The effects of selenium and other micronutrients on the antioxidant activities and yield of corn (*Zea mays* L.) under drought stress

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Abstract The effects of selenium (Se) on plant growth under drought stress and in the presence of micronutrients are yet to be investigated. Hence, in a field experiment in 2007 the effects of Se and micronutrients including iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), boron (B) and molybdenum (Mo) were evaluated on corn (*Zea mays* L.) grain yield under drought stress. Main- and sub-plots were devoted to irrigation (control and water stressed at the eight-leaf, blister and grain filling stages) and micronutrients treatments, respectively. Micronutrients were foliarly applied at 2 l ha^{-1} at the six-leaf stage, one week before tasseling, using a corn fertilizer, called biomin

containing (on the basis of dry weight percentage) Fe (2.6), Zn (4.1), Cu (1.5), Mn (2.6), B (1.5), Mo (0.5) and Mg (4.1). Se was used as sodium selenite (Na_2SeO_3), at the rate of 20 g ha^{-1} two weeks before treating the plants with drought stress. Effects of drought stress on plant growth were determined based on the activity or level of antioxidants. With increasing the stress level, addition of Se or micronutrients significantly enhanced the antioxidant activity and level as well as corn grain yield. The interaction effects between Se and micronutrients adversely affected antioxidant activity as well as corn grain yield. Se addition at the grain filling stage resulted in the highest grain yield under drought stress. The single but not the combined use of Se or micronutrients can alleviate the unfavorable effects of drought stress on corn yield by affecting plant metabolism including antioxidant activity.

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Abbreviations

SOD, EC 1.15.1.1	Superoxide dismutase
CAT, EC 1.11.1.6	Catalase
GPX, EC 1.11.1.9	Glutathione peroxidase
MDA	Malondialdehyde
N	Nitrogen
P	Phosphorous
Mg	Magnesium
Fe	Iron
Zn	Zinc
Mn	Manganese
Cu	Copper
B	Boron
Mo	Molybdenum
Se	Selenium

Introduction

Environmental stresses, including drought, affect plant growth and yield production in different parts of the world (Chaves et al. 2002). Drought stress may increase the formation of free oxygen radicals, hydrogen peroxide and hydroxyl radicals resulting in the degradation of membrane components, the oxidation of protein sulfhydryl (-SH) group and the loss of membrane functions (Yao et al. 2009). Drought stress can adversely affect root and shoot growth resulting in the reduction of leaf surface area (Sajedi et al. 2010).

The production of various forms of active oxygen, due to stress, can also damage cellular constituents such as lipids, carbohydrates, proteins and nucleic acids. The oxidative stress of drought may also adversely affect the resistance of chlorophyll and carotenoid membrane and hence prevents the plant from photosynthetic and respiratory processes and the subsequent growth. By controlling the production and adverse effects of active oxygen in plant, enzymatic and non-enzymatic mechanisms can enhance plant resistance to drought (Bowler et al. 1992; Yao et al. 2009).

The activity of enzymes such as SOD, especially under stress, can affect the structure of activated species such as superoxide and hydrogen peroxide. This may eventually result in the degradation of cellular components. High amounts of such species are produced under stress. CAT, SOD and ascorbate peroxidase are enzymes, which are vital for the activities of organism. Under different conditions, including stress, these enzymes can continuously collect free radicals, which are the products of aerobic metabolites. Peroxidases are a collection of enzymes that catalyse the breakdown of H_2O_2 (Mittler 1993), preventing cellular peroxidation and other possible damages. Reduction of glutathione by GPX decreases H_2O_2 activity (Dixon 1998), especially under stress. Therefore, the collection of antioxidants can help the plant handle the stress.

Se is among the beneficial micronutrients, which is essential for the growth and activities of different organisms, except plants. Previous research has indicated the effects of Se on plant growth under different conditions including drought (Germ et al. 2007). Elemental Se is poisonous to the plant, however after absorption by root system and through metabolic processes it is converted to selenomethionine, and selenocysteine amino acids, which are not poisonous to the plant (Pilon-Smits et al. 2009). Dhillon (2002) found that Se affected the defense mechanism of living organisms.

