mycorrhizal

Table 2. Analysis of variance for the characteristics of corn

Source of variance	Df	Shoot dry weight	The number of stomata on the lower surface of leaves	The number of stomata on the upper surface of the leaves	Chlorophyll b content	Carbohydrate content	Protein content
Replication	2	507.194 ^{ns}	35.361 ^{ns}	3.444 ^{ns}	1.734 ^{ns}	2.333 ^{ns}	1.028 ^{ns}
Water deficiency	2	5009.694*	298.778 ^{ns}	144.444**	15.170*	120.583*	68.528*
main error	4	463.611	54.403	6.528	1.007	7.792	4.444
Mycorrhiza	1	992.250**	28.444 ^{ns}	72.250**	0.234 ^{ns}	156.250**	28.444**
Water deficiency*Mycorrhiza	2	36.75 ^{ns}	32.704 ^{ns}	19.000*	0.289 ^{ns}	33.083 ^{ns}	0.861 ^{ns}
Cultivar	1	140.028 ^{ns}	5.444 ^{ns}	0.028 ^{ns}	1.247 ^{ns}	1.361 ^{ns}	0.444 ^{ns}
Water deficiency*cultivar	2	675.694**	3.444 ^{ns}	5.444 ^{ns}	3.217**	4.528 ^{ns}	0.861 ^{ns}
Mycorrhiza*cultivar	1	318.028 ^{ns}	196.000**	3.361 ^{ns}	0.047 ^{ns}	4.694 ^{ns}	4 ^{ns}
Water deficiency *Mycorrhiza*cultivar	2	201.194 ^{ns}	16.333 ^{ns}	10.704 ^{ns}	1.742 ^{ns}	9.028 ^{ns}	1.083 ^{ns}
Secondary error	18	78.509	16.019	4.278	0.517	13.454	2.972
The coefficient of Variance (percent)		7.84	9.75	8.17	12.58	4.82	20.15

Continue Table 2. Analysis of variance for the corn characteristics

Source of variance	Df	Malondialdehyde content	The amount of catalase	Peroxidase	Proline	100-kernel weight	Grain yield
Replication	2	1.194 ns	9.194 ns	7.694 ns	0.394 ns	1.861 ns	93.361 ns
Water deficiency	2	99.528*	152.861**	330.361**	2.787 ns	54.361*	8229.861**
main error	4	12.028	4.319	16.444	0.51	6.903	93.704
Mycorrhiza	1	58.778 ns	160.444**	400.000*	0.704 ns	21.778**	5353.361**
Water deficiency* Mycorrhiza	2	13.194 ns	5.361 ns	0.083 ns	0.22 ns	1.361 ns	143.028 ns
Cultivar	1	9 ns	0.704 ns	121 ns	0.704 ns	13.444*	2320.028*
Water deficiency* cultivar	2	4.083 ns	37.194*	108.583 ns	0.344 ns	2.528 ns	2163.361*
Mycorrhiza* cultivar	1	5.444 ns	16 ns	49 ns	0.028 ns	0.444 ns	84.028 ns
Water deficiency* Mycorrhiza* cultivar	2	35.194 ns	7.583 ns	15.083 ns	1.047*	0.861 ns	1011.861 ns
Secondary error	18	20.269	7.537	51.639	0.18	2.407	428.157
The coefficient of Variance (percent)		17.06	12.51	20.21	10.53	6.63	18.93

** and * denote significance at probability level of five percent.

fungi increase root length, the number of roots and root thickness (Anjum *et al.,* 2011). Similarly, Parniske (2008) also reported that the application of mycorrhizal fertilizers increases root number and root length in corn plants.

The Number of Stomata on The Lower Surface of Leaves

The highest number of stomata on the lower surface of the leaf was obtained with the application of mycorrhiza fertilizer and in cultivar 640. Application of mycorrhiza fertilizer caused a 15.7 percent increase in the number of stomata in the leaves of this cultivar (Table 4). Various studies have reported the effect of an increase in the number of mycorrhiza fungi on the stomata of plants. Abdallah *et al.* (2013) investigated the impact of mycorrhiza fungi on physiological characteristics of sunflowers and found that the use of mycorrhiza bio-fertilizer increases the number of stomata on the leaf surface in sunflower.

