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Changes in Antioxidant Enzymes Activity and Physiological Traits of Wheat Cultivars in Response to Arbuscular Mycorrhizal Symbiosis in Different Water Regimes

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ABSTRACT- This study was conducted to evaluate changes in antioxidants, free proline, relative water content and determination of root colonization of four commercial wheat (*Triticum aestivum* L.) cultivars (Azar2, Darab2, Shiraz, and Falat) inoculated with the fungus *Glomus intraradices*, under four water regimes of 100, 75, 50, and 25% of field capacity in the year 2010 at the School of Agriculture, Shiraz University. The means for leaf area, shoot fresh weight, root fresh weight, shoot dry weight and root/shoot ratio were 6.0, 10.2, 15.6, 25.2, and 10.31% respectively higher in the mycorrhizal as compared to non-mycorrhizal cultivars. Water deficit stress reduced root colonization percentage and the highest root colonization (28.10%) was observed in the cultivar Azar2. Compared to the non-mycorrhizal plants, inoculation increased the average values for relative water content, proline content, total chlorophyll content, total protein, superoxide dismutase, peroxidase, and catalase activities of mycorrhizal plants by 5.5, 35.6, 13.8, 21.6, 22.5, 22.7, and 15.5%, respectively. The highest peroxidase (9.77 U mg^{-1}), catalase (9.82 U mg^{-1}), and super oxide dismutase (19.80 U mg^{-1}) activities were obtained by Azar2. The results indicated that inoculation with *Glomus intraradices* alleviated the deleterious effects of water deficit stress on wheat cultivars via proline accumulation and increased antioxidant activities. The cultivars Azar2 and Darab2 had higher values for most of the antioxidants and root colonization. Consequently, these cultivars could be used in wheat breeding programs for better symbiosis and drought tolerance.

Keywords: Antioxidant enzymes, *Glomus intraradices*, Symbiosis, Water regime, Wheat

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INTRODUCTION

Being the most important cereal, the global production of wheat is severely affected by drought in many parts of the world. It has been proven that drought stress and water deficit conditions significantly affect photosynthesis, chlorophyll content and enzymatic and photochemical activities in Calvin cycle and consequently, the productivity of crop plants (9, 27, 33, 49 and 49). Water deficit condition changes the balance between the reactive oxygen species (ROS) production (including, superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-)) and antioxidant defense systems. As a consequence, these changes result in oxidative stress and damage to proteins, membrane lipids, and other cellular components (27). The antioxidant defense systems in plant cells include enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) and also proline as a non-enzymatic antioxidant. The toxic superoxide radical is usually dismutated by SOD to H_2O_2, a product which is relatively stable and detoxified by CAT and POD (25).

In the recent years, using biological methods and considering the potential of beneficial bacteria and fungi to elevate defense mechanisms in plants dealing with stress conditions has received increased attention (9, 19, 22, 39, 43, 45 and 47). Mycorrhizal symbiosis is known to alleviate the adverse effects of abiotic stresses. Arbuscular mycorrhizal (AM) fungi have been shown to enhance plant growth and drought tolerance by altering the physiochemical properties of the host plant, increasing water absorption capacity, increasing root hydraulic conductivity, and adjusting osmotic balance (2, 10, 14, 22, 23 and 43). Penetration to the root and the intracellular growth of the AM fungi involve complex sequences of biochemical and cytological events and intracellular modifications (14). The AM fungi are obligatory symbiotic soil organisms that colonize plant roots in some of the crops and improve their performance (5) by increasing nutrient supplies to the plants and reducing water stress effects (46 and 10).

The inoculation of plants with AM fungi increases antioxidant enzymes' activities in plant shoots and roots (4). On the other hand, mechanisms such as enhanced osmotic adjustment and leaf hydration, reduced oxidative damage, and improved nutritional status have been linked to AM-host plants symbiosis under drought conditions (4 and 10). Plants' response to water stress is complex and includes molecular and biochemical changes in the whole plant (18). It is well-known that osmotic regulators such as proline are relevant to the evaluation of the osmotic adjustment ability and drought resistance in plants (16). Higher activities of several enzymes during drought stress periods have been found in mycorrhizal compared to non-mycorrihzal plants (10). In addition, antioxidant enzymes scavenge ROS and decrease oxidative damages to plant cells; hence mycorrhizal cultivars are more tolerant than their non-inoculated counterparts. The significant role of AM fungi in enhancing POD activity and shoot and root dry matter has been reported for *Juniperus oxycedrus* (37). AM fungi have beneficial effects on wheat growth under drought conditions (2). Evaluating two wheat cultivars, Talaat and Shawky (45) indicated increased proline accumulation, POD and CAT activities in mycorrhizal wheat plants under salinity stress. Therefore, reviewing the literature suggests that using AM fungi improves the growth of plants including wheat in stress and water limited conditions, alleviates the adverse effects of drought and shows the necessity of evaluating the genetic variability of plants in order to screen varieties with better adaptability to AM fungi symbiosis.

