



ORIGINAL ARTICLE

## Response of Water Use Efficiency to Mycorrhizal Biofertilizer in Maize under Water Stress Conditions

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### ABSTRACT

Optimal exploitation of water resources plays an important role in agricultural sector. In order to achieve this object two field experiments were conducted in 2011 and 2012 (June 7<sup>th</sup>). The experiments were carried out as split-plot factorial based on randomized complete block design with three replications. Irrigation was imposed at three levels based on 70, 50 and 30% field capacity. Mycorrhizal biofertilizer was applied at two levels; control and 100 kg ha<sup>-1</sup>. Phosphorus fertilizer was applied at three levels; 0, 75 and 150 kg ha<sup>-1</sup> triple superphosphate. The results of combined variance analysis showed that different irrigation treatments, different P fertilizer levels and mycorrhizal biofertilizer significantly affected water use efficiency of grain yield (WUE<sub>GY</sub>) and water use efficiency of biological yield (WUE<sub>BY</sub>). Mycorrhizal biofertilizer application led to improved WUE<sub>GY</sub> and WUE<sub>BY</sub> as much as 4.2 and 7.9% respectively. Measured traits as affected by different irrigation regimes were decreased by increasing drought stress. Water use efficiency in AM plants had priority than non AM plants, as a consequence of enhancing nutrient uptake, extension of the root system and water status of the host plants.

Keywords: Maize, Mycorrhiza, Water stress, Water use efficiency

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### INTRODUCTION

Water deficit and drought are the most serious obstacles to agricultural development [1]. Higher yield may be achieved through improvements in water use, water use efficiency and harvest index [2]. Water use efficiency refers to the amount of losing water during the production of biomass or the CO<sub>2</sub> fixation in photosynthesis [3]. Irrigation management at the field scale can improve water use efficiency. Decrease irrigation is one strategy for maximizing WUE [4]. Arbuscular mycorrhizal symbiosis can protect host plants against detrimental effects of water scarcity [5] through direct uptake and transfer of water by the fungal hyphae to the host plant [6], retention properties through changes in soil water [7] and better osmotic adjustment [8]. Sharif and Claassen [9] concluded that the application of P increased shoot dry matter yield of *Capsicum annuum* L. the treatment of AM inoculation increased the shoot yield and shoot P content. Cozzolino *et al.* [10] reported that the leaf and root dry weight significantly increased with AMF inoculation and P application. When both factors were combined, the yield was 57.6% higher compared to non-inoculated plants. No significant differences were observed between inoculated and non-inoculated plants in biomass production, when P was not added. Kohler *et al.* [11] concluded that the shoot fresh biomass of inoculated plants was about 34% higher than that of non-inoculated plants. Water deficit caused a significant decrease in the shoot fresh and dry biomass and shoot water content of all plants. Shoot dry biomass and mycorrhizal colonization were decreased significantly under water-stress conditions. Ruiz-Sanchez *et al.* [12] reported that AM colonization increased rice shoot biomass by 50%, and this effect was also attributed to enhancement of rice photosynthetic efficiency. Ruiz-Sanchez *et al.* [13] reported that AM and non-AM plants were remarkably different in plant size. Erman *et al.* [14] conducted an experiment on chickpea and observed that AMF inoculation resulted in increased plant growth and nutritional parameters. Efeoglu *et al.* [15] conducted an experiment on maize under drought conditions and observed that maize cultivars exposed to drought had a lower fresh and dry biomass than

their controls due to a significant drought-induced reduction in growth. Fresh biomass of cultivars was significantly reduced under drought stress conditions. In addition, dry biomass was significantly decreased under drought stress. Celebi *et al.* [16] conducted an experiment on maize and reported that the effect of different irrigation levels and AMF applications on the plant height. The aim of this study was to assess the effects of mycorrhizal biofertilizer and drought stress on water use efficiency to apply optimal exploitation of water resources.

## MATERIALS AND METHODS

### Site of experiment

The experiments were conducted at the Agricultural Research Station in Khorramabad, Iran in 2011 and 2012 (June 7<sup>th</sup>), with Lat. 33°, 29' N; Long. 48°, 21' E; Alt. 1171 m above sea level; mean temperature during the growth season in the first and second year were 24.90°C and 25.92°C, respectively.

