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RESEARCH ARTICLE

Can arbuscular mycorrhizal fungi ameliorate the adverse effects of deficit irrigation on tall fescue (*Festuca arundinacea* Schreb.)?

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Abstract

With increasing concerns over global warming, the shortage of water supplies has turned to be a disputable problem in the world. Drought stress is one of the most prevalent abiotic stresses that affect turfgrass growth and quality in landscapes of arid or semi-arid regions. Among the common cool-season turfgrasses, tall fescue can tolerate drought more than other grasses. Fungal bio-fertilizers, especially Arbuscular Mycorrhizal Fungi (AMFs) are able to alleviate the harmful effects of abiotic stresses in the plant. This study was conducted in two consecutive years, 2014 and 2015, to evaluate how two types of AMFs (*Funneliformis mosseae* and *Rhizophagus irregularis*) would affect the growth characteristics of two cultivars ('H-d' and 'J-r') of tall fescue (*Festuca arundinacea* Schreb.) in the greenhouse and field conditions. Three irrigation intervals were applied, i.e. 7, 14 or 21-days. Results showed that by irrigating less frequently, the visual quality of turfgrass decreased, and there were reductions in the fresh weight, chlorophyll content, and relative water content. However, there were increases in the amount of phosphorus in the shoots, root colonization, compatible solutes (proline and glycine betaine) and antioxidant enzymes (superoxide dismutase and peroxidase). However, these changes were more pronounced in turfgrasses that were treated with AMFs. Qualitative and quantitative characteristics were substantially more promising in the greenhouse than in the field. This is the first report on the morpho-physiological and biochemical responses of tall fescue to AMFs. Results of this investigation could inspire further such works on other turfgrasses, given that the inoculation of turfgrasses with AMFs is a new approach with uncharted potentials that aims at reducing water consumption in the landscape environment.

Keywords: Arbuscular mycorrhizal, fungi, drought tolerance, enzyme activity, osmoprotectant, phosphorus, turfgrass

1. Introduction

The beauty and elegance of a landscape can partly depend on the pleasant green color of turfgrass since it comprises the main background of the landscape. The scarcity of water can influentially limit the irrigation of turfgrass and thus would reduce turfgrass quality, especially in arid and semi-arid zones. Therefore, water management is the main concern with regard to the management of turfgrass (McCann and Huang, 2007). During the past few decades, bio-fertilizers such as AMFs, make plants tolerant against abiotic stresses under unsuitable climatic conditions, have become the most applicable materials in nutritional programs and have drawn the attention of many researchers (Rai, 2006).

AMFs constitute the most popular mutual symbiosis that can occur between plant roots and the rhizosphere microorganisms (Smith and Read, 2008). Symbiosis thereby affects some morphological, anatomical and cell aspects of the plant via AMFs. These can promote the absorption of nutritional mineral elements and consequently the plants' growth and water uptake efficiency. AMFs can cause to increase photosynthesis and water absorption and further contribute to the production of osmoregulators. They cause phosphates in the rhizosphere to become more absorbable and induce stress genes that counter stressful conditions. In addition to being known as bio-fertilizer, AMFs can serve as bio-control agents or bio-productive agents and thereby improve the plant's tolerance to biotic and abiotic stresses as when plant hormones would act accordingly (e.g., Jasmonic acid (JA), salicylic acid (SA), ethylene, auxin, gibberellin (GA), cytokinin (CK), Abscisic acid (ABA) and Strigolactone (SL)) (Garg and Chandel, 2010; López-Ráez, 2015; Pozo *et al*., 2015). Moreover, AMFs can reduce the harmful effects of abiotic factors, which is to some extent known as the bioremediation of contaminated soils and the bio-monitoring of soil quality (Cornejo *et al*., 2017; Jeffries *et al*., 2003).

The most tolerant to heat and drought among the cool-season turfgrasses is the tall fescue (*Festuca arundinacea* Schreb.). Its exceptional drought tolerance results from its extensive root system that allows the plant to maintain the natural physiological state via its drought avoidance mechanisms (Fry and Huang, 2004). In spite of reports demonstrate the positive effects of AMFs on the physiological function of turfgrasses, there have been few studies on how AMFs affect qualitative and quantitative characteristics of turfgrasses (Nikbakht and Pessarakli, 2014). To the best of our knowledge, this research is the first to investigate how AMFs affect the tall fescue growth under drought condition.

2. Materials and Methods

This experiment was conducted under greenhouse conditions and also in the field environment (Bajgah) close to the Department of Horticultural Sciences, School of Agriculture, Shiraz University, Shiraz, Iran (52°32′E and 29°36′N, 1810 m asl). The research was done in 2 consecutive years from 2014 to 2015. The tillers of two cultivars of tall fescue were used. These cultivars were namely 'J-r' as drought-tolerant and 'H-d' as less tolerant to drought. Biochemical and physical characteristics of water and soil of the Bajgah region, Fars province were measured. Data are shown in Table 1. The plastic pots used in the experiment measured 25 liters in volume, 36 cm in height and 33 cm in diameter. Field capacity (FC) and permanent wilting point (PWP) of each pot were calculated by pressure plate apparatus and the results were joined to determine irrigation intervals (IIs). Average temperatures of the day and night were 30±3 and 24±3 °C, respectively, and the relative humidity was 50±5% in the greenhouse. Table 2 further elaborates on data pertaining to the climate. Maximum and minimum average of monthly temperatures in July and March were 25.76 and 8.16 °C, respectively. The average temperatures and relative humidity during the experiment were 17.3 °C and 35.2%, respectively.