Many reports concerning stress tolerance focus on the potential effects of AM fungi on plant's growth using single or a few cultivars (2, 3 and 45) and specific traits, but in the present study both physiological characteristics and antioxidant activities in addition to the root colonization (RC) percentage in four various wheat cultivars and different water regimes were considered simultaneously. In other words, this study was carried out to (1) evaluate the effect of an AM fungus on changes in antioxidant enzyme activities, free proline, water status parameters, and some physiological characteristics of different wheat cultivars and (2) to determine the variations in the wheat cultivars in response to mycorrhizal inoculation and different water regimes.

MATERIALS AND METHODS

Experimental procedures

The experiment was carried out in a greenhouse at the Agricultural Experiment Station of the Crop Production and Plant Breeding Department, College of Agriculture, Shiraz University, Shiraz, Iran in 2010. A factorial experiment based on a completely randomized design with three replications was used to evaluate the effects of the AM fungus and different water regimes (100, 75, 50, and 25% of field capacity (FC)) on four commercial wheat cultivars (Azar2, Darab2, Shiraz and Falat that provided by the Seed and Plant Improvement Institute, Karaj, Iran).

For each cultivar a number of plants were inoculated with the fungus, *Glomus intraradices* Schenck and Smith and some of plants were kept without any spore inoculation for preserving the naturally-occurring microbial association to be used as control. The inoculants of *G. intraradices* were obtained from the Department of Soil Science, Shiraz University. This fungus is abundant in Iranian soils (6, 28, 40, 50, 51, 52 and 53). Mycorrhizal inoculants were prepared through the trap culture of maize (*Zea mays* L.) (32). The trap culture medium was composed of autoclaved soil/quartz-sand (< 1 mm) (1: 4, v/v). The soil samples used for the pot experiment were collected from Bajgah, Shiraz, Fars, Iran. The physical and chemical properties of the soil samples (Table 1) were determined based on Page et al.'s (34) procedures. The 5 kg pots were filled with 4mm-sieved air-dried soil. In order to be close to real field conditions, the soil samples were not sterilized. 150 mg N kg^{-1} soil was used as urea 46% N fertilizer in all pots. The seeds were treated with ethanol 98% for about 20 s and were then washed three times with distilled water and kept at 20 °C. In mycorrhizal plants, 50 g of AM inoculants (containing spore numbers of 8 g^{-1} substrate and a root colonization of 85%) were added to the pots just below the seeds at the time of sowing. The potential of the inoculants was measured based on the methods described by Zarei et al. (51), for spore extraction and counting, and evaluating root colonization. Eight seeds were sown at a 3 cm depth in each pot. After germination, seedlings were thinned to four plants in each pot.

The water regimes were applied during tillering and maturity stages of the plants growth. For each water regime, the pots were daily weighed and watered until they reached the desired FC level. The temperature during the experiment ranged from 15 to 28 ˚C, with a 16/8 h light/dark photoperiod.

Five months after the sowing date and at the beginning of the reproductive stage, shoots were removed (cut at soil level) and the pots' contents maintained in polyethylene bags at 4 ˚C.

Table 1. Physical and chemical properties of the soil samples used for the greenhouse experiment

Sand (%)	Silt (%)	Clay (%)	EC (dS m^{-1})	pH		SP (%)	FC (%)	PWP (%)
18	49	33	0.5	7.9		54.7	25.3	9.1
OC (%)	**CaCO3 (%)**	**TKN (%)**	**Olsen P (mg kg^{-1})**	**K (mg kg^{-1})**	**Fe * (mg kg^{-1})**	**Zn* (mg kg^{-1})**	**Mn* (mg kg^{-1})**	**Cu* (mg kg^{-1})**
1.3	11	0.06	15	240	5	1.7	11.3	2

EC: electrical conductivity of saturated paste, OC: organic carbon, SP, FC, and PWP: soil moisture at saturation, field capacity, and permanent wilting point, TKN: total Kjeldahl N, K: NH_4OAc-extractable potassium and, *DTPA-Extractable Fe, Cu, Mn, and Zn.

