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Influence of Mycorrhizal Fungi and Water Stress on Growth and Yield of Two Onion Cultivars

تأثير فطر المايكورايزا والاجهاد المائي على نمو وانتاجية صنفين من البصل

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Abstract: A field study was conducted to determine the effect of arbuscular mycorrhizal (AM) fungi inoculation on bulb yield and mineral acquisition of two onion (*Alium cepa* L.) cultivars (Giza 20 and Texas Grano) grown under well-watered and water-stressed conditions. Onion seedlings were transplanted into planting furrows after treatment with or without the AM fungi *Glomus mosseae* or *G. fasciculatum.* Root colonization with AM fungi occurred in both cultivars under water-stressed and well-watered conditions, but the extent of AM fungi root colonization was higher under well-watered than under water-stressed conditions. Water stress had significantly reduced bulb yields and mineral acquisition in both cultivars either inoculated or un-inoculated plants. However, inoculation with AM fungi has improved onion bulb yield and mineral acquisition (P, Cu, Fe and Zn concentrations) irrespective soil moisture. The results indicated that Texas Grano cultivar benefited more than Giza 20 cultivar from AM fungi inoculation especially under water-stressed conditions. The improved yield and mineral acquisition due to AM fungi inoculation demonstrated the importance of mycorrhizal inoculation to reduce the effects of drought stress on onion grown under field conditions in dry and semi-dry areas.

Keywords: *Arbuscular mycorrhiza, Nutrition, Allium cepa, Water stress, arid region.*

المستخلص: تم إجراء دراسة حقلية لدراسة تأثير التلقيح بفطر المايكورايزا على إنتاج الأبصال وامتصاص العناصر المعدنية في صنفين من البصل تحت ظروف معاملتين من الري (جافة وغير جافة). حيث تم زراعة اشتال البصل في اتلام بعد تلقيحها او عدم تلقيحها بنوعين من فطر Glomus. دلت النتائج على إصابة الجذور بفطر المايكورايزا في كلا الصنفين سواءا تحت الظروف الجافة او غير الجافة. وكانت انتاجية النباتات الملقحة بالفطر من الابصال اعلى منها في النباتات غير الملقحة بالفطر. ادت معاملة الجفاف إلى تناقص إنتاجية الابصال وامتصاص العناصر المعدنية في كلا الصنفين، إلا ان التلقيح بفطر المايكورايزا ادى إلى زيادة إنتاج الابصال وامتصاص العناصر الغذائية بغض النظر عن معاملة الجفاف. إن زيادة إنتاج الابصال وامتصاص العناصر المعدنية نتيجة التلقيح بفطر المايكورايزا يدل على مدى قدرة هذا الفطر في التقليل من تاثير الجفاف على نباتات البصل المزروعة في حقول المناطق الجافة وشبه الجافة.

كلمات مدخلية : *فطر المايكورايزا، نمو النبات، تغذية النبات، البصل، الجفاف، المناطق الجافة.*

Introduction

In the arid and semi-arid regions of t world, drought conditions limit crop productivity. Incorporating or applying technologies in cropping systems that would enable plants to better withstand drought stress would improve crop production under dry conditions.

Arbuscular mycorrhizal (AM) fungi associated with plant roots enhanced crop growth and productivity under drought conditions by improving the mineral nutritional status of plants, especially low mobile nutrients such as P, Zn and Cu (Al-Karaki, *et al.* 2004; Morte, *et al.*

2000; Al-Karaki and Clark, 1998). This can be achieved by increasing the surface area of soil explored via fungal hyphae, and the much smaller hyphae can penetrate fissures in soil particles too small for roots (Marshner and Dell, 1994). AM fungi can excrete enzymes which solubilize non-available nutrients to the roots (Muchovej, 2004). AM fungi also enhances soil aggregation and water-holding capacity both by producing external hyphae and by exuding glomalin, a glycoprotein, from extraradical hyphae (Wright and Upadhyaya, 1998).