Table 1. Physico-biochemical properties of the soil and chemical analysis of the irrigation water used in the experiment

Soil				Water	
Physical property		Biochemical property		Chemical analysis	
Clay (%)	59.28	pH	7.76	pH	7.1
		EC (dS m^{-1})	0.76	EC (dS m^{-1})	0.49
Silt (%)	40.00	Organic matter (%)	1.2	HCO_3^- (mg l^{-1})	1.12
		N (mg kg^{-1})	6.55	CO_3^- (mg l^{-1})	0
Texture	Clay	P (mg kg^{-1})	25	$P_2O_4^+$ (mg l^{-1})	0.01
		K (mg kg^{-1})	600	NO_2 (mg l^{-1})	0.03
Field capacity (%)	24.3	Microbial respiration (mg CO_2 kg^{-1})	2.8	NO_3^- (mg l^{-1})	7.4
		Microbial biomass (mg C kg^{-1})	12.1	Cl^- (mg l^{-1})	43.6
		Most probable number (MPN g^{-1})	1.1×10^4	Mg^{2+} (mg l^{-1})	2.2

Table 2. Meteorological data of experimental region

Year	Month	Average monthly temperature (◦C)			Sunny hours	Evaporation (mm 24 h^{-1})	Rainfall (mm)	Average relative humidity (%)		
		Mean	Max	Min				Mean	Max	Min
1st (2014)	March	8.80	16.82	0.69	7.62	8.15	17.0	44.06	70.32	17.81
	April	12.07	20.44	3.71	8.51	5.39	41.5	41.95	67.98	15.91
	May	16.70	26.15	7.24	9.04	7.52	1.0	39.34	64.29	14.39
	June	22.15	33.07	11.23	13.73	10.33	0.0	29.52	47.48	11.55
	July	25.62	35.99	15.23	10.66	11.08	0.0	26.92	40.65	13.19
2nd (2015)	March	7.52	14.05	0.70	7.06	3.84	45.0	43.47	66.31	20.62
	April	13.64	20.55	6.74	7.50	5.62	39.5	43.10	67.31	19.00
	May	17.62	27.59	7.66	9.54	6.59	10.0	34.56	56.39	17.63
	June	22.98	33.46	12.50	10.67	9.08	0.0	24.65	37.71	11.58
	July	25.90	35.85	15.95	10.85	11.18	0.0	24.48	13.55	25.90

Source: Meteorological Station, School of Agriculture, Shiraz University.

2.1. Microorganisms

To evaluate the effects of 2 species of Fm and Ri, fungi were obtained from the Department of Soil Science, Shiraz University, Shiraz, Iran. These fungi were isolated from the Anguran Mine, Zanjan, Iran, during a research by Zarei *et al*. (2008a). Fifty g of sand-based inoculum of mycorrhizal fungi (containing spore numbers of 12-10 g^{-1} substrate and RC of 85-85%, respectively) were incorporated in the ground with AM sorghum's (*Sorghum bicolor* (L.) Moench) roots. The mixture was prepared through the trap culture of roots at the depth of 5 cm in the soil and was added to each pot. These were mixed thoroughly with the soil. For the control group, the pots were used containing an equal amount of sterilized sand-based inocula (Zarei *et al*., 2008b).

2.2. Treatments and experimental setup

To evaluate the effects of treatments, the greenhouse experiment was conducted in the factorial arrangement as a completely randomized design with 3 replications. The field experiment was based on a randomized complete block design with 3 replications. For appropriate growth and adaption, tillers were irrigated for 5 weeks to an extent that allowed the soil moisture to be at FC. The II treatments began to be performed when plants were well established. The II lasted for 12 weeks. Treatments were 2 cultivars of tall fescue ('H-d' and 'J-r'), there were 3 IIs (7, 14 and 21-days) and 3 fungi levels (the control group, *Funneliformis mosseae* and *Rhizophagus irregularis*). weeks. Treatments were 2 cultivars of tall fescue ('H-d' and 'J-r'), there were 3 IIs (7, 14 and 21-days) and 3 fungi levels (the control group, *Funneliformis mosseae* and *Rhizophagus irregularis*).

2.3. Measurements

2.3.1. Total fresh weight (FW) and visual quality (VQ)

At the end of the experiments, plants were harvested and separated into their root, clippings and verdure. Each part was weighed and total FW was calculated by summing their weights. Turfgrass VQ was measured according to shoot color, uniformity, and density, weediness and speed of establishment by being rated from 0 to 9, in which the highest quality acquired the highest score (9).

2.3.2. Leaf chlorophyll (Chl) and relative water content (RWC)

To determine Chl content, the samples were extracted with 80% acetone. Chl was measured by using the method of spectrophotometry (Esmaili and Salehi, 2012), using the following formula 1:

mg Chl/g f.w.= [(20.2(OD 645 nm) + (8.02(OD 663 nm)] × V / f.w. × 1000

where: OD is the optical density, V is the final solution volume in ml and f.w. is the plant tissue's fresh-weight in mg.

RWC was measured with González and González-Vilar (2001) method. For this reason, 0.2 g fresh leaf was weighed and placed in 50 ml distilled water for 4 h, and then the turgid leaves were weighed. Then, leaf samples were oven dried for calculating the dry weight at 70 °C for 48 h. The RWC was determined by the following formula 2:

RWC (%) = (f.w. –d.w.)/(s.w. –d.w.)×100

where: f.w. is fresh-weight, d.w. is dry-weight and s.w. is the saturated-weight.

2.3.3. Compatible solute content

Determination of proline (Pro) content was done by the method described by Bates *et al*. (1973). Briefly, 0.5 g dried leaf sample was shaken and homogenized with sulfo-salicylic acid. Thereafter, the extract was exposed to react chemically with glacial acid and acid-ninhydrin for 1 h at 100 °C in a Bain-marie. Then, samples were extracted with toluene and the samples' absorbance were measured at 520 nm.

The concentration of glycine betaine (GB) was determined using the method of Grieve and Grattan (1983). Briefly, 0.5 g dried leaf was homogenized with deionized water. Samples were mixed with sulfuric acid and potassium iodide-iodine (KI-I2), and were then centrifuged. To extract GB, the peridotite crystals that had formed were dissolved in 1, 2-Dichloroethane (DCA) and then sample absorbance was measured at 365 nm.

2.3.4. Enzyme assay

Superoxide dismutase (SOD) activity was determined according to the method described by Beauchamp and Fridovich (1971). The measurement was based on the ability of the SOD enzyme in preventing the reduction of nitro blue tetrazolium photochemical by the O_2●- radical as the most prominent reactive oxygen species (ROS) in the presence of riboflavin. Sample absorbance was measured at 560 nm.

Peroxidase (POX) activity in leaves of experimental plants was measured by method of Chance and Maehly (1955) with minor modifications. The enzyme activity measurement was based on the peroxidase enzyme and H_2O_2 radical ability. The absorbance of extract was read every 10 seconds for 1 min at 470 nm.

2.3.5. Phosphorus (P) content and root colonization (RC)

Determination of P concentration was done by the colorimetric assay (Watanabe and Olsen, 1965). To calculate P content, the standard curve was drawn for 0.1 to 1 mg/l P at 890 nm.