Root colonization (RC) and leaf area measurements

To assess the rate of RC, fresh root samples were fixed in formalin/acetic acid/alcohol solutions (FAA). After washing the roots in 8% KOH and staining with blue ink (Pelikan) and lactoglycerol (v/v) the grid-line intersect method was used to measure the percentage of the AM-root colonization (30). Leaf lengths and diameters of mycorrhizal and non-mycorrhizal plants were measured with a ruler and leaf area (LA) was calculated based on the following formula:

LA = maximum leaf length × maximum leaf width × 0.75.

Total chlorophyll (Chl) content

The total Chl content of the leaves was extracted using Arnon's (8) method. Total Chl was extracted in 80% cold acetone (4 °C) and the absorbance of the extract was measured spectrophotometrically at 645 and 663 nm, the total Chl being calculated based on the following formula (31):

Chl (mg mL^{-1}) = 20.2 × (A645) + 8.02 × (A663)

Where, A is spectrophotometer reading.

Antioxidants, proline and protein

Leaf samples (0.5 g) were homogenized in 10 mL of 3% (w/v) aqueous sulphosalicylic acid and the solution was filtered using Whatman No. 2 filter paper. Proline concentration (μmol g^{-1} fresh weight) was measured based on the procedure proposed by Bates et al. (11).

Total protein (Pr T) content was estimated using Bradford's (15) protocol, in which bovine serum albumin (BSA) was used as a standard. The activity of the SOD was determined based on its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (12 and 21). One unit (U) of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT at 560 nm (24).

POD activity was assayed (36) at 436 nm based on its ability to convert guaiacol to tetraguaiacol (ε = 26.6 mM cm^{-1}). The activity of CAT was determined by monitoring the disappearance of H_2O_2 at 240 nm (ε = 40 mM cm^{-1}) (1).

Relative water content (RWC)

Prior to measuring RWC, the root fresh weight (RFW) and the shoot fresh weight (SFW) of the plants were measured separately. Shoot dry weight (SDW) was also measured after drying at 65 ˚C for 72 h. Twenty-two days after the application of the

water regimes, the shoot of the smallest plant in each pot was sampled and weighed immediately for SFW determination. After being immersed in distilled water for 24 h, the turgid weight (TW) of the plants' shoot was measured. Then, the leaves were kept in an oven for 24 h at 60 °C and SDW was measured in each pot. The RWC was calculated using RWC= (SFW-SDW) ×100/ (TW-SDW) as a standard formula (54).

Statistical analyses

A test for normality of data was used to check normal distribution in Minitab software (v.14) and consequently the data for RC and RWC were log-transformed to obtain a normal frequency distribution. The data were analyzed based on a factorial experiment and a completely randomized design in SAS software (V. 9.1). A Least Significant Difference (LSD α= 0.05) test was used for mean comparisons. Mean comparisons were conducted for different water regimes and the cultivars regardless of mycorrhizal inoculation and performed separately for the means in inoculated and non-inoculated conditions. The difference ratio (D %) between mycorrhizal and non-mycorrhizal cultivars for all traits was calculated based on D= (mycorrhizal mean-non mycorrhizal mean)*100/non-mycorrhizal mean. In order to determine the variation among cultivars, the coefficients of variation (CV %) for all the traits were calculated based on the following formula:

$$CV = (\frac{\sqrt{MSE}}{\overline{X}})100$$

Where, MSE and $\overline{X}$ are the error mean square and the trait mean respectively.

RESULTS

Physiological traits and root colonization

The traits' means in the mycorrhizal cultivars were significantly greater than that of their non-inoculated counterparts (Table 2). As compared to non-mycorrhizal plants, mycorrhizal inoculation increased LA, SFW, SDW, RFW and the root/shoot ratio by 6.0, 10.2, 15.6, 25.2 and 10.31%, respectively (Table 2). RC was significantly much higher in mycorrhizal plants, but decreased as the severity of water deficit increased in mycorrhizal plants. The highest and the lowest RCs were found in Azar2 (28.10%) and Shiraz (23.81%) cultivars (Table 2). Azar2 plants treated with the AM fungus showed relatively higher RWC, SFW, RFW, SDW, and RC in 50% and also 25% FC levels as compared to those that were not treated (Table 3). A similar result was obtained for Shiraz, indicating that inoculated plants had higher amounts of SFW (3.41 g), RFW (0.67 g), SDW (3.28 g), and RC (32.4%) in 25% FC in comparison to the non-mycorrhizal Shiraz cultivar (Table 3). The root/shoot ratio was highest in mycorrhizal Azar 2 (0.28) and increased as the severity of drought increased (Table 2 and 3). Water deficit stress reduced LA, SFW, SDW, and RFW and also caused a significant decrease in RC in all cultivars (Table 2). The highest LA (95.08 cm^2), SFW (19.45 g), SDW (12.91 g), RFW (2.41 g), and RC (31%) were observed in well watered (100% FC) plants, while the lowest amounts were recorded in 25% FC as sever water deficit conditions (Table 2).

