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The effects of micronutrients foliar application on some biochemical and physiological characteristics of two wheat (*Triticum aestivum L.)* cultivars under water deficit conditions

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Key words: Wheat, relative water content, micronutrients foliar application, water deficit stress, antioxidant enzymes activity.

Abstract

Two field experiments were carried out in 2011-2012 on research farm of Islamic Azad University of Kermanshah and Mahidasht Agricultural Research Center to study the effects of foliar application of micronutrients (Mn, Zn and Mn + Zn) on some biochemical and physiological characteristics of wheat (Triticum aestivum L.)on different conditions of moisture. The experiments were conducted based on split factorial in randomized complete block with four replications. Three drought stress(I1= full irrigation,I2= with holding irrigation at the start of an thesis stage through the ripening,I3= with holding irrigation at the start of grain filling stage through the ripening) were randomized to the main plot units and 8 treatments from combination levels of two cultivars (C1=Bahar and C2=Pishtaz) and four foliar applications(F0=foliar application of water,F1=foliar application of Zinc(5000 ppm),F2=foliar application of manganese (5000 ppm) and F3= foliar application of Zinc and manganese (5000 ppm) were randomized to the sub plot units. The results showed that with increase severity of drought stress, Relative water content and cell membrane stability was decreased. With micronutrients foliar application, RWC and CMS increased on Pishtaz cultivar relative to Bahar cultivar. with increase severity of drought stress, activity of antioxidant enzymes (SOD and CAT) increased and foliar application Zn+Mn had higher significant effect on activity of antioxidant enzymes (SOD and CAT). Significant and positive correlation was found between grain yield and RWC.

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Introduction

In arid and semi- arid regions of the world, including Iran, drought stress is one of the most important factors that decrease agricultural crop production. Flowering and grain- filling stages are identified as among the most critical stages of wheat growth and development to drought stress , during which wheat exhibits the highest sensitivity to water deficit. Haydon and Cobbett.,(2007) reported that Heavy metals, such as Fe, Cu, Mn and Zn, function as micronutrients and are indispensable for plant growth. Broadley *et al.,(2007)* found that deficiencies of these heavy metals cause severe growth reduction. Several metabolic processes, such as nutrient uptake, cellular respiration, photosynthesis, and photolysis of water, may be affected by the presence of microelements like Zn, Cu, Fe, Mn and Mo (Sikora and Cieslik,1999).Soils of Iran are deficient in organic matter. Similarly, most of micronutrients, for example Zn and Mn are readily fixed in soil having alkaline pH. Plant roots are unable to absorb these nutrients adequately from dry topsoil (Foth and Ellis,1996).Foliar application of different micronutrients has been stated to be equally or more effective as soil application by different researchers (Torun *et al*.,2001).Activity of antioxidant enzymes increases in plant cells as a response to environmental stresses. Baby and Jini, (2011) and Rahimizadeh *et al.*,(2007) emphasized that environmental stresses can result in the production of Reactive Oxygen Species (ROS), including OH^- ,H_2O_2 and O^-_2 ,these ROS adversely affect crops yield and quality(Baby and Jini,2011;Rahimizadeh *et al*.,2007). ROS are highly reactive and can alert normal cellular metabolism through oxidative damage to nucleic acids , proteins and membranes; they also cause protein denaturation, lipid peroxidation, and DNA mutation(Baby and Jini,2011).To prevent damage to cellular components by ROS, plants have developed a complex antioxidant system. Baby and Jini,(2011) reported that the primary components of this system include carotenoids, ascorbate, glutathione and tocopherols, in addition to enzymes such as glutathione peroxidase (GPX) , superoxide dismutase (SOD), catalase (CAT), peroxidases and the enzymes involved in ascorbate-glutathion cycle such as glutathione reductase (GR) and ascorbate peroxidase (APX) (Baby and Jini,2011). Rahimizadeh *et al.*,(2007) stated that these enzymes have key role in the defense against oxidative stress. Bailly *et al.*, (2000) stated that in sunflower, the content of SOD and CAT in seeds will increase under drought stress condition. Also, Cakmak (2000) found that Zn deficiency stress may inhibit the activities of a number of antioxidant enzyme. Similarly, Rahmati *et al.*, (2004) reported that the activity of SOD and CAT in excess Mn treated cells increased compared with control treatment. Castro *et al.,* (2006) found that RWC is between 80.4 and 91.7 in non-stress conditions (full irrigation) in sunflower, while it is between 59.5 and 80.7 in the drought stress conditions. On the other hand, Talebi *et al.*,(2013) stated that Drought-tolerant chickpea cultivars have higher RWC in comparison to drought-susceptible cultivars. In addition, Majlesy *et al.*, (2012) stated that in forage Maize, Interaction of drought stress, potassium and non-application of micronutrients increased RWC 8% as compare with foliar application of micronutrients. It can be concluded that in drought stress, potassium application with micronutrients caused by the improvement of growth indeces. Pour mousavi *et al.*, (2007) reported that percentage of cell membrane stability affected by drought stress in flowering and ripening stages, as far as it increased by increasing drought stress intensity, however Covarrubias *et al.*, (1995) in their studies stated different results. Brown *et al.* (2004) stated that cell membrane stability and permeability of the marine invertebrate *Patella vulgata* are affected by copper, leading to changes in the physiological processes. Generally, researchers have indicated that micronutrients foliar application could compensate the impact of drought stress on plants(Ganji Arjenaki *et al.*, 2012; Hasheminasab *et al.*,2012; Waraich *et al.*, 2011) but it is not clear exactly, that elements application have the most impact on which of biochemical and physiological characteristics of plants, For this purpose, the objective of this research was to find out the effects of water deficit stress and micronutrients foliar application on some biochemical and physiological characteristics of two cultivars of wheat on west of Iran.