The method was used to determine RC described by Kormanik and McGraw (1982). Briefly, 0.5 g root was placed in a test tube containing potassium hydroxide (KOH) 8% for 24 hours. Then, the content of the tube was removed and roots were placed in hydrochloric acid (HCL) 2% for 15 minutes. Then, the acid was removed, and a colored solution (acid fuchsine) was added. These were stored at room temperature for 24 hours. In both cases, the dye solution comprised lactic acid, glycine and water with a ratio of 14:1:1. Fungal organs including arbuscule, hyphae, vesicles and spores were visible under the stereomicroscope.

2.3.6. Recovery period

When the field experiment had finished, measurements were taken to compare the recovery rate of plants under fungi treatments with plants of the control pots. For this purpose, plants that were treated under 21-day II were cut back by shoot clippings, and then pots were irrigated to maintain soil moisture at field capacity for 5 weeks.

3. Results

The two consecutive years of collecting data had significant differences with each other based on the Bartlett test. Therefore, the two years of results are presented separately.

3.1. Visual quality

For the greenhouse experiment, signs of yellowing and wilting (leaf rolling in tall fescue) where observed a month after applying drought stress. The VQ decreased significantly with increasing II in both cultivars. The best VQ was obtained in 'H-d', the 7-day II and in both fungal treatments. The best results were found in the interaction between 'H-d', the 7-day II and the Ri fungal treatment, while the worst VQ was observed in pots without fungi, the 21-day II for the 'J-r'.

Table 3. Main effects and interactions between different treatments on visual quality (0-9) and total fresh weight (g)

Cultivars	Fungi	Irrigation intervals	Interactions VQ In	Interactions VQ Out 1st	Interactions VQ Out 2nd	Interactions total FW In	Interactions total FW Out 1st	Interactions total FW Out 2nd
'H-d'	Control	7	6.66cd†	6.00bc	6.33bc	378.29e	154.95e	147.10f
		14	5.33ef	3.33hi	3.66f	316.35f	87.82h	81.27ij
		21	3.66gh	0.33l	1.00i	168.53h	32.86k	48.57l
	Rhizophagus irregularis	7	8.33a	7.33a	7.00ab	470.62c	176.07cd	179.32de
		14	7.33a-c	5.00d-f	6.33cd	395.50e	167.30de	160.95ef
		21	5.33ef	2.00jk	2.66gh	193.39gh	55.58ij	74.98jk
	Funneliformis mosseae	7	8.00ab	7.33a	7.33a	486.79c	204.63b	200.34bc
		14	6.33c-e	4.66ef	5.66c-e	386.54e	107.31f	117.01g
		21	5.66d-f	2.66ij	3.33fg	176.14h	91.90gh	95.29hi
'J-r'	Control	7	6.33c-e	5.33c-e	5.33de	483.43c	184.00c	186.66cd
		14	4.66fg	4.33fg	5.00e	392.77e	103.66fg	110.95gh
		21	2.66h	1.66k	2.33h	212.66g	46.53i-k	58.01kl
	Rhizophagus irregularis	7	7.33a-c	6.66ab	6.33bc	581.71b	260.95a	258..55a
		14	6.66cd	5.66cd	6.00cd	409.10de	159.64e	144.59f
		21	3.66gh	3.00hi	4.00f	210.88g	42.89jk	51.86l
	Funneliformis mosseae	7	7.00bc	7.33a	7.66a	650.32a	212.84jk	206.74b
		14	5.66d-f	6.00bc	6.33bc	435.61d	166.75de	167.46de
		21	5.66d-f	3.66gh	4.00f	195.79gh	58.34i	74.70jk

Main effects	TRTs	VQ In	VQ Out 1st	VQ Out 2nd	Total FW In	Total FW Out 1st	Total FW Out 2nd
Cultivars	Hd	6.29A	4.29B	4.77B	330.24B	119.82B	122.76B
	Jr	5.51B	4.85A	5.22A	396.92A	137.29A	139.95A
Fungi	CTL	4.88B	3.50C	3.94C	325.34B	101.63B	105.43B
	Ri	6.44A	4.94B	5.33B	376.87A	143.74A	145.04A
	Fm	6.38A	5.27A	5.72A	388.53A	140.29A	143.59A
Irrigation intervals	7	7.27A	6.66A	6.66A	508.53A	198.91A	196.45A
	14	6.00B	4.83B	5.44B	389.31B	132.08B	130.37B
	21	4.44C	2.22C	2.88C	192.90C	54.68C	67.23C

† Means having different letters (capitals for the main effects and lower cases for interactions) are significantly different at 5% level by LSD test.

* (VQ): visual quality; (FW): fresh weight; (In): greenhouse condition; (Out 1st): field environment in the 1st year; (Out 2nd): field environment in the 2nd year; (TRTs): treatments; (CTL): control; (Ri): *Rhizophagus irregularis*; (Fm): *Funneliformis mosseae*.

For the field experiment, the main effects of treatments were investigated. The best quality was observed in ‘J-r’ during both years, but the remarkable result was of the best quality in ‘H-d’ in combination with the 7-day II and also another remarkable result was for the ‘J-r’ in combination with the 14-day II and the 21-day II. In both years, the Fm caused the best VQ in the field experiment. Interaction between both cultivars and the 7-day II showed the best result. However, it should be noted that the 14-day II also exhibited an acceptable quality. Generally, the VQ decreased as the II increased in tall fescue. But, this reduction was more intangible when Fm was applied than when Ri was (Table 3).

3.3. Chlorophyll content

The Chl content in ‘H-d’ was more than the content in ‘J-r’ among plants in the greenhouse. Among fungi levels of treatment, the highest Chl content was found in the Fm treatment, while the control had the lowest Chl content. The Chl decreased as the II increased. By comparing the different interactions, the highest Chl content was found in the ‘H-d’ treated with the 7-day II by the Fm fungus, while the lowest Chl content was observed in the ‘J-r’ treated with the 21-day II and by no fungus, concerning the greenhouse environment.