Table 2. Mean comparison for measured traits in wheat cultivars under different water regimes and arbuscular mycorrhizal-symbiosis treatments

	RWC (%)	SFW (g)	RFW (g)	R/SH	SDW (g)	RC (%)	LA (cm^2)
Inoculation							
M	74.3 a*± 1.60	13.11 a± 1.03	1.54 a± 0.25	0.12 a± 0.014	8.74 a± 0.61	41.56 a± 1.17	80.80 a± 3.03
NM	70.5 b± 1.76	11.90 b± 1.01	1.23 b± 0.18	0.11 a± 0.009	7.56 b± 0.54	9.79 b± 0.60	76.23 a± 3.08
D (%)	5.4	10.2	25.2	10.31	15.6	324.5	6.0
Water regime (FC %)							
100	85.3a± 0.66	19.45a± 0.90	2.41 a± 0.45	0.10 b± 0.029	12.91 a± 0.55	31.04 a± 3.88	95.08 a± 4.00
75	80.1b± 0.77	16.96 b± 0.90	1.75 b± 0.32	0.09 b± 0.022	9.278 b± 0.61	29.35 b± 3.85	83.20 b± 4.79
50	70.2 c± 0.81	8.848 c± 0.73	0.85 c± 0.10	0.11 ab± 0.010	6.690 c± 0.34	23.14 c± 3.10	71.44 c± 2.53
25	55.5 d± 1.06	4.797 d± 0.54	0.55 d± 0.05	0.14 a± 0.013	3.735 d± 0.20	19.20 d± 2.85	64.37 d± 2.88
Cultivars							
Azar 2	75.1 a± 2.49	17.20 a± 1.36	3.152 a± 0.46	0.16 a± 0.035	10.4 a± 0.85	28.10 a± 3.72	95.07 a± 5.83
Darab 2	71.6 b± 2.25	10.90 b± 1.24	0.894b± 0.08	0.09 b± 0.012	7.95 b± 0.74	25.65 b± 3.36	69.72 bc± 2.63
Falat	72.4 b± 2.35	10.36 b± 1.22	0.877b± 0.08	0.11 b± 0.017	7.31 b± 0.88	25.16 b± 3.52	80.08 d± 2.76
Shiraz	70.8 b± 2.65	11.59 b± 1.53	0.638 c± 0.08	0.08 b± 0.011	6.95 b± 0.67	23.81 b± 3.60	69.22 b± 3.12
Coefficient of variation (%)	29.33	15.15	12.34	30.08	16.89	31.76	25.04

Table 2- continued

	Chl (mg mL^{-1})	Proline (µm g^{-1})	Pr T (mg mL^{-1})	POD (U mg^{-1})	CAT (U mg^{-1})	SOD (U mg^{-1})
Inoculation						
M	44.38a±2.01	6.4207a±0.54	9.07a±0.38	10.10a±0.54	9.11 a±0.45	18.23 a±0.69
NM	38.99b±2.11	4.7347b±0.43	7.46b±0.42	8.22b±0.54	7.89 b±0.42	14.89 b±0.69
D (%)	13.8	35.6	21.6	22.7	15.5	22.5
Water regime (FC %)						
100	59.95a±1.97	2.93a±0.19	6.274a±0.44	5.670a±0.44	5.74 a±0.32	13.06 a±0.74
75	43.84b±1.60	4.11b±0.51	7.306a±0.29	7.120b±0.46	6.61 a±0.38	14.51 a±0.88
50	34.25c±1.64	5.21c±0.42	9.307bc±0.58	11.05c±0.48	10.1 b±0.36	18.25 b±0.90
25	28.68d±1.58	10.1d±0.60	10.17c±0.64	12.82d±0.69	11.5 c±0.51	20.41 c±0.89
Cultivars						
Azar 2	49.46a±2.58	7.27a±0.88	6.63b±0.32	9.77a±0.61	9.82a±0.56	19.80a±1.14
Darab 2	41.02b±2.57	5.31b±0.69	8.97a±0.63	9.26a±1.03	8.03bc±0.82	16.81b±0.92
Falat	41.19b±3.48	5.05b±0.50	9.12a±0.55	8.85a±0.85	8.70b±0.48	15.24bc±0.93
Shiraz	35.05c±2.47	4.69b±0.66	8.35a±0.67	8.83a±0.60	7.46c±0.51	14.37c±0.82
Coefficient of variation (%)	12.56	8.78	6.12	19.89	11.24	14.44

