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Biochemical and Physiological Characteristics Changes of Wheat Cultivars under Arbuscular Mycorrhizal Symbiosis and Salinity Stress

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ABSTRACT: This study was performed to evaluate changes in antioxidant enzymes activity, free proline, relative water content (RWC) and physiological traits of four wheat (*Triticum aestivum* L) cultivars (Akbari and Darab) inoculated with Arbuscular Mycorrhizal (AM) fungus, *Glomus intraradices*, under three salinity stress including control (without salinity), 7 and14 ds m^{-1}. All growth parameters including shoot fresh weight (10.17%), shoot (15.6%) and root (25.2%) dry weight were higher in inoculated plants compared to non-inoculated ones. Salinity stress decreased root colonization percent and the highest root colonization observed in the cultivar Abari. Mycorrhizal inoculation enhanced RWC, proline content, pigment content, total protein, superoxide dismutase activity (SOD), peroxidase activity (POD) and catalase activity (CAT). The higher POD (9.77 Umg^{-1}), SOD (19.80 Umg^{-1}) and CAT (9.82 Umg^{-1}) obtained for Akbari cultivar than Darab cultivar. Salinity stress enhanced activity of all enzymes. The results indicated that *Glomus intraradices* inoculation can alleviate the deleterious effects of salinity stress on wheat cultivars through improving osmotic adjustment via accumulation of more proline and increasing the activity of antioxidant enzymes. The cultivar Akbari had higher antioxidant activity than other cultivar and consequently can be used in breeding programs for salinity stress.

Keywords: Antioxidant, *Glomus intraradices*, Osmotic adjustment, Antioxidant,

INTRODUCTION

The symbiosis of plants and microorganisms plays an important role in sustainable agriculture and natural ecosystems. Interactions between plants and AM fungi results in disease and/or the mutualistic symbiosis (García-Garrido and Ocampo 2002). Penetration to the root and the intracellular growth of the AM fungi involve complex sequences of biochemical and cytological events and intracellular modifications (Bonfante 2001).

It has been proven that AM fungi affect not only the plant growth but also contribute in plant tolerance to biotic and abiotic stresses (Augé 2001). These fungi are obligatory symbiotic soil organisms that colonize roots of most crops and improve their performance (Saed-moucheshi *et al.* 2013) by increasing nutrients supply to the plants and reducing abiotic stress's effects (Qiu-Dan *et al.* 2013).

It has been reported that plant inoculation with mycorrhizal fungi increases antioxidant enzymes in shoots and roots (Alguacil *et al.* 2003). On the other hand, mechanisms such as enhanced osmotic adjustment and leaf hydration, reduced oxidative damage and improved nutritional status have been proposed for the contribution of AM-host plants symbiosis in drought tolerance (Ghouchani *et al.* 2014). Plants response to abiotic stresses such as salinity is complex and include molecular and biochemical changes in whole plant (Condon *et al.* 2004). Salinity decreases the photosynthesis apparatuses of plants, causes changes in chlorophyll content and components, damage to photosynthetic apparatus (Iturbe-Ormaetxe *et al.* 1998) and also inhibits the enzymatic and photochemical activities in Calvin cycle (Monakhova and Chernyad'ev 2002). Environmental stresses change the balance between the production of reactive oxygen species (ROS) including super oxide radical (O_2-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and the antioxidant defense systems result in the accumulation of ROS and consequently oxidative stress to proteins, membrane lipids and other cellular components (Saed-Moucheshi, Shekoofa, and Pessarakli 2014b). The antioxidant defense systems in plant cells include enzymatic components such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) and also non-enzymatic constituents.

The toxic superoxide radical is usually dismutated by superoxide dismutase (SOD) to H_2O_2, a product which is relatively stable and detoxified by catalase (CAT) and peroxidase (POD) (Saed-Moucheshi *et al.* 2014a). Higher activities of several enzymes during drought stress period have been found in AM compared to non-mycorrihzal (NM) plants (Augé 2001). It is well-known that osmotic regulators such as proline, potassium and soluble sugar are small molecules relevant for evaluating osmotic adjustment ability and drought resistance in plants (Chen and Gallie 2004).

Due to importance of salinity stress issue, this study was performed to evaluate changes in antioxidant enzyme activity, free proline, relative water content and physiological traits of wheat during salinity stress conditions and to investigate the response of different cultivars under mycorrhizal inoculation and salinity stress.