Similar to the greenhouse, the field environment provided for the ‘H-d’ to have more Chl content during both years. Among fungi treatment levels, the best performance was observed by the Fm for the first year of the experiment, while the Ri caused the best performance in the second year. As expected, the Chl content decreased by increasing the II factor under field condition. In the first year of the experiment, the highest level of Chl was observed in the ‘H-d’ treated with the Fm fungus and by the 7-day II, and in the second year, the highest was observed in the ‘J-r’ treated with the Ri fungus and by the 7-day II. The second highest Chl content was belonged to the ‘H-d’ treated with the Fm fungus and by the 7-day II in the second year (Table 4).

3.4. Relative water content

In the greenhouse experiment, RWC decreased as the II increased. The ‘J-r’ is considered as a tolerant cultivar with high RWC. Both fungi treatments maintained the RWC.

For both years, there appeared no significant difference between the two cultivars in the field environment, but the ‘J-r’ had more RWC than ‘H-d’. Both fungi, especially Fm, had the greatest positive effect on the RWC. The interaction between different treatments resulted in the outcome that the ‘H-d’ is the more susceptible cultivar to drought and had the highest RWC in combination with the Fm fungus and the 7-day II. The lowest RWC was observed in the same cultivar by the 21-day II and with no fungal treatment (Table 4).

Table 4. Main effects and interactions between different treatments on chlorophyll content (mg/gr F.W.) and shoot relative water content (%)

Cultivars	Fungi	Irrigation intervals	Interactions Chl In	Interactions Chl Out 1st	Interactions Chl Out 2nd	Interactions RWC In	Interactions RWC Out 1st	Interactions RWC Out 2nd
'H-d'	Control	7	1.06cd†	0.83d	0.57f-h	83.23cd	67.70d	70.59d
		14	0.85h	0.63g	0.68ef	75.06e	53.16fg	55.96fg
		21	0.72k	0.50h	0.48h	58.10g	32.99k	34.73k
	Rhizophagus irregularis	7	1.12ab	0.92ab	0.86c	86.56bc	73.94b-d	77.83bc
		14	0.98e	0.71f	0.71de	84.43cd	57.53ef	60.55ef
		21	0.82hi	0.61g	0.56f-h	62.36fg	39.99ij	42.09j
	Funneliformis mosseae	7	1.15a	0.95a	1.13b	84.36cd	84.43a	88.86a
		14	1.04d	0.83d	0.75c-e	82.91cd	53.11fg	55.90fg
		21	0.92fg	0.71f	0.56f-h	67.50f	40.05ij	42.15ij
'J-r'	Control	7	1.03d	0.77e	0.88c	91.69ab	70.82cd	74.54cd
		14	0.74jk	0.50h	0.69ef	79.24de	47.62gh	50.12gh
		21	0.59l	0.29j	0.67ef	65.82f	34.47jk	36.28jk
	Rhizophagus irregularis	7	1.07b-d	0.85cd	1.38a	94.80a	78.43ab	81.22b
		14	0.87gh	0.62g	0.67ef	82.18cd	57.21ef	61.22ef
		21	0.72k	0.41i	0.84cd	62.92fg	45.98hi	48.40hi
	Funneliformis mosseae	7	1.11a-c	0.88bc	0.63e-g	93.70a	75.96bc	79.95bc
		14	0.93ef	0.64g	0.83cd	85.85c	60.15e	62.32e
		21	0.79ij	0.48h	0.50gh	63.16fg	48.61gh	51.14gh

Main effects	TRTs	Chl In	Chl Out 1st	Chl Out 2nd	RWC In	RWC Out 1st	RWC Out 2nd
Cultivars	Hd	0.86A	0.74A	0.70B	76.09B	55.88A	58.74A
	Jr	0.87B	0.60B	0.79A	79.93A	57.70A	60.58A
Fungi	CTL	0.83C	0.59C	0.66C	75.52B	51.13B	53.70B
	Ri	0.93B	0.68B	0.84A	78.87A	58.85A	61.88A
	Fm	0.99A	0.75A	0.74B	79.64A	60.39A	63.39A
Irrigation intervals	7	1.09A	0.87A	0.91A	89.11A	75.21A	78.83A
	14	0.90B	0.65B	0.72B	81.61B	54.80B	57.68B
	21	0.76C	0.50C	0.60C	63.31C	40.35C	42.47C

† Means having different letters (capitals for the main effects and lower cases for interactions) are significantly different at 5% level by LSD test. * (Chl): chlorophyll; (RWC): relative water content; (In): greenhouse condition; (Out 1st): field environment in the 1st year; (Out 2nd): field environment in the 2nd year; (TRTs): treatments; (CTL): control; (Ri): *Rhizophagus irregularis*; (Fm): *Funneliformis mosseae.*

Based on the obtained results, the 'J-r' exhibited the highest Pro content in the field environment. The Fm fungus caused the best effect on the Pro content of tall fescue in both years. The lowest Pro was observed in the control plants. Pro content increased parallel to the increase in II for the field environment. The lowest Pro content was observed in the interaction between 'H-d', no fungus and the 7-day II treatments. The highest content was shown in the 'J-r', Fm and the 7-day II treatments (Table 5).

3.6. Glycine betaine

The highest GB content was observed in the 'J-r' inside the greenhouse. In total, the fungal treatment had the highest GB content compared to the control. Drought stress increased the GB content. The highest r', Ri and the 21-day II in the greenhouse environment.The 'J-r' had the highest GB content in the field environment.

Similar to the greenhouse experiment, the control had the lowest GB content and the Ri had the highest level of GB. In the first year, by increasing the II to 14-day interval, the GB increased and thereafter exhibited a downward trend when treated by the 21-day II. The interaction between treatments showed that in the first year, the 'J-r' that was treated with the Fm fungus and the 14-day II had the highest GB content under field condition. A high content also observed in the case of 'J-r' being treated with Ri and the 21-day II. On the other hand, the lowest GB content was observed in the 'H-d' that was treated with no fungus by the 21-day II. Results of the second year showed that the 'J-r' treated with the Ri fungus exhibited the highest GB content when irrigated by the 21-day II. Second to the highest, the treating of 'J-r' with the Fm fungus by the 21-day II also caused a high GB content. However, the lowest content was observed when the 'H-d' was treated with no fungus by the 21 and 14-day IIs (Table 5).

Table 5. Main effect and interaction between different treatments on proline and glycine betaine content (µmol/gr F.W.)