Irrigation water enriched with Se significantly affected rice and sunflower growth. Se foliar application enhanced the production of plant antioxidants and the resistance to drought stress. Se alleviated the adverse effects of drought on plant growth and leaf water content in spring wheat (*Triticum aestivum* L.) (Kuznetsov et al. 2004). Addition

of 18 g ha^{-1} Se solution as sodium selenite at the flowering stage increased the activities of SOD, GPX and CAT antioxidant enzymes (Rahimizadeh et al. 2007; Dadnia et al. 2008).

Some nutrients, including Fe, Zn, Cu, Mn, B and Mo are required for plant growth at micro amounts. Ionic forms of Fe, Zn, Cu, Mn, and Mg act as co-factors in many antioxidant enzymes. Under micronutrient deficiency the activity of antioxidant enzymes decreases, which in turn increases plant sensitivity to environmental stresses (Cakmak 2000). Similar to the other crop plants, corn (*Zea mays* L.) growth and production is influenced by environmental stresses such as drought. In addition, a variety of nutrients are necessary for corn optimum growth at different stages. Corn is among the crop plants, which are very sensitive to micronutrient deficiency. Movahed Dehnavi et al. (2002) indicated that application of micronutrient fertilizers can enhance plants resistance to environmental stresses such as drought and salinity.

Application of micronutrient fertilizers under severe drought stress increased the activities of SOD, GPX and CAT by 22 %, 79 % and 58 %, respectively (Rahimizadeh et al. 2007). Nutrient deficiency can adversely affect plant growth through inducing oxidative stress on plant growth (Yu and Rengel 1999). More specifically, researchers have very recently indicated the importance of metal ions in the activity of cell mitochondria. For example, their deficiency can influence mitochondria activity through inducing oxidative stress on the metal binding proteins (Tan et al. 2010). The beneficial effects of Se on plant growth under drought have been previously indicated, however its effects, in the presence of micronutrients, on corn growth under drought are yet to be investigated. Hence, this research work was conducted to address such effects on corn growth, with respect to the activity of antioxidants.

Materials and methods

Experimental procedure

A field experiment was performed in the Research Station of Islamic Azad University, Arak Branch, Iran, in 2007. Soil physical and chemical properties were determined for the depths of 0–30 and 30–60 cm, using the standard laboratory methods (Table 1). The acidity (pH) and electrical conductivity (EC) of a saturated paste was determined (Rhoades 1982). Using wet oxidation (Nelson and Sommers 1982) and Kjeldahl method (Nelson and Sommers 1973) soil organic carbon and nitrogen (N) were measured, respectively. Phosphorous (P) using bicarbonate extraction method (Olsen 1954) and potassium (K) using flamephotometer (emission

Table 1 Soil physical and chemical properties

Depth (cm)	EC ds m ⁻¹	pH	OC%	N %	P (ppm)	K (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Sand %	Silt %	Clay %
0–30	1.20	7.5	0.82	0.080	5	150	0.8	4.6	10.6	1.14	29	35	36
30–60	1.70	7.4	0.61	0.061	3.6	120	0.4	4	6.6	0.88	27	29	44

EC: electrical conductivity, OC: organic carbon

spectrophotometry, Knudsen et al. 1982) were determined. Using atomic adsorption spectrometry (Model Perkin Elmer 3110) soil micronutrients including iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) were measured according to the diethylenetriaminepentaacetic acid (DTPA) method (Baker and Amachar 1982). Soil texture was also determined using the hydrometry method (Gee and Bauder 1986).

The experiment was a split plot factorial on the basis of a completely randomized block design with four replicates. The main plots were four irrigation levels including control (full watering), drought stress at the vegetative (V8), blister or seed formation and at the pre mature seed stage or dough stage with the subplots being the combination of Se and micronutrients treatments. Plants were subjected to the drought stress treatment at each growing stages by not watering the plots for a certain period of time and re-irrigating when 180 mm water had been evaporated from the basin pan. Full irrigation was arranged based on the amount of crop water requirement and the measured daily evaporation from the basin pan. Plots were irrigated using polyethylene tubes in which the amounts of water were controlled using water contour.