Plant growth responses to symbiotic root-AM fungi depend on such factors as AM fungi isolate, plant species/cultivar, and growing conditions (Al-Karaki, 2006; Ruiz-Lozano, *et al.* 1995; Jacobsen, *et al.* 1992). Since individual AM isolates may infect wide range of unrelated plant species, the lack of AM fungi specificity may result in considerable variation in symbiotic root-AM fungi responses (Ruiz-Lozano, *et al.* 1995; Ianson and Linderman, 1991). Knowledge about specific responses to given fungal isolate on plant productivity is important for successful utilization of the symbiotic root-AM fungi relationship. If tolerance of the plants to drought differs with AM fungi isolate with which plants are associated (Al-Karaki, *et al.* 2004; Ruiz-Lozano, *et al.* 1995), it is important to determine effective host AM fungi combinations for practical use in the field. The objective of this study was to compare the effects of two AM fungi isolates on bulb yield and nutrient acquisition in two onion cultivars grown under well -watered and water-stressed under field conditions.

Materials and Methods

a. Inoculum production

Glomus mosseae and *Glomus fasciculatum* were produced in green house pot culture with chickpea (*Cicer aritinum* L.) as a host plant. Spores of both isolates were collected from a wheat field (Al-Karaki and Al-Raddad, 1997), (See Fig. 1).

b. Production of transplants

Seeds of onion cultivars [Texas Grano (white skin) and Giza 20 (red skin)] were germinated in polystyrene trays filled with soilless mixture (2: 1 (v/v) peat moss: perlite). Seedlings were grown under green house conditions and transplanted after 60 days.

c. Cultural practices and experimental treatments

A field experiment was conducted on a silty clay soil, in the vegetable farm, Jordan University of Science and Technology, Irbid, Jordan. Composite soil samples were taken to a depth of 30 cm and analyzed for major soil properties and indigenous AM fungal spores. Soil properties before planting were 1.2% organic matter, pH 8.1, and 100 N, 8.1 P ($NaHCO_3$-exracted), 1.7 Cu, 11.2 Fe, and 1.5 Zn in mg kg^{-1} soil. Raised planting beds were prepared with four 10-cm-deep planting furrows in each plot. Plots dimensions were 2m x 2m. All treatments received a recommended dose of Nitrogen (N) fertilizer at a rate of 200 Kg N ha^{-1} as a urea. Fifty percent of added fertilizer was applied at transplanting and the remaining was top-dressed in two equal splits at 30 and 70 days after transplanting. A basal dose of phosphorus was applied just prior to transplanting at a rate of 20 Kg P_2O_5 ha^{-1} as triple super phosphate.

AM fungal treatments included no inoculation (control) or inoculation with *G. fasciculatum* (G_{fs}) or *G. mosseae* (G_{ms}). Before transplanting, furrows 0.30 m apart were opened to a depth of approximately 10 cm and mycorrhizal inoculum was evenly distributed along the bottom of the furrows of the whole plot. AM fungi inoculum was placed in the furrows below the onion seedlings and covered with soil from the furrow on the day of transplanting. The AM fungi inoculum was added at a rate of about 50400 and 28800 spores per longitudinal meter for G_{ms} and G_{fs}, respectively. The inoculum added consists of root colonized fragments with AM fungi and spores mixed with soil. Field soil contained indigenous AM fungi spores <1 g^{-1} soil. Onion seedlings of the two cultivars were transplanted on December (in 4 rows of 3 meter length) by hand. Seedlings were planted at a spacing of 30 cm between rows and 15 cm between plants. Weeds were removed manually.

Water-management treatments were: (i) water-stressed (WS) plants grown under rainfed conditions; and (ii) well-watered (WW) plants grown under rainfed conditions with supplemental irrigation scheduled to prevent symptoms of