FC: field capacity, M: mycorrhizal plants, NM: non-mycorrhizal plants, D: difference between M and non-NM cultivars, RWC: Relative water content; SFW: Shoot fresh weight: RFW: Root fresh weight; SDW: Shoot dry weight; RC: Root colonization; LA: Leaf area; Chl: Chlorophyll content; Pr T: Total protein content; POD: Peroxidase; CAT: Catalase; SOD: Superoxide dismutase, *: digits with different letter are significantly different

Table 3. Mean of physiological traits in the mycorrhizal and non-mycorrhizal cultivars under different water regimes

Water regime (FC %)	Cultivar	RWC (%)		SFW (g)		RFW (g)		R/SH	
		M	NM	M	NM	M	NM	M	NM
100%	Azar 2	88.70	84.00	25.40	24.00	7.13	4.74	0.28	0.20
	Darab 2	84.70	82.00	17.07	16.50	1.40	1.43	0.08	0.09
	Shiraz	86.47	84.03	15.95	14.97	1.26	1.32	0.06	0.06
	Falat	82.50	83.87	20.67	21.07	1.16	0.82	0.08	0.05
75%	Azar 2	84.57	82.53	22.90	19.00	4.78	3.83	0.21	0.20
	Darab 2	78.97	75.10	15.90	15.00	1.30	0.85	0.09	0.07
	Shiraz	79.17	76.10	16.33	15.10	0.93	0.83	0.06	0.06
	Falat	80.47	80.23	16.27	15.17	0.75	0.72	0.05	0.05
50%	Azar 2	75.03	69.07	15.07	13.47	1.61	1.57	0.11	0.12
	Darab 2	72.40	68.80	8.170	7.000	0.69	0.61	0.09	0.09
	Shiraz	73.20	67.67	7.900	6.330	0.86	0.60	0.12	0.11
	Falat	70.00	65.73	7.310	5.530	0.53	0.35	0.07	0.06
25%	Azar 2	61.57	54.17	9.570	8.230	0.80	0.76	0.09	0.10
	Darab 2	58.30	52.57	4.200	3.400	0.46	0.42	0.11	0.12
	Shiraz	57.53	54.87	3.410	2.870	0.67	0.55	0.24	0.20
	Falat	55.27	48.63	3.800	2.900	0.44	0.34	0.13	0.13
	$LSD_{0.05}$	1.39		1.14		0.16		0.097	

Table 3- continued

Water regime (FC %)	Cultivar	SDW (g)		RC (%)		LA (cm^2)	
		M	NM	M	NM	M	NM
100%	Azar 2	16.23	14.36	52.2	16.4	124.4	122.5
	Darab 2	14.32	11.42	47.6	15.1	81.40	85.74
	Shiraz	13.13	12.49	48.5	12.0	91.59	91.41
	Falat	11.38	9.98	46.5	10.0	86.34	77.13
75%	Azar 2	14.57	10.32	50.1	12.8	122.6	103.7
	Darab 2	9.710	7.49	45.8	13.9	71.40	62.55
	Shiraz	8.410	8.25	45.7	9.80	66.11	89.47
	Falat	8.350	7.13	48.1	8.50	75.08	74.54
50%	Azar 2	9.060	8.50	40.5	10.7	82.69	81.13
	Darab 2	6.910	6.39	36.2	7.80	62.76	62.42
	Shiraz	5.260	4.81	37.9	8.90	88.32	66.07
	Falat	6.620	5.98	35.7	7.30	59.91	69.20
25%	Azar 2	5.220	4.95	35.9	6.10	68.91	54.34
	Darab 2	4.030	3.36	32.6	6.20	66.42	66.09
	Shiraz	3.280	2.85	32.4	6.10	81.97	65.67
	Falat	3.370	2.82	29.1	5.20	63.87	47.70
	$LSD_{0.05}$	0.70		4.40		13.50	

FC: field capacity, M: mycorrhizal, NM: non-mycorrhizal, RWC: Relative water content, SFW: Shoot fresh weight, RFW: Root fresh weight, R/SH: root/shoot weight ratio, SDW: Shoot dry weight, RC: Root colonization, LA: Leaf area

The effect of AM fungus on RWC was significant and inoculation increased RWC by 5.4% (Table 2). The inoculation of plants with AM fungus significantly increased the RWC mean in the cultivar Azar 2 (61.57%) as compared with its non-mycorrhizal (54.17%) plants in the 25% FC water regime (Table 3). Under inoculation treatments and the 25% FC, the RWC values for the two drought sensitive cultivars, Shiraz and Falat, were 57.53% and 55.27%, respectively; while in non-inoculated conditions their corresponding values were 54.87 and 48.63% (Table 3). Water deficit stress treatments decreased RWC since the highest (85.3%) and the lowest (55.5%) RWCs were recorded in the 100% and the 25% FC, respectively. Among the cultivars, the highest (75.1%) RWC was recorded for Azar 2.