Corn plants were foliarly fertilized with micronutrients one week before tasseling at the six-leaf stage at two liter ha⁻¹ using a corn fertilizer, called “biomin” (JH Biotech Inc.) containing (on the basis of dry weight percent) Fe (2.6), Zn (4.1), Cu (1.5), Mn (2.6), B (1.5), Mo (0.5) and Mg (4.1). Se was used as sodium selenite (Na₂SeO₃), at the rate of 20 g ha⁻¹ two weeks before subjecting the plants to the drought stress treatment (Dadnia et al. 2008).

Corn seeds were planted 20 cm apart in 6-m rows, with a 75-cm spacing. To prevent any likely side effects, the two side lines between the plots were not planted. The amounts of fertilizers were determined according to soil testing analysis and one third of N and all of P was applied at seeding and the remaining N was fertilized twice during the vegetative stage. Ten sample plants were harvested from the middle of each plot at physiological maturity when a black layer was formed on the seed base.

Enzymatic bioassay

Sampling

For the measurement of enzyme activity and content, three leaf sample were collected from the sampled plants, and were

then washed, frozen and stored using liquid N₂ at –80 °C for further analyses.

Extract preparation

Using a mortar and pestle, leaf samples were homogenized with ice-cold extraction buffer (25 mM sodium phosphate, pH 7.8). The homogenate was then centrifuged at 18,000g for 30 min at 4 °C (the temperature for all operations) and the supernatant, which was used as a crude extract for the enzyme bioassay, was filtered using a filter paper.

Antioxidant bioassay

Catalase activity was measured by the method of Cakmak and Horst (1991). The reaction mixture contained 100 µL crude enzyme extract, 500 µL 10 mM H₂O₂, and 1,400 µL 25 mM sodium phosphate buffer. The decrease in the absorbance at 240 nm was recorded for 1 min by spectrophotometer (model Cintra 6, GBC Scientific Equipment, Dandenong, Victoria, Australia). CAT activity of the extract was expressed as CAT units per milligram of protein. Superoxide dismutase activity was determined with the reaction mixture containing 100 µL 1 µM riboflavin, 100 µL 12 mM L-methionine, 100 µL 0.1 mM EDTA (pH 7.8), 100 µL 50 mM Na₂CO₃ (pH 10.2) and 100 µL 75 µM nitroblue tetrazolium (NBT) in 2,300 µL 25 mM sodium phosphate buffer (pH 6.8), and 200 µL crude enzyme extract with the final volume of 3 mL.

SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT glass test tubes with the mixture as were illuminated with a fluorescent lamp (120 W); identical tubes, which were not illuminated were used as blanks. After illumination for 15 min, the absorbance was measured at 560 nm. One unit of SOD was defined as the amount of enzyme activity that was able to inhibit the photo reduction of NBT to blue formazan by 50 %. The SOD activity of the extract was expressed as SOD units per milligram of protein. Peroxidase activity was determined by the oxidation of guaiacol in the presence of H₂O₂. The increase in absorbance was recorded at 470 nm (Hernandez et al. 2000). The reaction mixture contained 100 µL crude enzyme, 500 µL H₂O₂ 5 mM, 500 µL guaiacol 28 mM, and 1,900 µL potassium phosphate buffer 60 mM (pH=6.1).

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content in 1 g leaf fresh weight according to Madhava Rao and Sresty (2000). MDA is a product of lipid peroxidation by thiobarbituric acid reaction. The concentration of MDA was calculated from the absorbance at 532 nm (correction was done by subtracting the absorbance at 600 nm for unspecific turbidity).

Statistical analysis

Data were subjected to the analysis of variance using SAS (SAS Inc. 1988) and means were compared using Duncan's multiple range test (Steel and Torrie 1980).

Results

According to the analysis of variance the effects of drought stress on the activity of antioxidants and corn grain yield were significant at different growth stages at $P=0.01$. However, Se addition just significantly affected the activity of antioxidants and not crop yield. The results also indicated that the activity of SOD, MDA content and corn grain yield was significantly affected by the interaction effects of drought and Se. Micronutrients significantly affected the activity of antioxidants and corn grain yield. The two way interaction effects of drought and Se significantly affected MDA content and in the case of Se and micronutrients, CAT and GPX were significantly affected. Their three way interaction significantly affected just corn grain yield (Table 2).