Cultivars	Fungi	Irrigation intervals	Interactions Pro			Interactions GB		
			In	Out 1st	Out 2nd	In	Out 1st	Out 2nd
‘H-d’	Control	7	3.27ef†	11.27l	7.27g	1.73g-i	3.46hi	3.60ij
		14	3.84ef	11.84j-l	7.84g	2.97bc	3.44hi	3.45j
		21	17.75bc	25.74e	22.07cd	1.72g-i	3.33i	3.42j
	Rhizophagus irregularis	7	4.64ef	12.87h-l	7.52g	2.08fg	3.58g-i	3.64h-j
		14	6.28e	15.61gh	10.61fg	2.25d-f	3.81e-g	3.85f-i
		21	20.41b	33.07c	18.86d	2.16ef	4.28a-c	4.28bc
	Funneliformis mosseae	7	5.75ef	12.25i-l	9.25fg	2.22d-f	3.93d-f	3.79f-i
		14	13.39d	20.06f	14.99e	2.63cd	4.04b-e	4.02d-f
		21	13.48d	36.81b	27.81b	1.98f-h	3.61g-i	3.63h-j
‘J-r’	Control	7	2.38f	11.38kl	9.04fg	1.66hi	3.83e-g	3.78f-i
		14	2.76ef	14.43g-k	10.43fg	1.98f-h	3.95d-f	3.93fg
		21	24.66a	29.66d	22.32cd	3.28ab	3.68f-h	3.73g-i
	Rhizophagus irregularis	7	4.55ef	14.88g-j	12.23ef	2.56c-e	4.29ab	4.27b-d
		14	3.24ef	16.91g	14.57e	2.70c	4.17a-d	4.23c-e
		21	18.47bc	33.47c	29.67b	3.68a	4.38a	4.78a
	Funneliformis mosseae	7	2.78ef	15.11g-i	12.45ef	1.44i	3.81e-g	3.87f-h
		14	4.41ef	27.74de	24.07c	3.52a	4.43a	4.49b
		21	15.37cd	40.70a	36.04a	3.63a	3.97c-f	3.98e-g

Main effects	TRTs	Pro			GB		
		In	Out 1st	Out 2nd	In	Out 1st	Out 2nd
Cultivars	Hd	9.87A	19.95B	14.03B	2.19B	3.72B	3.74B
	Jr	8.73A	22.70A	18.98A	2.72A	4.06A	4.12A
Fungi	CTL	9.11A	17.39C	13.16C	2.22B	3.62B	3.65C
	Ri	9.60A	21.14B	15.58B	2.57A	4.09A	4.18A
	Fm	9.20A	25.45A	20.77A	2.57A	3.97A	3.96B
Irrigation intervals	7	3.89C	12.96C	9.63C	1.95B	3.82B	3.82B
	14	5.65B	17.77B	13.75B	2.67A	3.97A	4.00A
	21	18.35A	33.24A	26.13A	2.74A	3.88AB	3.97A

† Means having different letters (capitals for the main effects and lower cases for interactions) are significantly different at 5% level by LSD test.

* (Pro): proline; (GB): glycine betaine; (In): greenhouse condition; (Out 1st): field environment in the 1st year; (Out 2nd): field environment in the 2nd year; (TRTs): treatments; (CTL): control; (Ri): *Rhizophagus irregularis*; (Fm): *Funneliformis mosseae*.

3.7. Superoxide dismutase

There was no significant difference between the two cultivars of Festuca cultivated in the greenhouse. The highest SOD activity was observed in the Fm fungal treatments. The lowest SOD activity was obtained via the Ri fungal treatment which had no significant difference with the control. The 21 and 7-day IIs had the highest and the lowest SOD activities, respectively. Interaction between treatments showed that ‘H-d’, Fm, 21-day II treatment had the highest SOD activity and ‘H-d’, the control and 7-day II had the lowest SOD activity in the greenhouse environment.

The ‘J-r’ had the highest SOD activity in both years in the field experiment. In the first year, the highest SOD activity was observed in Ri treatment. There was no significant difference between the control and the Fm but, in the second year under field condition, the Ri had the highest SOD activity, whereas the lowest SOD activity was observed in the control treatment. The highest SOD activity in both years was observed in the 14-day II. However, the SOD activity decreased as a result of the 21-day II. In the second year, the SOD activity in the 21-day treatment was lower than in the 7-day II. In the first year, the highest SOD activity pertained to the interaction between ‘J-r’, Ri and the 21-day II treatment. In the second year, it was in ‘J-r’, Ri, 14- and 7-day IIs. The lowest SOD activity was observed in ‘H-d’, control, 21-day II (Table 6).

Table 6. Main effect and interaction between different treatments on superoxide dismutase and peroxidase activity (u/g F.W.)

Cultivars	Fungi	Irrigation intervals	Interactions SOD In	Interactions SOD Out 1st	Interactions SOD Out 2nd	Interactions POX In	Interactions POX Out 1st	Interactions POX Out 2nd
'H-d'	Control	7	34.00h†	54.00g	42.00h	82.43c-e	27.89f	31.33bc
		14	74.00fg	54.00g	60.00fg	69.97e-g	46.66e	52.63bc
		21	93.33ef	114.67de	8.33j	96.19a-d	10.23h	14.14c
	Rhizophagus irregularis	7	58.67bcgh	89.00f	54.00gh	82.56c-e	56.55c-e	54.66bc
		14	148.67bc	108.00d-e	101.00d	74.49e-g	60.69cd	35.06bc
		21	136.00b-d	128.33cd	26.00i	101.73a-c	12.97gh	7.67c
	Funneliformis mosseae	7	49.33gh	100.00ef	64.00fg	106.14a	121.53a	123.16a
		14	164.33b	100.67ef	71.33ef	37.99h	108.12a	84.96ab
		21	284.67a	20.67h	17.00ij	17.39i	25.60fg	36.52bc
'J-r'	Control	7	169.33b	145.33bc	78.67e	84.39b-e	29.20f	35.76bc
		14	148.33bc	166.67ab	145.33b	61.53fg	49.62de	41.25bc
		21	137.67b-d	130.00cd	65.33fg	97.32a-d	8.51h	36.62bc
	Rhizophagus irregularis	7	105.33d-f	104.33ef	172.67a	102.58ab	10.30h	36.32bc
		14	54.67gh	139.33c	181.33a	58.37g	61.35cd	49.62bc
		21	122.33c-e	168.67a	80.67e	108.20a	48.18de	40.88bc
	Funneliformis mosseae	7	122.00c-e	146.67a-c	147.67b	58.27g	86.39b	83.06ab
		14	145.67bc	139.33c	128.67c	78.25d-f	64.92c	83.89ab
		21	125.00c-e	108.67s-f	93.67d	104.16a	14.10gh	10.53c