Chlorophyll content and total protein

A 13.8% and 21.6% increase was observed in Chl content and Pr T in the AM-treated plants compared to the non-AM counterparts (Table 2). Total Chl decreased as the severity of the water deficit increased in both inoculated and non-inoculated conditions, but the mycorrhizal plants had higher Pr T in the severe drought conditions (Table 4). Although water deficit stress reduced Chl content in all plants, a marked increase was observed in the leaves' Pr T contents under inoculation conditions. The highest Chl (59.95 mg mL^{-1}) and Pr T contents (10.17 mg mL^{-1}) were observed in the 100% and the 25% FC water regimes, respectively (Table 2). A significant increase in Chl and Pr T was observed for most of the mycorrhizal cultivars as compared with their non-mycorrhizal counterparts. Azar 2 and Darab 2 cultivars showed the highest Chl and Pr T contents, respectively, in all water regimes under inoculation treatments (Table 4).

Proline and antioxidants

Proline content was significantly influenced by the effect of inoculation (Table 2) and the mycorrhizal plants had much higher proline content (6.42 μm g^{-1}) than their non-inoculated counterparts (4.73 μm g^{-1}). In both mycorrhizal and the non-mycorrhizal plants, free proline contents significantly increased as drought levels increased, specifically, under 25% FC. Azar 2 had the highest free proline (7.27 μm g^{-1}), while the lowest (4.69μm g^{-1}) amount was found in the Shiraz cultivar (Table 2). Also, the difference between mycorrhizal and non-mycorrhizal cultivars was significant in most water regimes.

All antioxidant enzymes increased as the FC levels decreased in both mycorrhizal-treated and non-mycorrhizal plants, but inoculation had more effect on increasing the enzymes' activities (Table 4). The mean activities of CAT, SOD, and POD were 15.5%, 22.5%, and 22.7%, respectively, higher in the inoculated plants as compared with their non-inoculated counterparts (Table 2). The mycorrhizal Azar 2 plants had the highest values for SOD in all water regimes, and also for POD and CAT, except at the 25% FC (Table 4) under inoculation conditions. Based on the means of the inoculated and non-inoculated treatments, Azar 2 showed the highest activity for all antioxidant enzymes including POD (9.77 U mg^{-1}), SOD (19.80 U mg^{-1}) and CAT (9.82 U mg^{-1}) (Table 2). The activities of the POD and the CAT enzymes in Darab 2 were higher than in the other cultivars in the 25% FC (Table 4).

Table 4. Mean of biochemical traits in the mycorrhizal and non-mycorrhizal cultivars under different water regimes

Factor		Chl (mg mL^{-1})		PrT (mg mL^{-1})		Proline (µm g^{-1})	
Water regime (FC %)	Cultivar	M	NM	M	NM	M	NM
100%	Azar 2	69.03	65.90	6.21	5.33	3.22	2.16
	Darab 2	60.10	57.15	6.25	6.10	4.05	1.58
	Shiraz	65.86	60.27	8.41	6.06	3.12	3.28
	Falat	51.54	49.77	7.01	4.82	2.93	3.07
75%	Azar 2	53.28	49.07	8.00	5.97	8.91	3.18
	Darab 2	44.31	38.28	8.71	5.99	3.06	2.03
	Shiraz	49.53	41.80	8.67	6.92	6.89	4.22
	Falat	40.13	34.32	7.71	6.49	2.98	1.62
50%	Azar 2	44.68	39.41	6.78	5.55	9.30	5.23
	Darab 2	36.19	32.05	11.59	10.97	6.60	4.53
	Shiraz	37.23	26.28	10.57	9.91	3.84	3.51
	Falat	31.39	26.79	10.87	8.22	3.53	5.11
25%	Azar 2	38.48	35.83	8.81	6.39	13.99	12.13
	Darab 2	32.70	27.39	12.41	9.73	10.88	9.72
	Shiraz	27.71	20.87	11.50	10.90	8.46	7.05
	Falat	27.86	18.64	11.70	9.97	10.97	7.34
	LSD0.05	2.67		0.93		0.56	