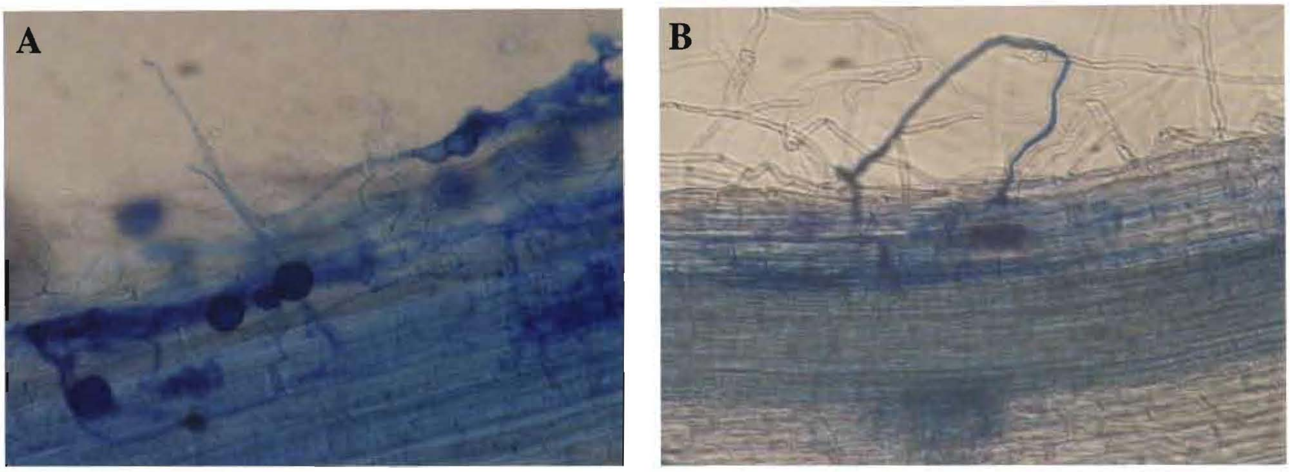

Fig. 1. Root sections of onion in which arbuscular mycorrhizal fungi structures appeared. **A**= Arbuscules and vesicles; **B**=hyphae.

water stress. Both treatments received about 50 mm irrigation water after planting, and another 50 mm were applied in two portions in March and April. The total seasonal water applied for the WW and WS treatments were 360 and 241 mm, respectively. Water was supplied to individual plots by a drip irrigation system. Total rainfall during the growing season was 141 mm.

d. Determination of mycorrhizal colonization
Onion roots were assessed for AM fungi colonization 140 days (mid bulb filling) and 180 days (at end of bulb filling) after transplanting. Root samples were cleared with 10% (w/v) KOH and stained with 0.05% trypan blue in lactophenol as described by Phillips and Hayman (1970), and microscopically examined for colonization using a gridline intercept method (Giovannetti and Mosse, 1980).

e. Bulb yield and components
Onion plants with bulbs were harvested at maturity (flaccid necks, inability to support leaf blades and falling down of foliage leaves). The harvested material was sun-dried and cleaned. Total bulbs harvested per plot were weighed to determine fresh bulb yield per unit area. Ten bulbs were randomly selected from each plot to determine mean weight per bulb. Two fresh bulbs were randomly selected from each plot, oven dried, and prepared for mineral analysis.

f. Mineral analysis
Dried shoots and bulbs were analyzed for mineral acquisition. Shoot and bulb concentrations of Zn, Cu, and Fe were determined by using the atomic absorption spectroscopy (Elmer-Perldn, 2380). Phosphorus concentration was determined according to the yellow phospho-vanado-molybdate complex method by using spectrophotometer (Watanabe and Olsen, 1965).

g. Experimental design and data analysis
The experiment was arranged in a split-plot design with water stress levels as main plots and AM fungi inoculum x onion cultivars combination as subplots with three replications. Data were statistically analyzed using analyses of variance (ANOVA). Probabilities of significance among treatments and interactions were used to compare means within and among treatments.

Results

The AM fungi root colonization was noted in roots of both mycorrhizal inoculated and non-inoculated plants, (See Fig. 2). Addition of G_{ms} *and* G_{fs} inoculums to the soil increased mycorrhizal colonization in the roots under both WW and WS conditions and for both samplings at mid- to end-bulbing stages (Table 1). Inoculation of plots with both AM fungal isolates increased total bulb yield and mean bulb weight of both cultivars regardless of water regime (Table 2). Water stress generally decreased the bulb yields and mean bulb weight in all plots. The G_{ms} plants had generally higher bulb yields and mean bulb weights than G_{fs} plants grown under both WW and WS conditions for both cultivars. The overall effects of AM fungi inoculation on the onion bulb yields and mean bulb weights (percentage-wise) of plants grown under WW and WS conditions are summarized in Table 3.