Mean comparison of main effects indicated that drought stress at different levels significantly enhanced the activity of antioxidants, compared with the control treatment and it resulted in significant reduction in corn grain yield. Averaged across the other two experimental parameters, addition of Se and other micronutrients decreased the activities of antioxidants (Table 3).

SOD activity ranged from 1,024 to 1,424 $\mu\text{mole/mg}$ protein at control to the highest level of drought. For CAT and GPX the corresponding ranges were equal to 79 to 101 and 12.7 to 21 $\mu\text{mol/mg}$ protein, respectively. MDA content on the basis of nmol/g fresh weight ranged from 0.78 to 1.78 and for the grain yield the corresponding values were in the range of 8,025 (control treatment) to 5,386 kg ha^{-1} (the highest drought level) (Table 3).

Se addition decreased the activity of SOD from 1,445 to 1,108, CAT from 97 to 84 and GPX from 18 to 14 $\mu\text{mol/mg}$ protein. Se reduced MDA content from 1.6 to 1.0 nmol/g fresh weight and the grain yield from 6,852 to 6,703 kg ha^{-1} . The reduction resulted by micronutrient addition was from 1,408 to 1,144 for SOD, 95.5 to 85.2 for CAT and 18 to 14 $\mu\text{mol/mg}$ protein for GPX. Micronutrients reduced MDA content from 1.5 to 1.1 nmol/g fresh weight and increased corn grain yield from 6,618 to 6,938 kg ha^{-1} . All the differences were statistically significant (Table 3).

Interestingly, while the addition of Se at control treatment decreased the activity of antioxidant enzymes, under stress Se significantly enhanced their activity. For example at the highest level of drought the activity of SOD, CAT and GPX increased from 1,129 to 1,718, 91 to 111, and 19 to 23 $\mu\text{mol/mg}$ protein, respectively. The corresponding increases for MDA and corn grain yield were equal to 1.33 to 2.23 nmol/g fresh weight and 4,458 to 6,314 kg ha^{-1} , respectively. The same trend was observed for micronutrient addition, although it decreased corn grain yield at different levels of drought. Micronutrient treatment at different levels of Se resulted in significant reduction of antioxidant activity or level as well as corn grain yield (Table 4).

The three way interaction effects interestingly indicated that Se can effectively alleviate the stress of drought on corn yield, especially at the highest level of drought as the amount of yield increased to 6,818 kg ha^{-1} , significantly different from the other treatments. At control, addition of

Table 2 Analysis of variance for the effects of experimental treatments on the activities of antioxidants and corn grain yield

S. O. V	df	SOD	CAT	GPX	MDA	Grain yield
Replication	3	21,928.52 ^{ns}	18,417 ^{ns}	0.46 ^{ns}	0.01 ^{ns}	20,634.1 ^{ns}
Water level (L)	3	567,191.97**	1,377.07**	212.38**	3.24**	18,890,861.9**
Error	9	15,982.00	27.58	1.69	0.08	512,256.9
Selenium (Se)	1	1,814,072.27**	2,618.88**	226.50**	5.71**	355,040.3 ^{ns}
L × Se	3	113,933.22*	91.10 ^{ns}	6.59 ^{ns}	0.21**	11,963,794.9**
Micronutrients (M)	1	1,115,400.07**	1,681.00**	236.39**	2.91**	1,647,468.5*
L × M	3	27,342.56 ^{ns}	23.07 ^{ns}	7.44 ^{ns}	0.29**	966,299.7 ^{ns}
Se × M	1	86,215.64 ^{ns}	216.83*	46.92*	0.06 ^{ns}	1,374,961.4 ^{ns}
L × Se × M	3	27,467.43 ^{ns}	14.01 ^{ns}	10.57 ^{ns}	0.12 ^{ns}	2,684,642.4**
Error	36	33,378.78	49.93	9.20	0.04	365,658.4
C V %	—	14.31	7.82	18.98	16.22	8.92

ns, * and ** : not significant, significant at 5 and 1 % level of probability, respectively. S.O.V.: source of variation.