MATERIALS AND METHODS

A. Experimental procedures

The experiment was carried out in the agriculture and natural resources center of Iazd, Iran in 2014-2015. A factorial experiment based on completely randomized design with three replications was used. The factors studied were salinity stress (three levels control, 7 and 14 ds m^{-1}) of soil; cultivars consist of two wheat cultivar Akbari (resistance) and Darab (susceptible); and mycorrhizal inoculation (inoculation and control). The fungus used in the present experiment was *Glomus intraradices* Schenck & Smith. Mycorrhizal inoculum was prepared through the trap culture in maize (*Zea mays* L.) with spores of *G. intraradices*. The mixture of trap culture medium was obtained from autoclaved soil/quartz-sand (<1mm) (4:1, v/v).

The soil samples were air dried, passed through 2 mm sieve and mixed uniformly. The physio-chemical properties of the soil were sandy loam, field capacity (%) 25.3, pH 7.9 (soil: distilled water, 1:1), electrical conductivity (dS m^{-1}) 0.5, carbonate calcium equivalent (%) 11.6, total organic matter (%) 1.34, total kjeldahl nitrogen (%) 0.06, Olsen phosphorus (mg kg^{-1}) 4.7 and 1M NH_4OAc-extractable potassium 240 (mg kg^{-1}), DTPA-Extractable of Fe, Cu, Mn, and Zn 5, 2,11.3 and 1.7 respectively (mg kg^{-1}) (Page *et al.*, 1982). In addition, field capacity (FC) of the soil samples was determined by pressure plate.

The pots with 240g weight, 23cm diameter, and 20cm height were filled with 5kg washed and sieved soil (mentioned above) without purification or sterilization. All pots received 150 mg N kg^{-1} soil (urea 46%) and 20 mg P kg^{-1} soil ($K_2HPO_4.3H_2O$) and some microelements up to 5 mg kg^{-1}. The seeds were treated with ethanol 98% for about 20s and then were washed three times with distilled water and kept at 20°C for a week. About 5cm of surface soil of each pot was removed and in mycorrhizal treatments, 50g inoculums (containing spore numbers of $8g^{-1}$ substrate and root colonization of 85 percent) was placed and incorporated with the remained soil and then 3cm of removal soil was added to the pots, after that eight seeds were planted at equal distances. Finally whole residual of removal soil was added to the pot. After germination, seedlings were thinned to four plants in each pot.

Pots weighed daily and according to decreased weight of each pot, decalcified water (for control treats) and water contain solved NaCl were added up to FC. The salinity treatments were applied at the tillering stage and the irrigation until this time was the same for all pots. The temperature during experiment ranged from 15 to 28°C, with a 16/8 h light/dark period.

After nearly 5 months from the sowing date and at the beginning of reproductive period, shoots were removed and content of pots (mycorrhizal roots plus soil possessing fungal spores and mycelia) were maintained in polyethylene bags at 4°C. Simultaneously, some pots were kept without any spore inoculation for preserving microbial association and used for control treatments.

B. Root colonization and leaf area measurements

Total fresh root and shoot weights of pots were measured separately. Shoot weight was also measured after drying at 65°C for 72. To assess rate of AMF colonization of root, sub-samples of fresh roots were fixed in formalin/acetic acid/alcohol solution (FAA). After washing roots in 8% KOH and staining with blue ink (Pelican) and lactoglycerol (v/v) (based on Kormanik and McGraw (1982) method) the grid-line intersect method was used to measure percentage of AMF colonization of root. Plants leaves were measured with the ruler and leaf area was calculated using following equation:

Leaf area = maximum leaf length × maximum leaf diameter × 0.75.

C. Relative water content (RWC)

Twenty-two days after applying water regimes, the plant shoot of the smallest plant in each pot was sampled and immediately weighed as shoot fresh weight (FW). After being immersed in distilled water for 24h, turgid weight (TW) of plant shoot was measured. Then, leaves were kept in oven for 24h at 60°C and shoots dry weight (DW) were measured. Relative water content was calculated by following standard formula (Zhou and Yu 2010):

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

D. Pigments content

Twenty two days after applying water stress treatments, one plant sample was randomly selected in each pot and total chlorophyll, chlorophyll a, chlorophyll b and carotenoid content of flag leaf were determined according to Arnon (1949).