### Experimental design and agronomic applications

Two experiments were carried out as split-plot factorial based on randomized complete block design with three replications. Irrigation was imposed at three levels; (a) well-watered conditions (I<sub>1</sub>), based on 70% field capacity; (b) moderate drought stress conditions (I<sub>2</sub>), based on 50% field capacity; (c) severe drought stress conditions (I<sub>3</sub>), based on 30% field capacity, as the main plot. Mycorrhizal biofertilizer (species *Glomus intraradices*) was applied at two levels; (a) control or without application of mycorrhizal biofertilizer (M<sub>1</sub>); (b) application of mycorrhizal biofertilizer (M<sub>2</sub>) 100 kg ha<sup>-1</sup>, as the sub plot. Phosphorus fertilizer was applied at three levels; (a) control (P<sub>1</sub>); without application of phosphorus fertilizer; (b) application of 75 kg ha<sup>-1</sup> triple superphosphate (P<sub>2</sub>); (c) application of 150 kg ha<sup>-1</sup> triple superphosphate (P<sub>3</sub>), as the sub plot (values were used according to the soil testing).

The experimental field was ploughed in fall and disked twice in spring. Each plot was 8 m in length and consisted of 4 rows separated by 0.75 m, with 0.20 m on-row spacing [17]. The studied hybrid was NS-640. According to the soil testing (Table 1), nitrogen and potassium fertilizers were determined, including 250 kg ha<sup>-1</sup> urea and 100 kg ha<sup>-1</sup> potassium sulfate. One third of nitrogen (N), all of mycorrhizal biofertilizer, phosphorous (P) and potassium (K) fertilizers were applied at planting and the remaining N was applied during the vegetative growth [18]. Farm operations for two years were the same.

Table 1- Chemical characteristics of the soil in the experimental site

Year	Depth (cm)	EC × 10 <sup>3</sup>	pH	T. N. V	O. C	P (av.) (mg kg <sup>-1</sup> )	K (av.) (mg kg <sup>-1</sup> )
2011	0-30	0.55	7.48	32.2	1.13	3.5	455
	30-60	0.67	7.70	35.0	0.95	2.2	340
2012	0-30	0.50	7.40	33.6	1.20	3.2	500
	30-60	0.62	7.40	35.2	0.85	2.5	370

### Soil water content measurement

Soil water content was measured by weighting the soil before and after drying at 105°C for 24 h. Moisture weight percentage was calculated by using the following equation proposed by Kirkham [19].

$$\theta m = \frac{W_1 - W_2}{W_2} \times 100$$

where  $\theta m$ ,  $W_1$  and  $W_2$  are water content (moisture content) percentage, soil wet weight (g) and soil dry weight (g) respectively. Samples were collected from the 0 – 30 and 30 – 60 cm depths. The soil texture was clay loam. Bulk density was 1.35 g cm<sup>-3</sup>. Moisture weight percentage in field capacity was 26.5 and 24.2 in 2011 and 2012 respectively. The soil pH was 7.5.

Irrigation time was determined by weighting soil samples (taken by Auger from the root extension depth) to obtain moisture weight percentage. Then by using the following equation proposed by Doorenbos and Pruitt [20] irrigation water volume was calculated.

$$V = \frac{(FC - \beta m) \times \rho b \times Dr \times A}{100}$$

where  $V$  is the irrigation water volume (m<sup>3</sup>),  $FC$  is the gravimetric soil water content at field capacity (%),  $\beta m$ , is the soil water content before irrigation by weight (%),  $\rho b$  is the bulk density of the soil (g cm<sup>-3</sup>),  $Dr$  is the root extension depth (m),  $A$  is the irrigated area (m<sup>2</sup>).

Table 2-Number of irrigation and irrigation water volume in 2011 and 2012

	2011		2012	
	Number of irrigation	Water volume (m <sup>3</sup> )	Number of irrigation	Water volume (m <sup>3</sup> )
Well-watered	19	7050	22	7580
Moderate stress	13	6560	15	6930
Severe stress	10	5590	12	6280

**Water use efficiency measurement**

Water use efficiency was estimated by the following equation[21]:

$$WUE = \frac{Y}{ET}$$

Where *WUE* is the water use efficiency (kg m<sup>-3</sup>), *Y* is the biological/grain yield (kg ha<sup>-1</sup>) and *ET* is the evapotranspiration (mm).