The Number of Stomata on The Upper Surface of The Leaves

The highest number of stomata on the upper surface of the leaves with a number of 30.8 was achieved at the irrigation treatment after 70 mm evaporation and mycorrhiza application. Whereas the lowest number with 22.6 was reached at irrigation after 150 mm

Table 3. Interactive effects of irrigation and cultivars on study traits

Irrigation surfaces (mm evaporation)	Cultivar	Dry weight per shoot (g)	B (mg per g on the wet weight)	The amount of catalase (absorption per mg protein)	Grain yield per unit area (g)
70	704	120.8 bc	6.100 b	17.67 c	152.8 a
70	640	139.5 a	7.467 a	18.00 c	108.0 bc
110	704	124.2 b	6.167 b	26.17 a	123.3 b
110	640	112.8 c	5.467 bc	22.33 b	704.7 b
150	704	88.17 d	4.317 d	22.17 b	75.83 d
150	640	92.67 d	4.767 cd	25.33 ab	84.17 cd

Superscripts (a-d) show significant differences in each column (p<0.05).

Table 4. Interactive effects of mycorrhiza and cultivars on study traits

Mycorrhiza	Cultivar	The number of stomata on the lower surface of leaves
Non-applicable	704	42.11 [ab]
Non-applicable	640	38.22 [b]
Applicable	704	39.22 [b]
Applicable	640	44.6 [a]

Superscripts (a-b) show significant differences in each column (p<0.05).

evaporation and when mycorrhiza was not applied. The application of mycorrhiza under complete irrigation conditions undoubtedly increases the number of stomata on corn leaves' surface. However, the increase in the number of stomata at the upper surface of the leaf and the sensitivity was higher in water shortage conditions. That can have a positive impact on the reduction of evaporation from the leaf surface (Table 5). The number of stomata in each plant is a genetic trait, but they usually interact with environmental and nutritional factors. Studies have shown that hormonal changes affect the number of stomata (Šantrůček *et al.,* 2014). Likewise, some studies have reported that mycorrhiza fungi and phosphate fertilizer increased levels of the hormone in plants (Sivagurunathan, 2014).

Chlorophyll b

In this study, unlike the amount of chlorophyll a, chlorophyll b was not affected by mycorrhiza application; however, the irrigation levels and the cultivar had a significant impact on the content of chlorophyll b (Table 6). The comparison of chlorophyll b under the influence of irrigation levels and cultivar showed that the highest content of chlorophyll b was obtained in irrigation after 70 mm evaporation by cultivar 640. Under full irrigation, cultivar 640, in comparison with 704 cultivars, showed more content of chlorophyll b, but under drought conditions, no significant difference was observed in chlorophyll b content (Table 3). Robinson *et al.* (2014) also showed a significant increase of chlorophyll b in sesame plants using mycorrhiza bio-fertilizer. Despite the fact that under the irrigation condition, there was more amount of chlorophyll b in cultivar 640 compared to cultivar 704, cultivar 640, however, showed a higher sensibility to drought when compared to cultivar 704. Both levels of water shortage irrigation after 110 and 150 mm evaporation caused a significant reduction of 27 and 36.4 percent in the content of chlorophyll b in cultivar 640. However, in cultivar 704, water shortage after irrigation of 150 mm evaporation was the only one with a significant chlorophyll b reduction (29.5 %).

Carbohydrate Content

The present study showed that carbohydrate content was affected by irrigation after 110 and 150 mm evaporation. A decrease of 5 and 8 percent, respectively, was observed (Table 6). Initially, during the grain filling stage, the primary compounds stored in the grain are protein and then later, carbohydrate compounds become stored in the grain as well. Therefore, since grain filling will be shortened due to the water deficit, accumulated carbohydrate percentage will be lower while protein percentage will be higher (Ghobadi *et al.,* 2011). Similarly, Ghobadi *et al.* (2011) found a significant reduction in carbohydrate content of grains under the effect of water shortage. In this study,

Table 5. Interactive effects of mycorrhiza and irrigation on study traits

Irrigation surfaces (mm evaporation)	Mycorrhizal	The number of stomata on the leaf surface
70	Non-applicable	25.33 [b]
70	Applicable	30.83 [a]
110	Non-applicable	26.17 [b]
110	Applicable	26.67 [b]
150	Non-applicable	20.17 [d]
150	Applicable	22.67 [c]

Superscripts (a-d) show significant differences in each column (p<0.05).