Pigments were extracted in 80% cold acetone and the absorbance of the extractions was measured spectrophotometrically at 645, 663, and 470 nm wavelength and subsequently, pigments content were determined based on the following standard formulas (Lichtenthaler and Buschmann 2001):

Total chlorophyll (mg/ml) = 20.2 (A_{645}) + 8.02 (A_{663})

Chlorophyll a (mg/ml) = 12.7 (A_{663}) - 2.69 (A_{645})

Chlorophyll b (mg/ml) = 22.9 (A_{645}) - 4.68 (A_{663})

Carotenoid (mg/ml) = (1000A_{470} - 3.27[Chl a] - 104[Chl b])/227

Where, A is recorded number in spectrophotometer and Chla and Chl b denote for chlorophyll a and chlorophyll b content.

E. Proline measurement

Free proline was extracted from fresh leaves according to the method of Bates et al (1973). Leaf samples (0.5g) were homogenized in 10mL of 3% (w/v) aqueous sulphosalicylic acid and the solution filtered using a Whatman No. 2 filter paper. Two mLs of solution was then mixed with 2mLs acid ninhydrin and 2mLs glacial acetic acid in a test tube, and incubated at 100°C water bath for 1 h. The reaction was terminated by placing the mixture in an ice bath. Free proline of solution was finely extracted with 4 mLs toluene. The absorbance was recorded at 520 nm and proline concentration was determined as µmol g^{-1} fresh weight using a standard curve.

F. Measurement of total protein and antioxidant enzymes activity

Leaf samples were frozen in liquid nitrogen and kept refrigerated at -80°C. Frozen leaves were ground to fine powder with a mortar and pestle in liquid nitrogen andwere extracted with ice-cold 0.1M Tris-HCl buffer (pH 7.5) containing 5% (w/v) sucrose and 0.1% 2-mercaptoethanol (3:1 buffer volume/fresh weight). The homogenate was centrifuged at 12000 × g for 20 min at 4°C and the supernatant was used to measure protein content and enzymes activity. Enzyme extraction was carried out at 4°C.

The protein content was estimated according to the method of Bradford (1972), using bovine serum albumin (BSA) as a standard and observance of 595 nm.

Superoxide dismutase (SOD) inhibits the photochemical reduction of nitrobluetetrazolium (NBT) (Beauchamp and Fridovich 1971), this ability used to determine its activity (Dhindsa, Plumb-Dhindsa, and Thorpe 1981). For SOD assay, the reaction mixture contained 50mM K-phosphate buffer (pH 7.8), 13mM methionine, 75µM NBT, 0.1µM EDTA, 4µM riboflavin and extracted enzyme. The reaction started by adding riboflavin after which the tubes were placed under two 15 W fluorescent lamps for 15 min. A complete reaction mixture lacking enzyme, which gave the maximal colour, considered as control.

A non-irradiated complete reaction mixture was used as a blank. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitoredat 560 nm (Giannopolitis and Ries 1977).

Peroxidase (POD) activity was assayed (Polle *et al.* 1994) at 436 nm by its ability to convert guaiacol to tetraguaiacol (ε =26.6mM cm^{-1}). The reaction mixture contained 100m M K-phosphate buffer (pH 7.0), 20.1m Mguaiacol, 10mM H_2O_2 and enzyme extract. The increase in absorbance was recorded by adding H_2O_2 at 436 nm for 5 min. The activity of Catalase (CAT) was determined by monitoring the disappearance of H_2O_2 at 240 nm (ε = 40mM cm^{-1}) according to the method of Aebi (1984). The reaction mixture contained 50mM K-phosphate buffer (pH 7.0), 33mM H_2O_2 and enzyme extract.

G. Statistical analysis

Normality test for all data was done using Minitab software (v.14) and the data for colonization and RWC were ArcSin to achieve a normal distribution. The main effects of water stress, cultivars and mycorrhizal inoculation and their interactions were tested using analysis of variance (ANOVA). Least Significant Difference (LSD) was used for mean comparison of main effects. The mean comparisons for interactions were made using slice procedure and Pearson correlation test was performed to identify the correlations among traits using SAS (Statistical Analysis Software).