Table 4- continued

Factor		POD (U mg^{-1})		CAT (U mg^{-1})		SOD (U mg^{-1})	
Water regime (FC %)	Cultivar	M	NM	M	NM	M	NM
100%	Azar 2	8.08	6.32	8.47	5.05	19.12	12.85
	Darab 2	4.33	4.30	4.44	4.13	10.74	15.49
	Shiraz	6.25	3.95	7.14	6.51	12.01	10.61
	Falat	7.77	4.38	5.16	5.00	14.67	9.03
75%	Azar 2	9.88	6.94	9.15	7.57	21.90	14.13
	Darab 2	7.34	6.29	5.87	4.37	15.34	12.84
	Shiraz	8.18	4.43	7.55	6.64	14.37	11.04
	Falat	8.93	4.97	6.55	5.21	15.18	11.25
50%	Azar 2	11.22	11.08	12.19	11.31	24.31	19.08
	Darab 2	10.63	10.28	10.83	10.22	20.93	17.40
	Shiraz	12.68	10.87	10.95	8.68	17.70	16.55
	Falat	11.68	10.02	8.69	8.14	16.19	13.83
25%	Azar 2	13.18	11.48	12.91	11.88	24.97	22.02
	Darab 2	16.18	14.71	12.94	11.43	21.75	20.00
	Shiraz	13.14	10.98	12.40	9.77	21.24	18.44
	Falat	12.21	10.67	10.51	10.45	19.27	15.58
	LSD0.05	1.01		0.70		1.43	

FC: field capacity, M: mycorrhizal, NM: non-mycorrhizal, Chl: Chlorophyll content, Pr T: Total protein content, POD: Peroxidase, CAT: Catalase, SOD: Superoxide dismutase

DISCUSSION

From the results of the present study it is obvious that most of the evaluated traits were significantly higher in the mycorrhizal wheat cultivars as compared with their non-mycorrhizal counterparts under different water regimes. Other reports also support the beneficial effects of the AM fungi on plant growth under drought conditions in wheat (2) and in other plants (10 and 38). It has been well-established that AM symbiosis protects host plants against negative effects of drought stress due to nutritional, physical and cellular improvements (39). In addition, the AM symbiosis increases the host plant's growth due to the improved nutrient absorption and better water uptake via external hyphae in inoculated roots (44). Many drought-adapted species from arid environments have a highly developed root system which may be considered as a mechanism of drought tolerance (37). The present study showed that the fungus *G.intraradices*, effectively colonized wheat plants. In addition, RC decreased in the mycorrhizal wheat cultivars as the levels of drought stress increased. Decreased ratio of RC in AM-treated Azar 2 was lower than in the other cultivars in the severe water deficit conditions, indicating variation among cultivars in response to the AM colonization.

As one of the characteristics of the drought tolerant plants, a significantly higher RWC was observed in the mycorrhizal wheat cultivars. Higher RWC in the mycorrhizal cultivars could be due to increased water uptake by the mycorrhizal hyphae in the roots. Drought stress decreased RWC in all the cultivars, while the resistant (Azar 2) and semi-resistant (Darab 2) cultivars showed higher RWC than the sensitive plants (Shiraz and Falat) due to their mechanism of uptaking higher water or preventing water loss from their shoots. The root/shoot ratio indicated that the fungus *G. intraradices* contributed to increased root weight and therefore higher uptake by the root under drought and normal irrigated conditions. The AM symbiosis may postpone declines in the leaf RWC of wheat under drought conditions (35) and changes in shoot water content relationships (13). In addition, hyphae may increase the soil to root contact in the soils with limited water content.

Proline content, Pr T, and all antioxidant enzymes increased as the severity of water deficit stress increased in both the mycorrhizal and non-mycorrhizal cultivars, but increasing rates were much higher in the former. Therefore, it can be concluded that these traits can be used as criteria for drought tolerance screening. In the literatures, there are conflicting reports about proline, indicating its higher (41 and 42) or lower (42 and 47) concentration in mycorrhizal plants under drought stress conditions. For instance, Ruiz-Sánchez et al. (38) reported lower accumulation of free proline in mycorrhizal rice as compared to non-mycorrhizal plants. Results of the present study showed that the Chl content decreased in lower FC % levels, indicating damaged photosynthetic apparatus under water deficit conditions; meanwhile, Chl contents in the mycorrhizal plants were significantly higher than those of the non-inoculated ones. Higher Chl content in the leaves of the mycorrhizal plants under stress conditions has been reported by Auge (10), Colla et al. (17), Kaya et al. (29), and Hajiboland et al. (26).