SOD Superoxide dismutase; CAT Catalase; GPX Glutathione peroxidase; MDA Malondialdehyde

Table 3 Mean comparison of antioxidants and corn grain yield, as affected by the main effects of experimental treatments

Treatment	SOD ($\mu\text{mol}/\text{mg}$ protein)	CAT ($\mu\text{mol}/\text{mg}$ protein)	GPX ($\mu\text{mol}/\text{mg}$ protein)	MDA (nmol/g fresh weight)	Grain yield (kg ha^{-1})
L1	1,024.19c	79.04d	12.71d	0.78d	8,025a
L2	1,240.69b	87.94c	14.09c	1.07c	6,989b
L3	1,417.00a	93.21b	16.07b	1.54b	6,712b
L4	1,423.69a	101.13a	21.04a	1.78a	5,386c
Se0	1,444.75a	96.73a	17.86a	1.59a	6,852a
Se1	1,108.03b	83.93b	14.10b	0.99b	6,703a
M0	1,408.41a	95.46a	17.60a	1.51a	6,618b
M1	1,144.37b	85.21b	14.06b	1.08b	6,938a

SOD Superoxide dismutase; CAT Catalase; GPX Glutathione peroxidase; MDA Malondialdehyde.

Mean values, followed by the same letters within each column are not significantly different using Duncan's multiple rang test at $P=0.05$, L1: control L2: drought stress at V8 Stage, L3: drought stress at blister stage, L4: drought stress at grain filling stage, Se0: Without Selenium, Se1: with Selenium M0: without micronutrients M1: with micronutrients

Se or/and micronutrients decreased the activity or content of antioxidants, which was not the case for drought treatments. In addition, there were interactions effects between Se and micronutrients, as addition of micronutrients with Se significantly decreased the activity of antioxidants activities and corn grain yield at different

Table 4 Mean comparison of antioxidants and corn grain yield, as affected by the two way interaction effects of experimental treatments

Treatment		SOD ($\mu\text{mol}/\text{mg}$ protein)	CAT ($\mu\text{mol}/\text{mg}$ protein)	GPX ($\mu\text{mol}/\text{mg}$ protein)	MDA (nmol/g fresh weight)	Grain yield (kg ha^{-1})
Water level	Selenium					
L1	Se0	1,159.37d	85.09de	14.07cd	0.99d	9,144a
	Se1	889.00e	72.99f	11.35d	0.58e	6,905bc
L2	Se0	1,113.75d	83e	12.80cd	0.85d	6,618c
	Se1	1,367.62bc	92.89bc	15.39c	1.30c	7,361b
L3	Se0	1,300.12cd	88.49cde	13.56cd	1.22c	6,595c
	Se1	1,533.87ab	97.94d	18.57b	1.86b	6,829bc
L4	Se0	1,129.25d	91.26bcd	18.67b	1.33c	4,458d
	Se1	1,718.12a	111a	23.40a	2.23a	6,314c
Water level	Micro-nutrients					
L1	M0	1,148.25b	85.53cd	13.85cd	0.97d	7,841a
	M1	900.12c	72.54e	11.57d	0.60e	8,208a
L2	M0	1,162.87b	83.86d	11.96d	1.03cd	7,153b
	M1	1,318.50b	92.02bc	16.22bc	1.11cd	6,826bc
L3	M0	1,263.75b	87.45cd	13.32cd	1.19c	6,995bc
	M1	1,570.25a	98.97ab	18.81b	1.90a	6,429c
L4	M0	1,250.75b	96.97b	19.36b	1.51a	5,765d
	M1	1,596.62a	105.29a	22.71a	2.06a	5,007e
Selenium	Micro-nutrients					
Se0	M0	1,540.06a	100.01a	18.92a	1.78a	6,717b
	M1	1,349.44b	93.44b	16.79a	1.41b	6,690b
Se1	M0	1,276.75b	90.90b	16.87a	1.24c	7,159a
	M1	939.31c	76.97c	11.32b	0.75d	6,545b