RESULTS

A. Physiological traits and mycorrhizal colonization

Salinity stress decreased all growth parameters including Biological yield, shoot dry weight and root fresh weight and also caused significant decrease in root colonization (RC) for all cultivars (Table 1). Highest Biological yield, shoot dry weight and root fresh weight and also root colonization were observed when no stress (control) was applied while the lowest ones were recorded for 14 ds/m salinity level. Compared with control treatments, traits measured in inoculated plants were higher than non-inoculated counterparts. The interactions of mycorrhiza inoculation × drought stress for root colonization and mycorrhiza inoculation × cultivar, drought stress × cultivar and mycorrhiza inoculation × drought stress × cultivar for fresh root weight were significant ($P>0.05$; data not presented). Regardless of salinity treatments and cultivars, mycorrhizal inoculation increased biological yield about 14.17% in inoculated plants compared to non-inoculated ones. Shoot dry weight (15.6%) and root fresh weight (25.2%) were also higher in inoculated cultivars. Akbari cultivar showed a higher colonization with mycorrhiza.

B. Relative water content (RWC)

Analysis of variance showed significant difference ($P<0.01$) for all main factors related to RWC. In general, inoculated plants showed higher amounts of RWC (74.3%) compared to non-inoculated (70.5%) ones. Salinity stress treatments decreased RWC. To illustrate, the highest (83.3%) and lowest (55.5%) RWC were recorded for control salinity and 14 ds/m, respectively. Among cultivars, the highest RWC (75.1%) was observed for Akbari while the lowest one (70.1%) was obtained for the cultivar Darab (Table 1).

Table 1: Mean comparison for morpho- and physiological measured traits.

Fungus	Cultivar	Stress	Relative water content	Root weight (gr/plant)	Shoot dry weight (gr/plant)	Biologic yield (gr/plant)	Colonization (%)
Inoculated	Akbari	Control	0.86478	3.256	13.126	15.95	48.50
		7 ds/m	0.84559	4.783	14.570	22.90	50.14
		14 ds/m	0.61579	0.796	5.2160	9.570	35.91
	Darab	Control	0.88690	3.131	16.233	25.40	52.22
		7 ds/m	0.78937	1.299	9.7060	15.90	45.83
		14 ds/m	0.57541	0.672	3.2830	3.410	32.38
Non-Inoculated	Akbari	Control	0.82024	3.432	11.423	16.50	15.08
		7 ds/m	0.82542	3.834	10.316	19.00	12.83
		14 ds/m	0.69064	1.566	8.5000	13.47	10.68
	Darab	Control	0.83972	4.737	14.363	24.00	16.39
		7 ds/m	0.75105	1.846	7.4900	15.00	13.91
		14 ds/m	0.68806	0.611	6.3860	7.000	7.816
Least significant difference			0.09	0.89	2.03	3.65	7.98