Supplemental irrigation and AM fungal inoculation both had significant effects on shoot and bulb nutrient concentrations. Water stress generally decreased the concentrations of studied nutrients (P, Zn, Cu, and Fe) in shoots and bulbs in all sampling dates (Tables 4, 5, 6, and 7). AM fungal inoculation increased concentrations of P, Zn, Cu and Fe in shoots and bulbs at all samplings irrespective of soil moisture (Tables 4, 5, 6, and 7). Shoot and bulb concentrations of P, Zn, Cu and Fe were generally higher for G_m plants than G_f plants at all samplings (Tables 4, 5, 6, and 7). Shoot and bulb concentrations of P, Zn, Cu and Fe were generally higher in Texs Grano than Giza 20 cultivar, although these differences were only significant for bulb P and Fe concentrations at mid bulbing stage and for Cu concentrations at maturity stage.

Fig. 2. Arbuscular mycorrhizal fungi as appeared in roots, on right side spores of *Glomus mossae* (upper), and *Glomus fasciculatum* (lower).

Table 1. Mycorrhizal root colonization of arbuscular mycorrhizal fungi (AMF) inoculated and non-inoculated (nonAMF) onion cultivars grown under well-watered (WW) and water-stressed (WS) conditions.

AMF status	Cultivar	Root colonization			
		Mid Bulbing		End Bulbing	
		WW	WS	WW	WS
Non AMF	Giza 20	44	34	65	57
	Texas Grano	46	32	70	45
G. mosseae	Giza 20	53	45	78	72
	Texas Grano	55	43	85	67
G. fasciculatium	Giza 20	50	42	75	63
	Texas Grano	52	40	73	61
Significance					
WS		<0.01		<0.05	
AMF		>0.1		<0.01	
WSxAMF		>0.1		>0.1	
Cultivar (C)		>0.1		<0.05	
WSxC		>0.1		>0.1	
AMFxC		>0.1		>0.1	
WSxAMFxC		>0.1		>0.1	

Table 2. Total Bulb yield, bulb mean weight of non-inoculated (Non AMF) or AMF inoculated (AMF) onion cultivars grown under two water levels (at mid and end bulb filling stage).

AMF status	Cultivar	Bulb yield (ton ha^{-1})		Bulb mean weight(g bulb^{-1})	
		WW	WS	WW	WS
Non AMF	Giza 20	60.4	50.0	268	208
	Texas Grano	70.0	40.7	290	195
G.mosseae	Giza 20	90.0	60.4	380	265
	Texas Grano	100.3	60.0	428	252
G.fasciculatium	Giza 20	80.1	60.1	337	253
	Texas Grano	80.4	50.6	349	235
Significance					
WS		<0.05		<0.05	
AMF		<0.01		<0.01	
WSxAMF		<0.01		<0.01	
Cultivar (C)		>0.1		>0.1	
WSxC		<0.01		<0.01	
AMFxC		>0.1		>0.1	
WSxAMFxC		>0.1		>0.1	

Table 3. Percent change in bulb yield and bulb mean weight due to AMF (*Glomus* sp.) inoculation of onion cultivars grown under WW and WS conditions. (Yield $Y=Y_{AM}\cdot Y_{nonAM} \times 100/ Y_{nonAM}$)

Enhancement AMF status	Cultivar	Bulb yield (%)		Bulb mean weight (%)	
		WW	WS	WW	WS
G.mosseae	Giza 20	49.0	20.8	41.8	27.4
	Texas Grano	43.3	47.4	47.5	29.2
G.fasciculatium	Giza 20	32.6	20.2	25.7	21.6
	Texas Grano	14.9	24.3	20.3	20.5

Table 4. Shoot and bulb P concentration (mg/g) in shoots and bulbs of uninoculated (Non AMF) or AMF inoculated (AMF) onion cultivars grown under WW and WS conditions.

AMF status	Cultivar	Shoot Mid bulbing		Shoot End bulbing		Bulb Mid bulbing		Bulb End bulbing		Bulb Maturity	
		WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
Non AMF	Giza 20	2.31	1.76	2	1.6	2.5	2.3	4.8	3.7	5.77	4.4
	Texas Grano	2.9	1.41	2.5	1.2	3.2	2	5.2	3.1	5.83	3.76
G.mosseae	Giza 20	3.61	2.73	3.1	2.3	3.7	2.9	5.7	4.9	7.13	6
	Texas Grano	3.77	2.63	3.7	2.2	4.6	2.8	7.1	4.7	8	5.6
G.fasciculatium	Giza 20	3.1	2.21	2.9	2.1	3.4	2.8	5.5	4.2	6.5	4.8
	Texas Grano	3.33	2.1	3	1.7	3.3	2.7	5.7	3.9	6.71	4.46
Significance											
WS		<0.01		<0.05		<0.05		<0.01		<0.01	
AMF		<0.01		<0.01		<0.05		<0.01		<0.01	
WSxAMF		>0.1		>0.1		<0.01		>0.1		>0.1	
Cultivar (Computer)		>0.1		>0.1		<0.01		>0.1		>0.1	
WSxC		<0.05		<0.01		<0.01		>0.1		>0.1	
AMFxC		>0.1		>0.1		>0.1		>0.1		>0.1	
WSxAMFxC		>0.1		>0.1		>0.1		>0.1		>0.1	