SOD Superoxide dismutase; CAT Catalase; GPX Glutathione peroxidase; MDA Malondialdehyde

Mean values, followed by the same letters within each column are not significantly using Duncan's multiple rang test at $P=0.05$, L1: control L2: drought stress at V8 Stage, L3: drought stress at blister stage, L4: drought stress at grain filling stage, Se0: Without Selenium, Se1: with Selenium, M0: without micronutrients, M1: with micronutrients

drought levels, while their single application significantly increased the activity and level of antioxidants (Table 5).

Discussion

When plants are subjected to different stresses, the activity of antioxidants are increased to alleviate the stress through degrading the products of stress including free radicals such as superoxide and peroxide, which are not favorable to plant growth (Cakmak 2000; Habibi et al. 2004; Rahimizadeh et al. 2007). Therefore, determination of antioxidants activity in plants can be used as a useful indicator for plant response to the stress and accordingly the appropriate ways of alleviating stress can be suggested. In this experiment the effects of drought stress on the activity of antioxidants (as indicators of drought stress) and corn grain yield were evaluated and the single and combined effects of Se and micronutrients to alleviate the stress were tested. There is little data regarding such effects.

According to the results of this research work, Se had negative effects on the activity of antioxidants at the control level of drought; however it significantly increased the activity of antioxidants at the stressed treatments, which is also indicated by the significant interaction effect between

Se and drought on the activity of antioxidants (Table 2). These results are in agreement with Shen et al. (2008). Such enhancing effects of Se on the activity of antioxidants have been attributed to the antioxidant activity of selenoproteins and the upregulating effects of Se on the genes responsible for the related stress mechanisms (Hartikainen 2005).

Se can also affect plant growth through influencing the production of jasmonic acid and the stress hormone ethylene and the proteins, which affect the plant resistance versus pathogens and sulfate/selenite production (Tamaoki et al. 2008). GPX contains Se in its structure, which can influence the enzyme activity. The increase in the activities of antioxidants under drought stress with Se addition is in agreement with the results of other researchers (Bailly et al. 2000; Habibi et al. 2004; Rahimizadeh et al. 2007). Metal ions such as Fe, Mn, Cu, Zn, and Mg are essential for plant growth and production; because they are necessary for many plant activities and are the cofactors for most antioxidant enzymes. Based on the type of their metal cofactor (Cu or Mg, Mn or Fe) SOD enzymes are categorized (Yu and Rengel 1999).

CAT has iron in its structure and catalyses the turn of hydrogen peroxide to oxygen and water. CAT reduces hydrogen peroxide by reduced glutathione, resulting in the protection of cellular damage from oxidative processes

Table 5 Mean comparison of antioxidants and corn grain yield, as affected by the three way interaction effects of the experimental treatments

Treatment	SOD ($\mu\text{mol}/\text{mg}$ protein)	CAT ($\mu\text{mol}/\text{mg}$ protein)	GPX ($\mu\text{mol}/\text{mg}$ protein)	MDA (nmol/g fresh weight)	Grain yield (kg ha^{-1})
L1Se0M0	1,262.25cde	89.95cdef	14.72cd	1.20d	8,769ab
L1Se0M1	1,056.50ef	80.22fg	13.42cde	0.77e	9,519a
L1Se1M0	1,034.25ef	81.12fg	12.97cde	0.73e	6,914cde
L1Se1M1	743.75g	64.85h	9.72e	0.43f	6,898cde
L2Se0M0	1,293.50cde	90.95cdef	13.67cde	1.2n	6,146def
L2Se0M1	1,441.75bc	94.82bcd	17.10bc	1.37cd	7,089cd
L2Se1M0	1,195.25cde	89.22cdef	15.35c	0.86e	8,159b
L2Se1M1	1,032.25ef	76.77g	10.25de	0.85e	6,562cdef
L3Se0M0	1,405.25bcd	92.85bcde	16.27bc	1.55bc	7,243c
L3Se0M1	1,662.5ab	103.02ab	20.87ab	2.18a	5,947ef
L3Se1M0	1,478.00bc	94.92bcd	16.75bc	1.62bc	6,747cdef
L3Se1M1	1,122.25def	82.05efg	10.37de	0.83e	6,912cde
L4Se0M0	1,642.50ab	109.75a	23.80a	2.11a	4,712g
L4Se0M1	1,793.75a	112.25a	23a	2.36a	4,202g
L4Se1M0	1,399.50bcd	98.32bc	22.42a	1.75b	6,818cde
L4Se1M1	859.00fg	84.20defg	14.92cd	0.90e	5,810f