D

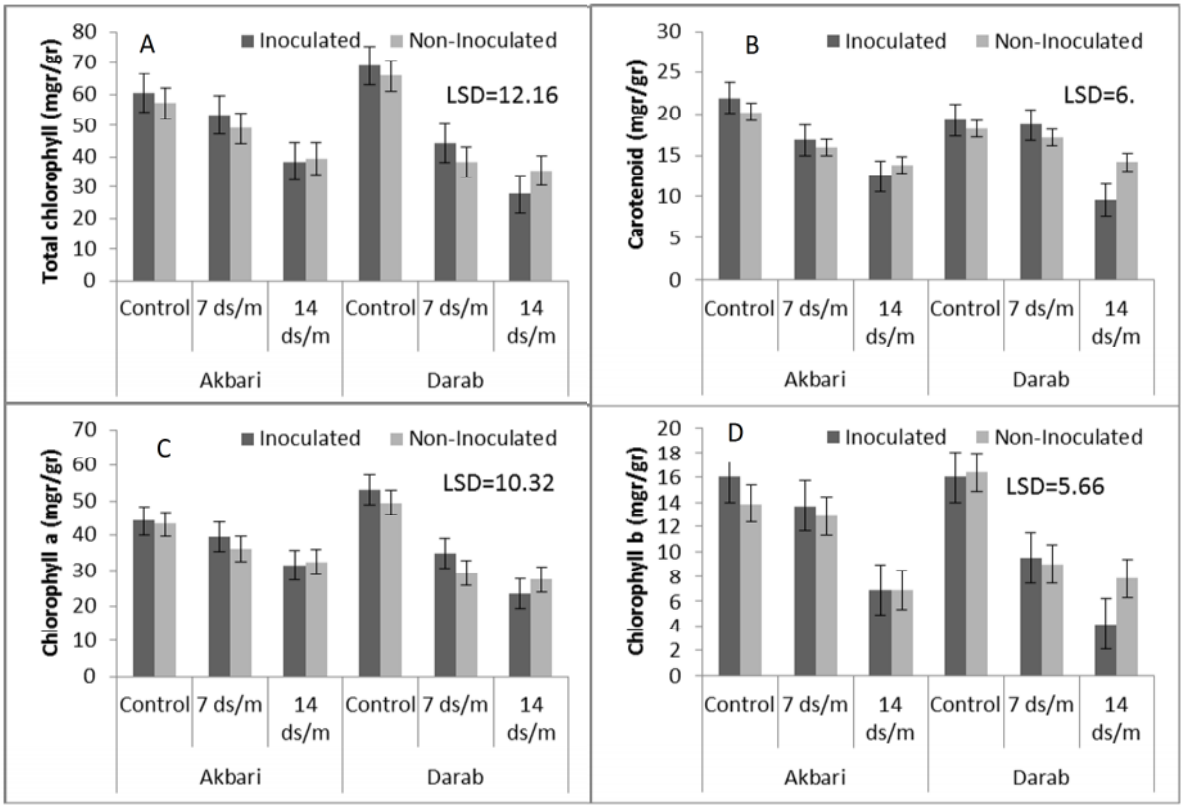

Fig. 1. Total chlorophyll content (A), Carotenoid (B) and Chlorophyll a (C) and chlorphyll b (D)for wheat cultivars under different salinity stress inoculated or non-inoculated with mycorrhizal fungi.

C. Pigments' content

Total chlorophyll: There were no significant different for inoculation and all interaction related to total chlorophyll content but effects of stress and cultivar were significant. Controls had maximum content of total chlorophyll (14.68) and sever salinity stress showed minimum content (6.76; Fig. 2A). Darab had higher (12.25) content of total chlorophyll than that in Akbari (11.71).

Carotenoids: content of carotenoids for all main effects of stress, inoculation and cultivar and also for stress × cultivar interaction were significant. Sever salinity showed maximum negative effect on carotenoids content that was 37.91% lower than control (Fig. 1B). Inoculated plants showed high content for carotenoids (19.79) while non-inoculated plant showed lower content (15.43). Darab showed higher amount of Carotenoids (18.30) than Akbari cultivar (16.16).

Chlorophyll a: Results of analysis of variance showed no significance difference ($p>0.05$) related to chlorophyll a (Cha) content inoculated and non-inoculated plant but the main effect of stress and cultivar was significant (Fig. 1C). In general, salinity stresses decreased the amount of cha and the lowest cha was recorded for sever salinity stress (14 ds/m). Darab cultivar showed higher content of Chathan Akbari cultivar.

Chlorophyll b: effects of stress ($p<0.01$), inoculation ($p<0.05$), cultivar ($p<0.01$) and stress × cultivar interaction ($p<0.01$) on content of chlorophyll b (Chb) were significant. Stresses caused decrease in Ch b but effect of sever salinity level was higher than others (Fig. 1D). Higher content of Ch b was recorded inoculation condition in compare with non-inoculation condition. Akbari had a higher Chb content than Darab.

C. Antioxidant enzymes activity and protein content

Except the main effect of cultivar on Peroxidase activity, the effects of other main factors(Stress and inoculation treatments) on antioxidant enzymes activity were significant. higher activity of POD (9.77 Umg^{-1}), SOD (19.80 Umg^{-1}) and CAT (9.82 Umg^{-1}) obtained for Akbari cultivarthan Darab (Fig. 2B,C,D). Salinity stress enhanced activity of all enzymes. The activity of Peroxidase, Catalase and Superoxide dismutase were 22.87, 15.46 and 22.43% respectively higher in inoculated plants than their non-inoculated counterparts.

Protein content in stresses condition was higher than control (Fig. 2A). In control (without stress) non-inoculated plants showed lower protein content than inoculated ones. There was no difference between stressful conditions and control, and also for Darab and Akbari cultivars related to total protein content.