Main effects	TRTs	SOD In	SOD Out 1st	SOD Out 2nd	POX In	POX Out 1st	POX Out 2nd
Cultivars	Hd	115.89A	85.48B	49.30B	74.32B	52.24A	48.90A
	Jr	125.59A	139.78A	121.56A	83.67A	41.39B	46.43A
Fungi	CTL	109.44B	110.78B	66.61C	81.97A	28.68C	35.28B
	Ri	104.28B	122.94A	102.61A	87.87A	41.67B	37.36B
	Fm	148.50A	102.67B	87.06B	67.03B	70.10A	70.34A
Irrigation intervals	7	89.78C	106.56B	93.17B	86.06A	55.30B	60.71A
	14	122.61B	118.00A	114.61A	63.43B	65.22A	57.89A
	21	149.83A	111.83AB	48.50C	87.49A	19.93C	24.39B

† Means having different letters (capitals for the main effects and lower cases for interactions) are significantly different at 5% level by LSD test.
* (SOD): superoxide dismutase; (POX): peroxidase activity; (In): greenhouse condition; (Out 1st): field environment in the 1st year; (Out 2nd): field environment in the 2nd year; (TRTs): treatments; (CTL): control; (Ri): *Rhizophagus irregularis*; (Fm): *Funneliformis mosseae*.

3.8. Peroxidase

Results showed that the POX decreased initially by the 14-day II, but the highest activity was observed in the 21-day II in the greenhouse experiment. Furthermore, 'J-r' had the highest SOD activity, while Ri and Fm had the highest and the lowest POX activity. Interaction between treatments showed that the highest POX activity was in 'J-r', both fungi treatments, 21-day II and also in 'H-d', Fm, 7-day II, concerning the greenhouse environment. However, unlike the greenhouse, the field environment showed that 'H-d' had the highest POX activity. Furthermore, the Fm among other treatments caused the highest POX activity. In the first year, the POX activity increased in 14-day II but decreased in 21-day II. In the second year, the POX activity increased as the II increased. The highest POX activity was observed in the interactions of 'H-d', Fm, 7- and 14-day IIs, whereas the lowest activity was observed in both cultivars, control, and the 21-day II (Table 6).

3.9. Phosphorus

The ‘J-r’ had the highest P content in the greenhouse. Fungi accounted for most of the P uptakes, while the lowest P content was observed in the control plants. The content decreased as the II increased. The interaction between ‘J-r’, Fm and 7-day II showed the highest P content, but the lowest content was observed in the interaction between ‘H-d’, the control and the 21-day II, in the greenhouse environment. The ‘J-r’ also exhibited the highest P content under field condition in both years. Ri had more effect on P content than others and the highest content was observed in this treatment. In both years, the field drought stress reduced the P content. The highest content was observed in the interaction between ‘J-r’, Ri, and 7-day II. The lowest P content was observed in the interaction between ‘H-d’, the control, and the 21-day II (Table 7).

3.10. Root colonization

Results showed that, in the greenhouse, the ‘J-r’ exhibits more RC of fungi in comparison with the ‘H-d’. Furthermore, both fungal treatments were observed to increase the magnitude of colonization. On the other hand, RC decreased when II increased in the greenhouse.
In both years for the field environment, ‘J-r’ had more colonization than ‘H-d’. Fungal treatments augmented RC significantly, and the control had the lowest RC. Like the greenhouse, RC in the field environment decreased by increasing the II (Table 7).

Table 7. Main effects and interactions between different treatments on phosphorus concentration (mg/ kg D.W.) and root colonization (%)

Cultivars	Fungi	Irrigation intervals	Interactions P			Interactions RC		
			In	Out 1st	Out 2nd	In	Out 1st	Out 2nd
‘H-d’	Control	7	2.24e-g†	2.24c	2.22b-e	32.08g-i	13.91f	9.16g
		14	2.12fg	1.62ef	1.59h	23.75ij	8.41fg	4.16hi
		21	1.86g	1.53f	1.57h	19.58j	2.08g	1.83i
	Rhizophagus irregularis	7	3.69ab	2.32bc	2.88a	63.33bc	45.16bc	37.58c
		14	2.51d-f	2.32bc	2.00e-g	55.00cd	26.25de	32.06d
		21	2.41d-f	2.05cd	2.12c-f	40.41fg	22.91e	21.83f
	Funneliformis mosseae	7	3.70ab	2.54b	2.44b	75.83a	39.25c	40.83c
		14	2.88cd	2.29bc	2.30b-d	46.66d-f	28.58de	26.51e
		21	2.51d-f	1.60ef	1.58h	38.33f-h	28.75de	19.00f
‘J-r’	Control	7	2.71de	2.28bc	2.31b-d	38.33f-h	8.41fg	6.83gh
		14	2.79d	2.05cd	2.07d-f	30.00hi	12.08f	6.66gh
		21	2.22e-g	1.75ef	1.75gh	23.75ij	3.33g	3.33hi
	Rhizophagus irregularis	7	3.48b	3.12a	3.17a	69.58ab	53.16a	55.25a
		14	3.46b	2.57b	2.49b	52.91de	47.91ab	37.75c
		21	2.56d-f	1.79d-f	1.88fg	44.58ef	26.58de	20.83f
	Funneliformis mosseae	7	4.18a	2.88a	2.41bc	73.75a	48.33ab	51.83a
		14	3.39bc	1.88de	2.47b	52.91de	42.91bc	45.83b
		21	2.55d-f	2.18c	1.91fg	38.33f-h	30.83d	27.08e

Main effects	TRTs	P			RC		
		In	Out 1st	Out 2nd	In	Out 1st	Out 2nd
Cultivars	Hd	2.66B	2.06B	2.08B	43.88B	23.92B	21.33B
	Jr	3.04A	2.28A	2.27A	47.13A	30.39A	28.38A
Fungi	CTL	2.32B	1.91C	1.92C	27.91B	8.04B	5.16B
	Ri	3.02A	2.36A	2.42A	54.30A	37.00A	34.19A
	Fm	3.20A	2.23B	2.18B	54.30A	36.44A	35.21A
Irrigation intervals	7	3.33A	2.56A	2.57A	58.81A	34.70A	33.58A
	14	2.86B	2.12B	2.15B	43.54B	27.69B	25.50B
	21	2.35C	1.82C	1.80C	34.16C	19.08C	15.48C

† Means having different letters (capitals for the main effects and lower cases for interactions) are significantly different at 5% level by LSD test.
* (P): phosphorus; (RC): root colonization; (In): greenhouse condition; (Out 1st): field environment in the 1st year; (Out 2nd): field environment in the 2nd year; (TRTs): treatments; (CTL): control; (Ri): *Rhizophagus irregularis*; (Fm): *Funneliformis mosseae*.