SOD Superoxide dismutase; CAT Catalase; GPX Glutathione peroxidase; MDA Malondialdehyde

Mean values, followed by the same letters within each column are not significantly using Duncan's multiple rang test at $P=0.05$, L1: control, L2: drought stress at V8 Stage, L3: drought stress at blister stage, L4: drought stress at grain filling stage, Se0: Without Selenium, Se1: with Selenium, M0: without micronutrients, M1: with micronutrients

(Marschner 1995; Cakmak 2000). Our results are also in agreement with Wang et al. (2004) and Rahimizadeh et al. (2007) who indicated that application of micronutrients can alleviate the adverse effects of stresses such as salinity and drought on plant growth. Micronutrient presence in the structure of antioxidants (Yu and Rengel 1999; Allen et al. 2007), their important effects on the cellular respiration (Tan et al. 2010), and their deficiency under drought make their application necessary under different conditions, including stress (Hu and Schmidhalter 2005).

At the control level of water stress, addition of Se or micronutrients decreased the antioxidants activities or corn grain yield. This may be due the toxic effects of Se or micronutrients on plant growth, especially at higher concentration. However, with increasing the level of stress the application of Se (Xue et al. 2001) or micronutrients significantly enhanced the antioxidants activities and corn grain yield indicating that there are interaction effects between the drought stress and Se (Shen et al. 2008) or micronutrients. Se or micronutrients under stress can help the plant to utilize the mechanisms, which eventually result in the production of antioxidants, more effectively. Researchers have indicated that under drought stress, in addition to enhancing the level of antioxidants, Se can also enhance plant resistance to the stress by increasing the amount of proline in plant (Khatab 2004).

The combined use of Se and micronutrients adversely affected the antioxidants activities and corn grain yield indicating that there are some kind of antagonistic effects between Se and micronutrients and their combined use is not recommendable under drought stress. It is likely that the presence of each may interrupt the functioning of the other (Singh and Singh 1978; Yang et al. 2007).

According to the mean comparisons Se addition resulted in the highest amounts of antioxidants during the grain filling stage. This can be attributed to the reduction in plant absorption and hence less water availability for metabolic activities during the final period of plant growth. Therefore, the antioxidant activities might have increased to alleviate probable damages caused by water stress (Rahimizadeh et al. 2007).

Conclusion

According to the results of this research work, addition of Se or micronutrients is recommendable for increased corn production under drought stress as such use can enhance the activities of antioxidants and hence protect the plant from oxidative damage. However, the combined use of Se and micronutrients are not suggested because the antagonistic effects between the two treatments decreased antioxidant activities and corn grain yield.