The crop evapotranspiration for the irrigation intervals was estimated by the water balance procedure using the following equation[22]:

$$ET = I + P - D \pm \Delta s$$

Where *I* is the irrigation amount (mm), *P* is the precipitation (mm), *D* is the deep percolation (mm) and  $\Delta s$  is the change of soil water depth between two irrigations in root zone.

**Statistical analysis**

The recorded data were statistically analyzed using the software MSTAT-C. Mean comparisons were calculated using Duncan's Multiple Range Test at  $P \leq 0.05$ .

**RESULTS**

The results of combined variance analysis showed that different irrigation treatments, different P fertilizer levels and mycorrhizal biofertilizer application significantly affected water use efficiency of grain yield and water use efficiency of biological yield (Table 3). The results of mean comparisons showed that the highest  $WUE_{GY}$  (1.270 kg m<sup>-3</sup>) and  $WUE_{BY}$  (3.354 kg m<sup>-3</sup>) were found under well-watered conditions. The lowest  $WUE_{GY}$  (0.829 kg m<sup>-3</sup>) and  $WUE_{BY}$  (2.434 kg m<sup>-3</sup>) were observed under severe drought stress conditions. The highest  $WUE_{GY}$  (1.120 kg m<sup>-3</sup>) and  $WUE_{BY}$  (3.070 kg m<sup>-3</sup>) was relevant to application of 150 kg ha<sup>-1</sup> triple superphosphate. The lowest  $WUE_{GY}$  (1.050 kg m<sup>-3</sup>) and  $WUE_{BY}$  (2.779 kg m<sup>-3</sup>) were found in relation to without application of phosphorus fertilizer (Table 4). Mycorrhizal biofertilizer application led to improved water use efficiency of grain yield and water use efficiency of biological yield, as much as 4.2 and 7.9% respectively.

Table 3-Combined analysis of variance (mean squares) for grain yield (GY), biological yield (BY) water use efficiency of grain yield ( $WUE_{GY}$ ) and water use efficiency of biological yield ( $WUE_{BY}$ )

S. O. V	df	MS			
		GY	BY	$WUE_{GY}$	$WUE_{BY}$
Year (Y)	1	33.376*	49.176	174.008**	521.291*
R(Y)	4	12.202	80.164	28.067	163.583
Irrigation (I)	2	179.632**	928.313**	187.770**	763.051**
Y×I	2	0.380 <sup>ns</sup>	5.909 <sup>ns</sup>	3.406 <sup>ns</sup>	37.594 <sup>ns</sup>
Error (a)	8	1.492	18.074	2.685	38.572
Phosphorus (P)	2	2.021**	38.290**	4.374**	81.951**
Y×P	2	0.083 <sup>ns</sup>	8.423 <sup>ns</sup>	0.161 <sup>ns</sup>	21.879 <sup>ns</sup>
I×P	4	0.282 <sup>ns</sup>	6.401 <sup>ns</sup>	0.549 <sup>ns</sup>	11.245 <sup>ns</sup>
Y×I×P	4	0.276 <sup>ns</sup>	2.140 <sup>ns</sup>	0.561 <sup>ns</sup>	5.622 <sup>ns</sup>
Mycorrhiza (M)	1	2.217*	72.696**	5.935**	155.969**
Y×M	1	0.080 <sup>ns</sup>	9.245 <sup>ns</sup>	0.131 <sup>ns</sup>	21.979 <sup>ns</sup>
I×M	2	0.191 <sup>ns</sup>	13.450 <sup>ns</sup>	0.324 <sup>ns</sup>	23.120 <sup>ns</sup>
Y×I×M	2	0.172 <sup>ns</sup>	8.394 <sup>ns</sup>	0.395 <sup>ns</sup>	16.856 <sup>ns</sup>
P×M	2	0.022 <sup>ns</sup>	0.152 <sup>ns</sup>	0.033 <sup>ns</sup>	0.276 <sup>ns</sup>
Y×P×M	2	0.122 <sup>ns</sup>	0.189 <sup>ns</sup>	0.226 <sup>ns</sup>	0.207 <sup>ns</sup>
I×P×M	4	0.083 <sup>ns</sup>	1.739 <sup>ns</sup>	0.160 <sup>ns</sup>	4.325 <sup>ns</sup>
Y×I×P×M	4	0.092 <sup>ns</sup>	1.001 <sup>ns</sup>	0.188 <sup>ns</sup>	2.527 <sup>ns</sup>
Error (b)	60	0.242	4.659	0.492	9.976 <sup>ns</sup>
C. V %	-	6.75	11.07	6.47	10.88