3.11. Recovery period

The best recovery was achieved in the AMFs treatment, whereas the control displayed no improvements (Figure 1).

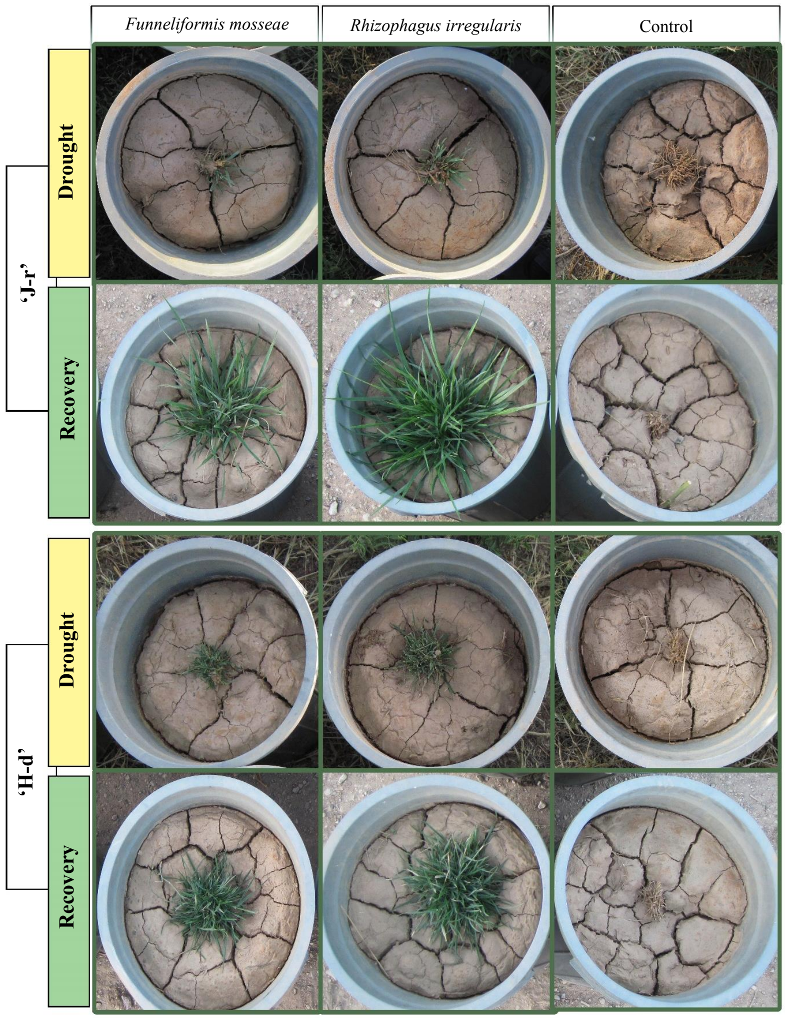

Figure 1. Analogy visual quality of two cultivars of tall fescue between fungi treatments and control at recovery period and severe drought stress.

4. Discussion

Perceptibly, decrease in plant quality i.e. turfgrass FW and RWC can resulted from the reduction in cell turgor pressure under drought stress. The decrease in osmotic pressure causes plant cells to shrink due to tissue water loss and it can culminate in plant weakness (Taiz *et al*., 2015). In this experiment, reductions in VQ, RWC and total FW were observed as the II increased. This reduction was less pronounced in the greenhouse than the field environment. Although the approximate quality features of 'H-d' was acceptable under the greenhouse condition,'J-r' showed better function in general. Many research projects have focused on the effects of drought stress on the tall fescue (Caturegli *et al*., 2015; Ebrahimiyan *et al*., 2013; Etemadi *et al*., 2015; Fu and Huang, 2001; Jiang and Huang, 2001; Manuchehri and Salehi, 2015; Pirnajmedin *et al*., 2015; Sarmast *et al*., 2015). It has been reported that shortage in the water supply reduces VQ, RWC and FW. Man *et al*. (2011) reported that the reduction in VQ and RWC – as a result of drought stress in tall fescue – is reversible in the recovery period. According to this research, the VQ, FW and RWC rendered better results when plants were treated with AMFs during the recovery period. Researchers have shown that AMFs improve plant-water relations and that the morphological conditions of the plant are enhanced by their roots. This enhancement can also be explained by the secretion of glomalin that contributes to a better uptake of water and nutrients by the plant (Cornejo *et al*., 2017; Durán *et al*., 2016; Miransari, 2014; Quiñones-Aguilar *et al*., 2016).

Leaf Chl content is one of the most important physiological characters that can change during stressful conditions. In fact, Chl content decreases as a result of accelerating lipid peroxidation and the demolition of chloroplast membranes (Fracheboud and Leipner, 2003). Many reports show that drought stress can substantially reduce the Chl content in the tall fescue, but in some cases the pattern of reduction could display an inconsistent trend, depending on environmental conditions, soil type and genetic traits of the cultivar. These may have sudden incremental effects on the Chl content or sometimes erratic changes in the initial period of stress or mild stress. Then, as the stress continues to persist, the Chl content will decrease subsequently (Ebrahimiyan *et al.*, 2013; Etemadi *et al*., 2015; Fu and Huang, 2001; Jiang and Huang, 2001; Manuchehri and Salehi, 2015; Pirnajmedin *et al*., 2015; Sarmast *et al*., 2015). The results of this study indicate that less frequencies of irrigation can cause reductions in the Chl and generally 'H-d' indicates better function than 'J-r' especially under the greenhouse condition, but when plants were treated with AMFs, the lower irrigation frequency was less detrimental in comparison to the control. AMFs increase the Chl concentration in the plant by enhancing the plant's N and Mg uptake, as these are inseparable components of the chlorophyll molecule (Miransari, 2014). Furthermore, the AMFs serve to increase the surface area between the roots and the soil. In this respect, they increase the leaf water potential and antioxidant enzyme activity in a manner that leads to the Chl being less damaged under stressful conditions (Augé, 2001, 2004; Durán *et al*., 2016).