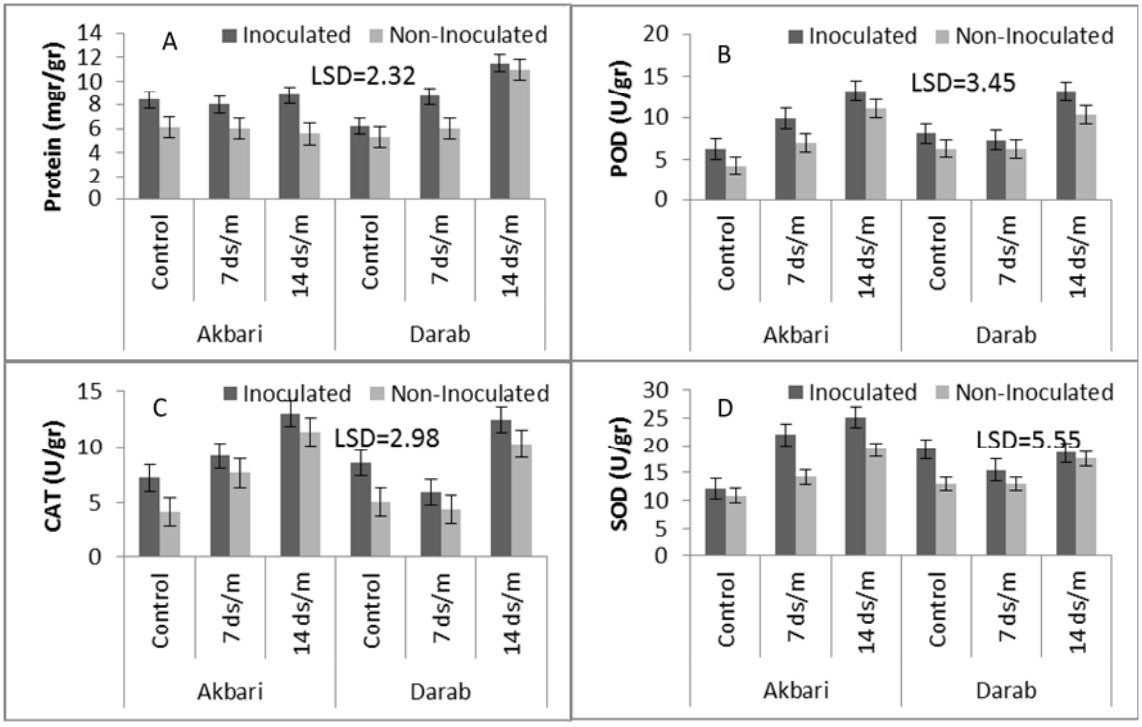

Fig. 2. Protein (A), POD (B), CAT (C), and SOD (D) activity for wheat cultivars under different salinity stress inoculated or non-inoculated with mycorrhizal fungi.

D. Proline content

The effect of main factors and all interactions except mycorrhizal inoculation × stress on proline content were significant. Salinity stress increased the amount of free proline especially under the severe salinity stress (10.1 µmg^{-1}). The amount of proline at this salinity level was about 2 fold higher than other treatments (Fig. 3). Significant difference was observed between Darab and Akbari cultivar for proline, where Akbari cultivar had higher amount of free proline (7.27 µm g^{-1}) than the other cultivar (4.69µm g^{-1}).

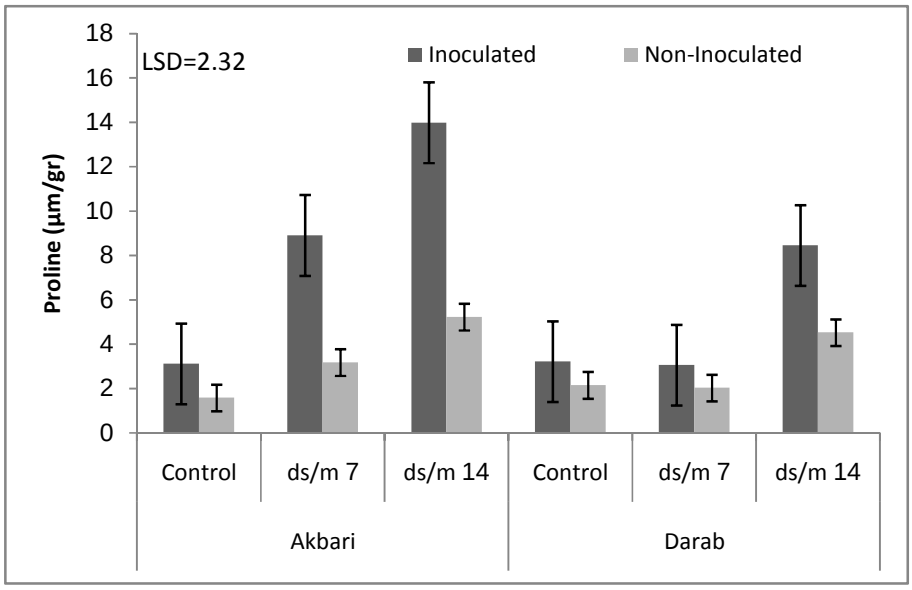

Fig. 3. Protein (A), POD (B), CAT (C), and SOD (D) activity for wheat cultivars under different salinity stress inoculated or non-inoculated with mycorrhizal fungi.