Table 5. Shoot and bulb Cu concentration (µg/g) in shoots and bulbs of uninoculated (Non AMF) or AMF inoculated (AMF) onion cultivars grown under WW and WS conditions.

AMF status	Cultivar	Shoot Mid bulbing		Shoot End Bulbing		Bulb Mid bulbing		Bulb End bulbing		Bulb Maturity	
		WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
Non AMF	Giza 20	9.8	8.4	9	8	6.1	5.4	8.4	6.2	11.4	8.7
	Texas Grano	10.4	7.3	10	6.7	7	5	8.7	5.7	12.37	7.5
G.mosseae	Giza 20	13	11.4	12.5	10.2	7.8	6.8	14	8.8	17	12.13
	Texas Grano	15.5	9.6	14	9.4	10.6	6	16.7	8	20.13	10.9
G.fasciculatium	Giza 20	12	10	11.5	9.3	7.4	6.3	10.9	8.5	13.9	11.13
	Texas Grano	12.8	9.2	12	8.4	7.5	5.8	11.8	7.3	14.8	10.3
Significance											
WS		<0.01		<0.01		<0.05		<0.01		<0.01	
AMF		<0.01		<0.01		<0.01		<0.01		<0.01	
WSxAMF		>0.1		>0.1		>0.1		<0.01		<0.01	
Cultivar (C)		>0.1		>0.1		>0.1		>0.1		<0.01	
WSxC		>0.1		<0.01		<0.05		<0.05		>0.1	
AMFxC		>0.1		>0.1		>0.1		>0.1		>0.1	
WSxAMFxC		>0.1		>0.1		>0.1		>0.1		>0.1	

Table 6. Shoot and bulb Fe concentration (µg/g) in shoots and bulbs of uninoculated (Non AMF) or AMF inoculated (AMF) onion cultivars grown under WW and WS conditions.

AMF status	Cultivar	Shoot Mid bulbing		Shoot End Bulbing		Bulb Mid bulbing		Bulb End bulbing		Bulb Maturity	
		WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
Non AMF	Giza 20	428	338	295	192	110	83.1	110	83.1	115	102
	Texas Grano	431	299	319	160	107	76	107	76	117	91
G.mosseae	Giza 20	461	417.7	427	252	120	103	120	103	124	114
	Texas Grano	500	389	490	247	128	96	128	96	131	109
G.fasciculatium	Giza 20	436	358	380	240	115	97	115	97	120	110
	Texas Grano	448	349	440	219	112	92	112	92	119	104
Significance											
WS		<0.01		<0.01		<0.01		<0.05		<0.01	
AMF		<0.01		<0.01		<0.01		<0.01		<0.01	
WSxAMF		<0.01		<0.01		<0.01		>0.1		<0.01	
Cultivar (C)		<0.01		>0.1		<0.01		>0.1		>0.1	
WSxC		<0.01		<0.05		<0.01		>0.1		<0.01	
AMFxC		<0.01		<0.01		<0.01		>0.1		>0.1	
WSxAMFxC		<0.01		>0.1		<0.01		>0.1		<0.05	

Table 7. Shoot and bulb Zn concentration (μg/g) in shoots and bulbs of uninoculated (Non AMF) or AMF inoculated (AMF) onion cultivars grown under WW and WS conditions.