Table 6. Effects of irrigation on study traits

Irrigation surface (evaporation mm)	Carbohydrate content (%)	Protein content (%)	Malondialdehyde	Peroxidase (absorbed per mg protein)	Hundredweight (g)
70	79.33 [a]	6.333 [b]	23.42 [b]	29.50 [b]	25.08 [a]
110	75.92 [b]	8.250 [b]	26.58 [ab]	38.42 [a]	24.08 [a]
150	73.00 [b]	11.08 [a]	29.17 [a]	38.75 [a]	21.00 [b]

Superscripts (a-c) show significant differences in each column (p<0.05).

the application of mycorrhiza significantly increased the carbohydrate content of corn.

Protein Content

By reducing the amount of irrigation water from irrigation after 70 mm evaporation to after 150 mm evaporation, the protein content increased up to 74.7 percent (Table 6). An inverse relationship between the amount of carbohydrates and the amount of protein in the grain is observed; that is, the amount of protein reduces as that of carbohydrates increases. Ghobadi *et al.* (2011) showed that the protein content of corn grain increases as the tension percent increases. So, the least amount of protein was obtained under full irrigation, and the highest amount of protein was obtained under severe drought tension. However, the percentage of protein content in grain under mild and severe moisture stress conditions did not show any significant difference. Water shortage tension does not decrease the remobilization of nitrogen from leaves to grains, increasing the protein content of the grain. The application of mycorrhiza had a significant effect on the protein content of corn grains. The protein content when mycorrhiza was applied is 9.4 percent, while under no-application, the protein content is 22 % more.

Malondialdehyde Content

In this study, reduction in the water available from irrigation after 70 mm evaporation to irrigation after 150 mm evaporation resulted in 24.3 % malondialdehyde (Table 6). Valentovic *et al.* (2006), in a study on corn, observed that water shortage leads to the increase of electrolyte leakage because the cell membrane stability decreases. Water shortage increases the production of active forms of oxygen, allowing the cell membranes to be attacked and thus generating malondialdehyde. The amount of malondialdehyde is an index of membrane damage. Anjum et al (2011) stated that the damage to cell membranes causes an increase in electrolyte leakage rate.

Catalase Content

According to the results of this study, the highest amount of catalase in irrigation treatment was obtained after 110 mm evaporation and cultivar 704. The lowest levels were obtained in irrigation after 70 mm evaporation and in both cultivars 704 and 640. For cultivar 704, both irrigation treatments after 110 and 150 mm evaporation caused a 48.2 and 25.5 percent increase in the catalase content of corn leaves, respectively. Similarly, for cultivar 640, both irrigation treatments after 110 and 150 mm evaporation caused a respective 23.8 and 40.5 % increase in the catalase of corn leaves (Table 3). Moussa and Abdel-Aziz (2008) showed that water shortage could cause an increase the amount of antioxidants in corns. In this study, the application of mycorrhiza had a significant increase in the catalase content of corn leaves. In a similar study, Wu (2011) also showed that the use of mycorrhiza significantly increased the catalase content.

Peroxidase

It was observed that water shortage caused a significant increase in the peroxidase content of corn leaves. Both irrigations after 110 and 150 mm evaporation significantly increased the peroxidase content of corn leaf by 31 %. No significant difference was observed in peroxidase content between irrigation treatments after 110 and 150 mm evaporation (Table 6). Li-Ping *et al.* (2006) observed similar results in corn, he noted an increase in peroxidase content in leaves of corn due to the water shortage impact. Wu (2011) also showed that the use of mycorrhiza significantly increased the peroxidase content of corn leaves.

Proline Content

The highest content of proline in irrigation treatment was achieved after 110 mm evaporation and the application of mycorrhiza in cultivar 704. The lowest Proline content was also related to irrigation after 70 mm evaporation and no-application of mycorrhiza in cultivar 640. According to the results, in irrigation after

Table 7. Comparison of the averages of the traits affected by irrigation surfaces and mycorrhizal application in corn cultivars

Irrigation surfaces (mm evaporation)	Mycorrhizal treatment	Cultivar	Proline
70	Non-applicable	704	3.467 ^{cd}
70	Non-applicable	640	3.167 ^{d}
70	Applicable	704	3.467 ^{cd}
70	Applicable	640	3.867 $^{b-d}$
110	Non-applicable	704	3.967 $^{b-d}$
110	Non-applicable	640	4.200 $^{a-c}$
110	Applicable	704	4.867 ^{a}
110	Applicable	640	3.633 ^{cd}
150	Non-applicable	704	4.567 ^{ab}
150	Non-applicable	640	4.467 ^{ab}
150	Applicable	704	4.167 $^{a-c}$
150	Applicable	640	4.500 ^{ab}

Superscripts (a-d) show significant differences in each column (p<0.05).