DISCUSSION

In the present study, growth characteristics including biological yield, shoot dry weight and root fresh weight were considerably higher in inoculated plants under all conditions. It has been well-established that AM symbiosis protects host plants against negative effects of abiotic stresses due to nutritional, physical and cellular improvements (Ruiz-Lozano 2003). In addition, the AM symbiosis increases host plant growth due to improved plant nutrition and also water uptake via external hyphae in inoculated roots (Sweatt and Davies Jr 1984). The beneficial effects of different mycorrhizal fungi on plant growth under stressful conditions have been demonstrated in wheat (Al-Karaki 1998) and other plant species (Ruiz-Sánchez *et al.* 2010). Higher growth characteristics are likely due to uptake more nutrient especially potassium (K) and phosphorus (P) (Roldán *et al.* 2008) and also water absorption in mycorrhizal plants. Potassium plays a key role in salinity stress condition and is known to be the cationic solute responsible for stomatal movements in response to changes in leaf water status (Ruiz-Lozano 2003). Many salinity-adapted species have a highly developed root system which may be considered as a mechanism of drought tolerance (Roldán *et al.* 2008).

Because of its extended root system, the cultivar Akbari can uptake more water and consequently, tolerate drought stress condition. By increasing salinity stress level, root colonization in inoculated plants decreased but decreasing ratio for Akbari was lower than other cultivar. It can be concluded that Akbari could be a suitable candidate for consideration inbreeding programs for achieving higher wheat-AM symbiosis and producing higher salinity tolerate cultivars. The declining of colonization rate in response to increasing salinity stress level indicates that salinity stress suppresses the colonization of AM fungi.

Proline content in plants leaves increased with the increase in the severity of salinity stress in both inoculated and non-inoculated cultivars, confirming the positive effects of proline in salinity tolerance. Osmotic adjustment is considered to be an important component of salinity tolerance mechanisms in plants. Under salinity stress conditions, higher plants accumulate small molecules including organic solutes and inorganic ions to increase osmotic adjustment (Wu and Xia 2006). Plants with higher osmotic regulators absorb more water from soil in water-deficit conditions (due to the salinity stress) than those with lower amounts of osmotic adjustment regulators. Many plants species decrease the osmotic potential of their cells by synthesizing and accumulating compatible osmolytes, such as proline participating in the osmotic adjustment (Ruiz-Sánchez *et al.* 2010). In present study, inoculated plants contained higher proline concentration (35%) than non-inoculated ones indicating the effects of mycorrhizal symbiosis on osmotic adjustments and increasing drought tolerance in plants.

The highest proline concentration in both inoculated and non-inoculated plants and all salinity levels was observed in the cultivar Akbari. There are also conflicting reports about higher (Subramanian and Charest 1995) or lower (Wu and Xia 2006) concentration of amino acids and proline in plant-AM fungi under abiotic stresses conditions. Ruiz-Sánchez *et al.* (2010) reported lower accumulation of free proline in mycorrhizal rice than non-mycorrhizal plants.

Changes in the protein and chlorophyll content, the concentration of antioxidants and the activity of oxidative enzymes are symptomatic for oxidative stress (Smirnoff 1993). In present study, total protein content of inoculated and non-inoculated cultivars increased with increasing the severity of salinity stress probably duo to plants response to enhance enzymatic and non-enzymatic protein to regulate the osmotic adjustment in plant cells. In the control level of salinity, the response of wheat cultivars to protein content was not significantly different but in the severe stresses levels, sensitive cultivar (Darab) showed lower protein than resistant one (Akbari) indicating the role of total protein content in enhancing drought tolerance. Total protein content of inoculated wheat cultivars showed that mycorrhizal symbiosis affects biochemical component concentrations in the host plants which can induceabiotic stress tolerance which is in agreement with the result of Subramanian and Charest (1995)and Ruiz-Sánchez *et al.* (2010).