		Shoot				**Bulb**					
AMF status	**Cultivar**	**Mid bulbing**		**End Bulbing**		**Mid bulbing**		**End bulbing**		**Maturity**	
		WW	**WS**	**WW**	**WS**	**WW**	**WS**	**WW**	**WS**	**WW**	**WS**
Non AMF	Giza 20	24.7	20.2	31.4	21.8	26.3	22.1	31.8	24.1	42	38
	Texas Grano	25.9	19	31.8	20.8	27.2	21.1	31.9	22	43	34
G.mosseae	Giza 20	30.7	25.7	36.6	28.3	37.5	28.4	37.2	30.9	50	41
	Texas Grano	33.6	24.8	44.1	28.3	33.2	25	43.7	28.1	53	39
G.fasciculatium	Giza 20	29.3	24	34.1	26.2	31.1	25	35	27.6	46.7	44.4
	Texas Grano	29.8	23.8	35.9	24.5	32.3	23.8	33.1	27.1	47.5	41
Significance											
WS		<0.01		<0.01		<0.01		<0.05		<0.01	
AMF		<0.01		<0.01		<0.01		<0.01		<0.01	
WSxAMF		>0.1		>0.1		>0.1		>0.1		>0.1	
Cultivar (C)		>0.1		>0.1		>0.1		>0.1		>0.1	
WSxC		>0.1		>0.1		>0.1		>0.1		>0.1	
AMFxC		>0.1		>0.1		<0.05		>0.1		>0.1	
WSxAMFxC		>0.1		>0.1		<0.05		>0.1		>0.1	

Discussion

Inoculation of onion plants with AM fungi increased the level of colonization in the roots of both cultivars. This increase was greater in plants grown under WW than under WS conditions. These data agreed with the general observation that AM fungi levels are lower under WS than WW conditions (Al-Karaki, *et al.* 2004; Al-Karaki and Clark, 1998; Ryan and Ash, 1996). The highest level of root colonization caused by AM fungal inoculation was attained at end-bulbing stage for both cultivars. Koch, *et al.* (1997) reported high AM fungal inoculum in disinfested soil under field conditions.

Inoculation with AM fungi provided an important enhancement to bulb yields in both cultivars. However, there were differences between tested cultivars in their response to AM fungi inoculation and water regime. The enhancement in bulb yields due to AM fungi inoculation was higher for Giza 20 cultivar grown under WW than under WS conditions, while the opposite occurred for Texas Grano cultivar. Texas Grano cultivar is considered more sensitive to drought stress than Giza 20 cultivar. The higher proportional increase in Texas Grano bulb yields in WS plants due to AM fungal inoculation might be attributed to increased dependence of this cultivar on AM fungi for mineral and water uptake. Similar results were obtained for wheat by Al-Karaki, *et al.* (2004).

Enhanced plant yield following AM fungal inoculation was related to improved uptake of P, Cu, and Fe, especially under WS conditions (Al-Karaki, *et al.* 2004; Sylvia, *et al.* 1993). Mycorrhizal fungi may improve nutrient uptake by improving the exploration of the soil pore space (Sylvia, *et al.* 1993). Davies, *et al.* (1992) found that external hyphal development and soil aggregation of mycorrhizal plants were enhanced by drought acclimation. O'Keefe and Sylvia (1993) observed that external hyphae adhere to soil particles, which would improve contact with the soil solution. Furthermore, they demonstrated that hyphae access smaller pore spaces than plant roots and root hairs. As soil water content decreases, the relative importance of these factors would increase.

Several factors such as host plant, AM fungal isolate, and soil environment can influence the effectiveness of root-AM fungi symbioses. It is important to understand and manipulate these factors to optimize plant growth responses to AM fungi. It is necessary to select AM fungal isolates best adapted to the environment in which a plant species is normally grown. Isolates of AM fungi differ in ability to enhance plant growth (Al-Karaki, *et al.* 2004; Ruiz-Lozano, *et al.* 1995). Specific AM fungal isolates may be related to the ability of AM fungi to colonize roots (Abbott and Robson, 1982) and for production of external hyphae to enhance P and water acquisition (Davies, *et al.* 1992).

The enhancement in bulb yields and mean bulb weights due to inoculation with AM fungi was higher for G_{ms} than G_{fs} for plants grown under both WS and WW conditions. Fungal isolates have been reported by many researchers to differ in their ability to ameliorate plant water stress (Al-Karaki *et al.* 2004; Al-Karaki, *et al.* 1998; Ellis, *et al.* 1985).

The improved yield and nutrient uptake in onion plants reported here demonstrate the potential of mycorrhizal inoculation to reduce the effects of drought stress on onion grown under field conditions of arid and semiarid regions.

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