Materials and methods

Treatments and experimental design

This research was conducted in 2011- 2012 in research farm of Islamic Azad University of Kermanshah(47°,20'E; 34°,20'N), 1351 m elevated from sea level and in Mahidasht Agricultural Research Center (46°,50'E; 34°,16'N), 1380 m elevated from sea level.

The layout of the experiments was a split-factorial with organized treatments following a randomized complete block design, with four replications. Three stress treatments (I_1=Full irrigation;I_2= with holding irrigation at the start of an thesis stage through the ripening;I_3= with holding irrigation at the start of grain filling stage through the ripening) were randomized to the main plot units. Subplot units consisted of 8 treatments from combination of two cultivars (C1=Pishtaz and C2=Bahar) and four foliar application (F0=foliar application of water;F1=foliar application of zinc(5000 ppm); F2=foliar application of manganese(5000 ppm) and F3= foliar application of Zinc and manganese (5000 ppm)).

Climatological classification

Based on Dumarten's climate classification method, climate of both stations is cold semi-arid (Table 1.).

Table1. Changes temperature and precipitation in two regions, during the (2011 to 2012) farming year.

	Mahidasht Agricultural Research Center (Mahidasht Soil Fertility Research Station)				Research farm of Islamic Azad University of Kermanshah			
Month	Precipitation (mm)	Minimum temperature (C°)	Maximum Temperature (C°)	Average temperature (C°)	Precipitation (mm)	Minimum temperature (C°)	Maximum Temperature (C°)	Average temperature (C°)
Oct.	0	5.8	28.1	16.9	0	8.8	28.2	18.7
Nov.	115.9	0.9	16.5	8.7	131	4.4	17.3	10.6
Dec.	0.5	-9.6	7.12	2.9	0.8	-4.2	12.6	3.1
Jan.	4.9	-6.8	11.1	2.2	10.4	-2.6	11.5	4.4
Feb.	76.7	-5.5	8.5	1.5	68.2	-2.5	9.3	3
Mar.	23.3	- 4.7	9.9	2.6	34.3	-1.8	11	4.4
Apr.	24.4	3.5	20.5	13.3	45.7	5	19.5	11.8
May.	15.6	6.7	26.7	17	17.9	9.4	27.3	18.4
Jun.	0	10.5	32.8	22.5	0	14.3	33.6	24.9
Jul.	0	13.3	37.5	27.5	0	17.3	37	28.1
Aug.	0	13.8	38.1	27.4	0	19.1	39	29.8
Sep.	0	10.5	34.9	23.2	0	14.6	35.7	25.9