110 mm evaporation and cultivar 704, the application of mycorrhiza caused a significant increase of 23 % in the proline content of the leaves of corn. However, for other irrigation levels, mycorrhiza had no significant impact on the proline content of corn leaves. Also, in a condition of no-application of mycorrhiza, irrigation after 150 mm evaporation of both cultivars 704 and 640 resulted in a respective 32.3 and 41.9 % reduction in proline content of corn leaves (Table 7). Studies have shown that proline increases under water shortage conditions, which plays an important role in osmotic adjustment (Chorfi and Taibi, 2011).

100-kernel Weight

According to this study's findings, irrigation after 150 mm of evaporation from the evaporation pan resulted in a 16 % loss of the 100-kernel weight of corn compared with that of irrigation after 70 mm of evaporation from the evaporation pan (Table 6). Kernel weight in plants is decided by two factors: the kernel filling rate and the duration of kernel filling. Water deficiency negatively affects both factors that determine the kernel weight. Water deficiency reduces the kernel filling rate by increasing the viscosity of phloem sap and reducing the rate of photosynthesis. It also reduces the filling duration, as reported by Lisanti *et al* (2013). Khoshvaghti *et al.* (2014) observed a 27 % loss of 100-kernel weight of corn after imposing water deficiency. Significant differences were observed between the cultivars in terms of the kernel weight. Farnia and Khodabandeloo (2015) reported that the application of mycorrhiza leads to a significant increase in the kernel number of corn. In the case of a limited number of assimilates, increasing the number of kernels leads to the reduction of assimilate supply per kernel, resulting in kernel weight reduction (Krupnova, 2010).

Grain Yield

The highest corn grain yield per unit area was obtained in irrigation after 70 mm evaporation in cultivar 704, and the least was obtained in irrigation after 150 mm evaporation in cultivar 704 likewise. When the irrigation levels was decreased from after 70 mm evaporation to irrigation after 150 mm evaporation, cultivar 704's grain yield decreased severely when comparison with yield in irrigation after 110 mm evaporation. Furthermore, cultivar 640 had a lower yield compared to the 704 cultivars under the specific conditions of irrigation after 70 mm evaporation. No significant difference was observed in grain yield under conditions of irrigation after 70 mm evaporation and irrigation after 150 mm evaporation. However, under irrigation after 150 mm evaporation, corn grain yield in cultivar 640 was 64.3% lesser when compared with irrigation after 110 mm evaporation. In general, it was observed that under a full irrigation system, the grain yield of cultivar 640 in comparison with 704 was 28.9% lower. Similarly, in drought conditions, there was no significant difference in

grain yield between the cultivars (Table 3). Mohammadai *et al.* (2012) studied the effect of irrigation on corn and reported that with decreasing levels of irrigation from after 130 mm evaporation to after 70 mm evaporation, corn grain weight decreased by 54%. Khoshvaghti *et al.* (2014), in a study of the impact of water shortage on corn, also found out that the decrease of water in irrigation from after 50 mm evaporation to after 90 mm evaporation caused a 25.8 % reduction in the corn grain weight. Water shortage in the flowering stage reduces the number of grains; the significant reasons behind this are the decrease of assimilates for corn growth and increase in the floret sterility (Anjum *et al.,* 2011). In other similar studies, Farnia and Khodabandehloo (2015) also obtained a significant increase in corn grain yield as a result of mycorrhiza fertilizers application.

CONCLUSION

According to the results, it can be stated that all three factors of water deficiency, mycorrhiza application and cultivar caused a significant change in grain yield by changing both the main components of grain number and the hundred-kernel weight. All three factors of water deficiency, mycorrhiza application and cultivars could therefore be related to grain yield number and 100-kernel weight. Due to the lack of significant differences between yield in cultivars under water deficient conditions and results showing that cultivar 704 produces the highest yield, planting cultivar 704 and Mycorrhizal treatment is therefore recommended in the studied area.

ACKNOWLEDGMENTS

We thank the Faculty of Agriculture, Malekan Branch, Islamic Azad University, Malekan, Iran for funding this research.

CONFLICT OF INTEREST

The author states that this article is original research that has not been published in another Journal and that there is no conflict of interest.

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