\*\* : Significant at  $P \leq 0.01$ , \* : Significant at  $P \leq 0.05$  and ns: Non- significant

## DISCUSSION

AM fungal are known to be effective in increasing nutrient uptake, particularly phosphorus. They do this through two important mechanisms. First, they are known to produce phosphatase enzymes that cleave ester bonds that bind P to C in organic matter, thereby releasing phosphate that can be taken up by the fungi and passed on to the plant. Second, they produce low molecular weight organic acids, such as oxalates, which enhance the availability of soil P by increasing weathering rates of P contained in clay minerals [23]. Arbuscular mycorrhiza may also promote P uptake by increasing its solubility in soil through pH changes or by exudation of P mobilizing compounds like organic acids and phosphatases [24]. Generally, enhanced plant P nutrition is still a major outcome of the AM symbiosis [25]. Phosphorus plays an important role in energy storage and transfer in crop plants [26]. Limitation of phosphorus led to decrease in ribulose biphosphate (RuBP) regeneration [27]. Briefly, phosphorus has an important effect on photosynthesis. Smith and Read [28] stated that AM symbiosis increases host plant growth as a result of improved plant nutrition. The mycorrhizal fungi and plant roots symbiosis can promote plant uptake water and improve plant growth [29]. Also, they are beneficial to improve the soil structure and aggregate stability [30]. Thus, improved soil aggregation can be expected to increase absorption of water by plants, which can also enhance plant growth [11]. Therefore, the increase of 4.2 and 7.9% water use efficiency of grain yield and water use efficiency of biological yield in this study associated with AM symbiosis can be due to nutrient and water uptake. The results of the present study are in agreement with the conclusions of Auge *et al.* [6] and Ruiz-Lozano *et al.* [5] in relation to water uptake, Cozzolino *et al.* [10], Sharif and Claassen [9] associated with nutrient uptake, Efeoglu *et al.* [15], Kohler *et al.* [11], Celebiet *et al.* [16], Erman *et al.* [14], Ruiz-Sanchez *et al.* [13] for the role of Arbuscular mycorrhizal symbiosis to plant growth.

Table 4-Mean comparisons of grain yield (GY), biological yield (BY) water use efficiency of grain yield (WUE<sub>GY</sub>) and water use efficiency of biological yield (WUE<sub>BY</sub>)

Factor	GY (kg m <sup>-2</sup> )	BY (kg m <sup>-2</sup> )	WUE <sub>GY</sub> (kg m <sup>-3</sup> )	WUE <sub>BY</sub> (kg m <sup>-3</sup> )
<b>Irrigation (I)</b>				
I <sub>1</sub>	0.926 a	2.448 a	1.270 a	3.354 a
I <sub>2</sub>	0.776 b	1.966 b	1.153 b	2.918 b
I <sub>3</sub>	0.487 c	1.433 c	0.829 c	2.434 c
<b>Phosphorus (P)</b>				
P <sub>1</sub>	0.706 b	1.863 b	1.050 b	2.779 b
P <sub>2</sub>	0.729 b	1.921 b	1.083 b	2.856 b
P <sub>3</sub>	0.754 a	2.063 a	1.120 a	3.070 a
<b>Mycorrhiza (M)</b>				
M <sub>1</sub>	0.714 b	1.867 b	1.061 b	2.781 b
M <sub>2</sub>	0.745 a	2.031 a	1.108 a	3.022 a

Means, in each column and for each factor, followed by at least one letter are not significantly different at the 5% probability level

## CONCLUSION

The results showed that water use efficiency of grain yield (WUE<sub>GY</sub>) and water use efficiency of biological yield (WUE<sub>BY</sub>) in maize plants have been affected greatly by water stress conditions. The data showed that the mycorrhizal biofertilizer application improved WUE<sub>GY</sub> and WUE<sub>BY</sub> in maize plants as a consequence of enhancing extension of the root system, water status of the plants and nutrients uptake, in particular phosphorus. Generally, AM plants have a greater effect than non-AM plants. Different P fertilizer levels have significantly affected WUE<sub>GY</sub> and WUE<sub>BY</sub> in maize plants. With respect to environmental problem associated with fertilizer and water limitation in future, it is essential that we apply water resources appropriately and decrease fertilizers application in order to improve soil fertility, productivity and water quality.

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