Characteristics of the soil of experiment

Soil type of Islamic Azad University of Kermanshah at test site was loamy – clay with EC= 3.5 ds.m^{-2} and pH=7.5, Mahidasht test site had loamy – clay texture with EC=3.15 ds.m^{-2} and pH=7.4.

Preparation and planting

Planting date was second weeks of November, 2011. Based on soil analysis, required fertilizers were used as follows: 200 kg urea per ha that one third prior to planting and two third were used as top dressing at tilling and stem elongation stages. Each plot consisted of 6 rows 20 cm apart, and 5 m long, 1 and 4 m distances were kept between test plots and blocks, respectively. Density was taken at 400 seeds per square meter. The first irrigation was applied immediately after seed sowing.

Grain yield

At the maturity stage, plants from 2th and 3th rows, 3 m long, were harvested from each plot center and grain yield, were measured.

Relative water content

Relative water content was determined according to Ardakani and Nadvar,(2010).

Cell membrance stability

Cell membrance stability was determined according to Khavarinejad and Nagafi,(2000).

Antioxidant enzymes activity

Superoxide dismutase and catalase were determined according to Giannopolities and Ries,(1977) and Chance and Maehly,(1955),respectively.

Statistical analysis

MSTATC and SPSS software's were used for statistical analysis. Combined variance analysis was performed after Bartlet test for checking uniformity of data variance (p=0.05) on targeted traits. Duncan multiple range tests were used to determine the significance of differences between treatment means at 0.05 levels. Table1. shows the changes temperature and precipitation in two regions, during the (2011 to 2012) farming year.

Results and discussion

The results of biochemical and physiological characteristics variations of wheat cultivars to water deficit stress and foliar application (Mn and Zn) are presented in table 2. Table3. and table 4.

Table2. Mean comparisons of interaction effects of water deficit stress and foliar application on two wheat cultivars at two regions of experiment.

Experimental treatments			RWC (%)	CML (μmohs.cm-1)	SOD (unit mg-1 frwt)	CAT (unit mg-1 frwt)
Full irrigation(I)	Bahar (C1)	Foliar application of water (F0)	75.50d	1439a	8.42a	4.22a
		Foliar application of Zn (F1)	85.38a	1409c	6.76b	3.40c
		Foliar application of Mn (F2)	78.38c	1430b	6.52b	3.70b
		Foliar application of Zn +Mn (F3)	82.38b	1416bc	6.45b	3.19d
	Pishtaz (C2)	Foliar application of water (F0)	75.90d	1417a	13.76a	4.67a
		Foliar application of Zn (F1)	89.38a	1385c	12.66bc	3.62b
		Foliar application of Mn (F2)	83.38c	1407b	13.22b	4.22a
		Foliar application of Zn +Mn (F3)	86.38b	1396bc	12.31c	3.31d
With holding irrigation at the start of an thesis stage through the ripening(I2)	Bahar (C1)	Foliar application of water (F0)	58.50c	1808a	13.57d	4.73d
		Foliar application of Zn (F1)	64.50a	1767d	14.49c	5.30c
		Foliar application of Mn (F2)	59.50bc	1793b	14.84b	5.72b
		Foliar application of Zn +Mn (F3)	62.50ab	1779c	15.32a	6.32a
	Pishtaz (C2)	Foliar application of water (F0)	61.13c	1754a	15.37c	4.85d
		Foliar application of Zn (F1)	68.50a	1736b	15.66c	5.39c
		Foliar application of Mn (F2)	64.50bc	1748a	16.24b	5.75b
		Foliar application of Zn +Mn (F3)	66.50ab	1746a	17.26a	6.41a