In the present study, chlorophyll concentrations were significantly reduced by increasing severity of drought treatments due to suppression of enzymes responsible for the synthesis of photosynthetic pigments. Chlorophyll concentrations have often been higher in the leaves of control and abiotic stressed mycorrhizal plants than non-mycorrhizal ones (Augé 2001). The results of this study showed higher chlorophyll content for inoculated plants relative to non-inoculated ones. Higher chlorophyll content and growth parameters of inoculated cultivars are likely due to alleviating water stress and increased mineral uptakes. Increased chlorophyll content in the leaves of mycorrhizal plants under stress conditions has been reported by Colla *et al.* (2008), Kaya *et al.* (2009) and Hajiboland *et al.* (2010).

Relative water content (RWC) of inoculated plants was significantly higher than that of control plants up to 9% regardless of salinity levels and cultivars. Higher RWC of inoculated wheat cultivars could be due to either increased water uptake by mycorrhizal hyphae or higher proline or other components which interfere in osmotic adjustment. Salinity stress decreased RWC of all cultivars while the resistant (Abari) showed higher RWC than sensitive one (Darab)maybe due to their mechanism to uptake higher water or preventing water loss from their shoots. As it was expected, water stress caused decreases in RWC for all cultivars and inoculated treatments. Safir, Boyer, and Gerdemann (1972) concluded that AM symbiosis probably affects the water relations of soybean plants indirectly through improved phosphorus (P) nutrition. There are reports thatAM symbiosis may postpone declines in leaf relative water content of wheat in abiotic conditions(Panwar 1993) and changes in shoot water content relationships (Bethlenfalvay *et al.* 1987).

Antioxidant enzymes such as POD, SOD and CAT are of known indicators to evaluate the status of oxidation-reduction in plants(Dhanda, Sethi, and Behl 2004).AM symbiosis affects reactive oxygen metabolism and antioxidant production, but the exact mechanisms involved are still unclear (Wu and Xia 2006). Salinity stress induces ROS, as a result of lipid peroxidation, cause oxidative damages to plants cells. Superoxide dismutases catalyze the dismutation of superoxide into oxygen and hydrogen peroxide, a product which is relatively stable and detoxified by CAT and POD (Grant and Loake 2000). Therefore, higher activity of antioxidant enzymes is related to higher stress tolerance in plants. Our results showed higher activity for enzymatic antioxidant such as POD, CAT and SOD for inoculated wheat plants compared with non-inoculated ones regardless of salinity stress and cultivars effects. Higher activity of antioxidant enzymes in inoculated plants is related to the role of mycorrhizal symbiosis in biochemical and molecular changes in plant cells. Higher activity of antioxidant enzyme caused removalof more ROS and decreased oxidative damages and stress in plants cells; hence inoculated cultivars are more tolerant than non-inoculated ones due to higher antioxidant activity. Increasing antioxidant enzymes activity with respect to increasing water stress levels indicates the role of these enzymes in drought tolerance mechanisms. In the preset study highest SOD activity in inoculated cultivars was recorded for Azar2 in all water regimes. The same results were also observed for CAT and POD in other cultivars. It is assumed that inoculated plants produce antioxidant enzymes as a defense mechanism simultaneously with starting mycorrhizal symbiosis that is beneficial response for plants and therefore, inoculated plants can quickly response to stresses.

In general, the activity of POD and CAT enzymes were lower compared to the activity of SOD enzyme. The activities of several enzymes have been compared in mycorrhizal and non-mycorrhizal plants during drought stress conditions (Augé 2001). An increase in several antioxidant enzymes has been reported in the shoots of mycorrhizal plants in semiarid conditions (Alguacil *et al.* 2003). In the results of Roldán *et al.* (2008) there was no significant effect on SOD activities for the AM-colonization compared to control plants of *Juniperus oxycedrus* while the effect of this fungi on POD activity, shoot and root dry mater was significant. In their research, SOD activity decreased as stress levels increased in inoculated plants but the POD activity was higher and remained nearly constant during the stress period.

CONCLUSION

The results showed that AM symbiosis had significant effects on wheat physiological traits and antioxidant enzymes activity. It seems that the AM symbiosis increases osmotic adjustment by enhancing accumulation of free proline in shoots that could contribute in maintaining more water in the shoots. Higher activities of antioxidant enzymes in inoculated cultivars caused more productivity and protect plants cells from deleterious effects of salinity stress. The results also indicated that inoculating wheat cultivars with *Glomus intraradices* can alleviate the deleterious effects of salinity stress through improving osmotic adjustment via accumulation of more proline and increasing the activity of antioxidant enzymes. The cultivar Abari had higher antioxidant activity than other cultivar (Darab) and consequently can be used in wheat breeding programs for salinity stress.

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