GB and Pro are the main organic osmolytes that accumulate in response to environmental stresses in plants. They regulate the osmotic pressure and also maintain the redox balance by regulating Mg and ROS metabolism (Chen and Murata, 2008; Szabados and Savoure, 2010). Different studies have investigated the effects of drought stress on the tolerance of tall fescue cultivars. Reports indicate that the Pro content increased in plants in response to drought (Ebrahimiyan *et al*., 2013; Etemadi *et al*., 2015; Man *et al*., 2011; Manuchehri and Salehi, 2015; Pirnajmedin *et al*., 2015; Sarmast *et al.*, 2015). According to previous evidence and this experiment, the tall fescue may be introduced as a specific model plant for its increase in Pro content when being exposed to drought stress. In this experiment, the GB concentration increased under drought stress. Our results confirm a few studies on monocotyledons such as the report concerning Corn (Quan *et al*., 2004). In the present study, the increase in amino acids content was observed parallel to the increase of II. The 'J-r' as drought-tolerant cultivar had higher levels of amino acid content than 'H-d'; AMFs treatments showed the same compared to the control, especially under field condition as a result of severe stresses. There is no report about AMFs mechanism in osmolytes, however some reports indicate positive relations between these microorganisms and the amount of amino acids against abiotic stresses (Pinior *et al*., 2005).

Researches show that stresses such as water deficiency can lead to cellular damage by ROS activation. The SOD accelerates O_2•- conversion to H_2O_2 and O_2 in plants which are exposed to abiotic stress. The POX improves plant growth under stressful conditions by oxidizing various substrates using H_2O_2 and by preventing the excess accumulation of H_2O_2 (Karuppanapandian *et al*., 2011). Many studies about the tall fescue showed that the SOD and POX enzymes activity increase in stress conditions. However, in some cases, enzyme-disorder activity was observed along with the decrease or erratic decrease-increase in enzyme activity due to the severity of stress and the special features of the cultivar in coping with stress (Etemadi *et al*., 2015; Fu and Huang, 2001; Jiang and Huang, 2001; Manuchehri and Salehi, 2015; Pirnajmedin *et al*., 2015; Sarmast *et al.*, 2015). In this study, the 'J-r' with fungal treatments had optimum enzymatic performance against stress, except for POX in the greenhouse condition. Most fungal treatments and for most of the IIs, either the greenhouse or the field condition increased enzymatic activity, but in some cases the Fm could not change the POX activity in the greenhouse in comparison with the control. Altogether, most treatments led to the increase in enzymatic activity parallel to the increase in II, but we found instances of decrease-increase and erratic changes in enzymatic activity. This erratic change is in agreement with previous studies. It seems that mycorrhizal colonization and the arbuscule have important roles in O_2•- reduction and facilitate the degradation of H_2O_2 in the plant against abiotic stresses. Plant symbiosis with AMFs in the short-run can increase enzyme activity and induce enzyme genes and new isoforms (Durán *et al*., 2016; Wu *et al*., 2014).

Phosphorus is an essential macroelement for the plant and plays crucial roles in some main cell functions in the plant such as sugar-phosphate intermediate of respiration and photosynthesis and phospholipids that make up the plant membrane. Under stress conditions, the P content and uptake decrease and lead to a less vigorous growth, narrow shoot production, leaf senescence and leaf firing. In this experiment, the P content decreased as the II increased, which is in agreement with a previous report by Huang (2001). Moreover, this reduction was lesser in 'J-r' compared to 'H-d' and occurred less in AMFs treatments, as well, especially when treated by Ri. The main role of AMFs is to make available the inactive macroelements in the soil to the plant. The uptake of P is improved by AMFs under stress because of the integrity maintenance of vacuolar membranes and different enzymes production such as phosphatase (Smith and Read, 2008; Miransari, 2014).

Researchers have shown that by increasing the RC, the total surface area covered by the fungus increases as well (including root length, root architecture, and root/shoot ratio) which prompts the improvement in relations of nutrient acquisition and hydration. Many studies have shown that RC could decrease in response to the scarcity of moisture in the soil, however the percentage of RC may differ based on the soil type and plant or fungi species (Augé, 2001, 2004; Grümberg *et al*., 2015; Wu *et al*., 2013). In this study, the RC percentage decreased with increasing the II while in general, this reduction was lesser in 'J-r' compared to 'H-d'. According to the results, there is a direct relationship between plant growth and RC. Under stress conditions, plant growth decreases. However, by increasing the RC, the plant growth increases relatively in comparison with the control. Due to controlled condition in the greenhouse, especially in terms of moisture, turfgrasses which were in the greenhouse had higher levels of growth and RC than the field-grown ones.

5. Conclusions

Management against drought stress requires novel approaches of which the AMFs can generate promising results. Turfgrass is commonly replaced with other drought-tolerant plants in places where the water consumption of turfgrass is deemed too excessive. The question remains whether any solutions exist to reduce turfgrass water requirement so as to prevent the turfgrass from being replaced with other plants. This study was the first extensive research that investigated plant biomass, the amino acid and antioxidant enzymes activity on a quantitative and qualitative basis, in the light of turfgrass treatment with AMFs. Characteristics of the tall fescue can be improved as a result of its interaction with AMFs – besides monitoring water relationship by applying different IIs. Our results proved that 'J-r' and 7-day II with Fm treatment is the best in terms of inducing plant tolerance against drought. In regions where limitations to water resources are prevalent, the 14-day II could also yield acceptable quality in the tall fescue. This research concluded that AMFs can save vital organs of the tall fescue, especially the crown, when being under severe and long stressful conditions. As a matter of consequence, then, most of the organs can be revived in the recovery period. Where drought stress is problematic, this research recommends the turfgrass be inoculated with AMFs during the rainy season in order to increase turfgrass compatibility and to strengthen RC. This research can provide a methodological template for more studies on other species of turfgrass, since turfgrass have seldom been treated with AMFs.

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