With holding irrigation at the start of grain filling stage through the ripening(I3)	Bahar (C1)	Foliar application of water (F0)	66.50c	1629a	10.37c	4.36d
		Foliar application of Zn (F1)	73.50a	1605c	10.69c	4.62c
		Foliar application of Mn (F2)	69.50bc	1615b	11.42b	4.94b
		Foliar application of Zn +Mn (F3)	71.50ab	1603c	12.67a	5.28a
	Pishtaz (C2)	Foliar application of water (F0)	68.50bc	1646a	11.46d	4.10d
		Foliar application of Zn (F1)	78.50a	1609d	12.20c	4.65b
		Foliar application of Mn (F2)	71.50c	1636b	13.10b	5.02a
		Foliar application of Zn +Mn (F3)	74.50b	1623c	13.73a	4.43c

*Within column, means followed by the same letter are not significantly different at p=0.05 RWC: Relative water content ;CML: Cell membranes leakage; SOD: Superoxide dismutase CAT; Catalase.

Table 3. Mean comparisons of main effects on relative water content, cell membranes stability and activity antioxidant enzymes(SOD and CAT) at two regions of experiment.

Traits	RWC (%)	CML (μmohs.cm^{-1})	SOD (unit mg^{-1} frwt)	CAT (unit mg^{-1} frwt)
I1	82.53a	1412c	10.01c	3.79c
I2	63.20c	1766a	15.35a	5.56a
I3	71.75b	1621b	11.95b	4.67b
C1	70.64b	1607b	10.96b	4.65a
C2	74.35a	1591a	13.92a	4.70a
F0	68.27d	1615a	13.53a	5.22a
F1	76.63a	1585d	12.08c	4.49c
F2	71.13c	1605b	12.56b	4.89b
F3	73.96b	1594c	11.59d	4.09d

Within column, means followed by the same letter are not significantly different at p=0.05. I1,I2 and I3 ; Full irrigation; withholding irrigation at the start of an thesis stage through the ripening and withholding irrigation at the start of grain filling stage through the ripening. ;C1, and C2: Pishtaz and Bahar cultivars . F0,F1,F2 and F3;foliar application of water; foliar application of zinc(5000 ppm) ; foliar application of manganese(5000 ppm) and foliar application of Zinc and manganese (5000 ppm).

Table 4. Correlation coefficient between studied traits.

Traits	1	2	3	4	5
1.RWC	1				
2.CML	-0.917^{**}	1			
3.SOD	-0.500^{**}	0.663^{**}	1		
4.CAT	-0.658^{**}	0.791^{**}	0.778^{**}	1	
5.GY	0.890^{**}	-0.836^{**}	-0.427^{**}	-0.647^{*}	1

* and **: significant at 5 and 1% levels of probability, respectively. RWC:Relative water Content ; CML:Cell membrance leakage ; SOD:Superoxide dismutase ; CAT:Catalase ; GY:Grain yield.

Relative water content

At I1,I2 and I3 levels(Full irrigation, with holding irrigation at the start of an thesis stage, with holding irrigation at the start of grain filling stage) and in C1cultivar (Bahar) and C2 cultivar (Pishtaz),F1(Foliar application of zinc) significantly increased RWC compared to F0(Foliar application of water).Generally micronutrients foliar application increased RWC relative to F0. I2 significantly decreased RWC compared to other stress levels infact with increase rate drought stress (from I3 to I2), Relative water content was decreased. Alizade,(2002) reported that Leaf RWC is of the best growth/biochemical indices revealing the stress intensity. C2 cultivar (Pishtaz) had the Maximum RWC relative to C1 cultivar(Bahar) and C2 cultivar (Pishtaz) is resistance to terminal season drought stress conditions . Ganji Arjenaki *et al.*,(2012) stated that rate of RWC in plant with high resistance against drought is higher than others. Also these results correspond to those of (Hemantranjan, 1996; Singh and Patel, 1996).