Our results showed higher activities of CAT, POD, and SOD in the inoculated wheat cultivars compared with the non-inoculated ones. It is assumed that increase in the production of antioxidant enzymes in inoculated plants under stress conditions is a defense mechanism. The activities of POD and CAT were lower compared to the activity of SOD, indicating the higher role of SOD in stress conditions. Antioxidant enzymes such as CAT, POD, and SOD are known to scavenge ROS in plants (20).

AM symbiosis effects reactive oxygen metabolism and antioxidant production, but the exact mechanisms involved are unclear (47 and 48). It has been proposed that SODs act as the first line of defense against ROS, dismutating superoxide to H_2O_2 and subsequently detoxified by CAT (7). In the preset study, the highest SOD activity was recorded for the mycorrhizal Azar 2 plants in all water regimes.

CONCLUSIONS

The results showed that there was noticeable variation among the wheat cultivars in response to AM symbiosis and that the fungus, G. intraradices, alleviated the deleterious effects of water stress through accumulating more proline and increasing the antioxidant enzymes' activities.

According to the results it can be concluded that among the antioxidant enzymes, SOD may act as a better criterion for drought tolerant screening since its activity in different cultivars and water regimes was in concordance with other physiological characteristics. The response of the cultivars to AM inoculation was different, the mycorrhizal Azar 2 plants showing the highest values for LA, RWC, SFW, RFW, SDW, RC and SOD as relevant traits for increasing drought tolerance in wheat.

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تغییرات درکارکرد آنزیم‌های آنتی‌اکسیدان و صفات فیزیولوژیک ارقام گندم در پاسخ به همزیستی *Arbascular mycorrchizal* در رژیم‌های مختلف آبیاری

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چکیده- این تحقیق به منظور ارزیابی تغییرات در کارکرد آنزیم‌های آنتی اکسیدان، پرولین، محتوای نسبی آب و تعیین کلنیزاسیون ریشه در چهار رقم تجارتی گندم (*Triticum aestivum* L) شامل آذر-۲، داراب-۲، شیراز و فلات در همزیستی با قارچ *Glomus intraradices* تحت چهار تیمار آبیاری۱۰۰، ۷۵، ۵۰ و ۲۵ درصد ظرفیت مزرعه‌ای (FC) در سال ۱۳۸۹-۱۳۸۸ در دانشکده کشاورزی دانشگاه شیراز طرح‌ریزی گردید. در ارقام تلقیح شده میانگین سطح برگ، وزن تر اندام هوایی، وزن تر ریشه و وزن خشک اندام هوایی به ترتیب ۶/۰، ۱۰/۲، ۱۵/۶ و ۱۵/۲ درصد بیشتر از ارقام تلقیح نشده بود. کمبود آب درصد کلنیزاسیون ریشه را کاهش داد و بیشترین مقدار کلنیزاسیون (۲۸/۱۰ درصد) در رقم آذر-۲ مشاهده گردید. در مقایسه با ارقام تلقیح نشده، تیمار تلقیح با قارچ میانگین محتوای آب نسبی، پرولین، مقدارکلروفیل، پروتئین کل، آنزیم‌های سوپراکسید دیسموتاز، پرواکسیداز وکاتالاز را در ارقام میکوریز به ترتیب به مقدار ۵/۵، ۳۵/۶، ۱۳/۸، ۲۱/۶، ۲۲/۵، ۲۲/۷ و ۱۵/۵ درصد افزایش داد. بیشترین کارکرد آنزیم‌های پراکسیداز (۹/۷۷ U mg^{-1})، کاتالاز (۹/۸۲U mg^{-1}) و سوپراکسید دیسموتاز (۱۹/۸۰U mg^{-1}) در رقم آذر-۲ به دست آمد. نتایج تحقیق نشان داد که تلقیح با قارچ *Glomus intraradices* آثار مضر تنش کمبود آب بر ارقام گندم را از طریق افزایش تجمع پرولین و کارکرد آنتی‌اکسیدان‌های آنزیمی کاهش داد. ارقام آذر-۲ و داراب-۲ مقادیر بالاتری را برای آنزیم‌ها و کلنیزاسیون ریشه در مقایسه با سایر ارقام نشان دادند. بنابراین، از این ارقام می‌توان در برنامه‌های به‌نژادی برای همزیستی بهتر و تحمل خشکی در گندم استفاده کرد.

واژه‌های کلیدی: آنزیم‌های‌آنتی‌اکسیدان، رژیم‌های‌آبیاری، گندم، همزیستی، *Glomus intraradices*

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* به ترتیب دانشجوی سابق کارشناسی ارشد، استادیار، استادیار، استاد و استاد

** مکاتبه کننده