Cell membranes stability

At I1, I2 and I3 and in C1 and C2 cultivars, F1 significantly increased cell membranes stability compared to F0. The highest and lowest Cell membranes stability pertained to the F1 and F3 relative to F0, respectively.I2 significantly decreased Cell membranes stability compared to other stress levels. C2 cultivar had the Maximum Cell membranes stability. Ingram and Bartels, (1996);Cakmak *et al.*,(1999) and Waraich *et al.*,(2011) reports are in agreement with findings of this research.

Antioxidant enzyme activity of superoxide dismutase

At I1 and in C1 and C2 cultivars,F1and F2 significantly increased SOD activity compared to F0,respectively. At I2 and I3 and in C1 and C2 cultivars, F3 significantly increased SOD activity to F0, respectively. Foliar application levels significantly increased SOD activity. The highest and lowest SOD activity pertained to the F3 and F1 relative to F0, respectively. Waraich *et al*, (2011) reported that Zn application reduces the activity of membrane-bound NADPH oxidase which in turn decreases the generation of ROS and reduces photo-oxidation damage while the activities of SOD, POD, and CAT are enhanced indicating that Zn lowers the ROS generation and protect cells against ROS attack under water stress. The maximum activity of SOD found in I2 and the minimum activity of the enzyme found in I1. SOD activity also increased, with increasing severity of drought (Hasheminasab *et al.*,2012).C2 cultivar had the Maximum SOD activity. Hasheminasab *et al.*, (2012) Stated that drought tolerante genotypes of wheat had a highest SOD activity under drought condition. In this case, In many experiments tolerant cultivars had higher antioxidant activity (Abdelsamad *et al.*, 2007; Selote and Khanna-Chopra,2004).

Antioxidant enzyme activity of catalase

At I1, I2 and I3 and in C1 cultivar, F2, F3 and F2 significantly increased CAT activity compared to F0, respectively. At I1, I2 and I3 and in C2 cultivar, F1, F3 and F3 significantly increased CAT activity compared to F0, respectively. I2 and I1 had the highest and the lowest CAT enzyme activity, respectively. There were no significant differences between C1 and C2 cultivars, but different foliar application levels had significant difference on this trait. The highest and lowest CAT activity pertained to the F3 and F1 relative to F0, respectively. Results of this research are in agreement with findings of bottom researchers: Luna *et al.*,(2004) stated that total leaf CAT activity was significantly increased only in response to severe drought. But Jiang and Huang,(2001) reported that increased, decreased or unchanged CAT activities under drought stress have been observed. The exact cause of increased activity of CAT is not known, but the increased CAT activity as a hydrogen peroxide scavenging enzyme may indicate the enhanced rate of hydrogen peroxide formation (Jiang and Huang, 2001).Waraich *et al*,(2011) found that application of some micro- nutrients like Zn, Si and Mg increase antioxidants concentration and improves drought tolerance in plants. On the other hand ,results of this research is parallel with results of other works reporting the increased CAT activity in response to drought stress in barley (Salekjalali *et al*.,2012) ,wheat (Ahmadizadeh *et al*.,2011)and maize(Ahmadi *et al*,2010).

Correlation between traits of wheat

Significant and positive correlation was found between grain yield and RWC, and significant and negative correlation was found between grain yield and cell membrane leakage and activity of antioxidant enzymes (SOD and CAT). Lascano *et al*.,(2001) did not find a clear correlation between drought stress tolerance and antioxidant system activity.

Conclusions

Results of this study showed that under drought stress condition, activity of antioxidant enzymes (superoxide dismutase, catalase) increased. The plant to cope with drought stress, activity of antioxidant enzymes (SOD and CAT) increased; it was a defense mechanism that reduced grain yield on wheat. With increase severity of drought stress, Relative water content and cell membrane stability were decreased. With micronutrients of foliar application, RWC and CMS increased on Pishtaz cultivar relative to Bahar cultivar. Foliar application of micronutrients (manganese + zinc) also increased enzymes activity and foliar application of zinc increased relative water content and cell membranes stability.

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