References

- Allen MD, Kropat J, Tottey S, Del Campo J, Merchant S (2007) Manganese deficiency in *Chlamydomonas* results in loss of photosystem II and MnSOD function, sensitivity to peroxides, and secondary phosphorus and iron deficiency. *Plant Physiol* 143:263–277
- Bailly C, Benamar A, Corbineau F, Come D (2000) Antioxidant systems in sunflower seeds as affected by priming. *Seed Sci Res* 10:35–42
- Baker DE, Amachar MC (1982) Nickel, copper, zinc and cadmium. In: Page AL, Miller RH, Keeney DR (eds) *Methods of soil analysis, part 2*. American Society of Agronomy, Madison, pp 323–338
- Bowler C, Van Montago M, Inze D (1992) Superoxide dismutase and stress tolerance. *Ann Rev Plant Physiol Plant Mol Bio* 43:83–116
- Cakmak I (2000) Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *New Phytol* 146:85–200
- Cakmak I, Horst W (1991) Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tip of soybean (*Glycine max* L.). *Plant Physiol* 83:463–468
- Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osório ML, Carvalho I, Faria T, Pinheiro C (2002) How plants cope with water stress in the field? Photosynthesis and growth. *Ann Bot* 89:907–916
- Dadnia MR, Habibi D, Nourmohammadi G, Ardakani MR (2008) Antioxidative response of sunflower (*Helianthus annuus* L.) varieties under water deficit and selenium foliar application. *Crop Res* 35:195–200
- Dhillon K (2002) Selenium enrichment of the soil plant system for a seleniferous region of North-West. *Ind J of Hydrol* 272:120–130
- Dixon DP (1998) Glutathione mediated detoxification systems in plants. *Curr Opin Plant Biol* 1:258–266
- Gee GW, Bauder JW (1986) Particle-size analysis. In: Klute A (ed) *Methods of soil analysis, part 1*. Agronomy monograph, vol. 9, 2nd edn. ASA and SSSA, Madison, pp 383–411
- Germ M, Kreft I, Stibilj V, Urbanc-Bercic O (2007) Combined effects of selenium and drought on photosynthesis and mitochondrial respiration in potato. *Plant Physiol Biochem* 45:162–167
- Habibi D, Boojar MM, Mahmoudi A, Ardakani MR, Taleghani D (2004) Antioxidative enzymes in sunflower subjected to drought stress. 4th International Crop Science Congress, Australia
- Hartikainen H (2005) Biogeochemistry of selenium and its impact on food chain quality and human health. *J Trace Element Med Biol* 18:309–318
- Hernandez JA, Jimenez A, Mullineaux P, Sevilla F (2000) Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defenses. *Plant Cell Environ* 23:853–862
- Hu Y, Schmidhalter U (2005) Drought and salinity: a comparison of their effects on mineral nutrition of plants. *J Plant Nutr Soil Sci* 168:541–549
- Khatab H (2004) Metabolic and oxidative responses associated with exposure of *Eruca sativa* (rocket) plants to different levels of selenium. *Inter J of Agricul Biol* 6:1010–1106
- Knudsen D, Peterson GA, Pratt PF (1982) Lithium, sodium and potassium. In: Page AL, et al (eds), *Methods of soil analysis: Part 2. Chemical and Microbiological Properties*. ASA Monograph Number 9, pp. 225–246
- Kuznetsov V, Kidin VP, Vladimir V (2004) Protective effect of selenium on wheat plant under drought stress. Abstract of articles Symposium of Plant Biology 2004 - Lake Buena Vista, FL USA.
- Madhava Rao KV, Sresty TVS (2000) Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* L. Millspaugh) in response to Zn and Ni stresses. *Plant Sci* 157:113–128

- Marschner H (1995) Mineral nutrition of higher plants. Academic, New York
- Mittler R (1993) Detection of ascorbate peroxidase activity in native gels by inhibition of the ascorbate dependent reduction of nitroblue tetrazolium. *Ann Biochem* 212:540–546
- Movahed Dehnavi M, Modarres Sanavi AM, Soroushzade A, Jalali M (2002) Effect foliar application of zinc and manganese on yield and yield components of three safflower cultivars under drought stress in Isfahan. *Proceeding book of 8th Congress of Agronomy and Plant Breeding*. Rasht, Iran
- Nelson DW, Sommers LE (1973) Determination of total nitrogen in plant material. *Agron J* 65:109–112
- Nelson DW, Sommers LE (1982) Total carbon, organic carbon, and organic matter. In: Page AL, Miller RH, Keeney DR (eds) *Methods of soil analysis*, part 2, 2nd edn. American Society of Agronomy, Madison, pp 539–573
- Olsen RS (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Department of Agriculture, No. 939
- Pilon-Smits EA, Quinn C, Tapken W, Malagoli M, Schiavon M (2009) Physiological functions of beneficial elements. *Curr Opin Plant Biol* 12:267–274
- Rahimizadeh M, Habibi D, Madani H, Mohammadi GN, Mehraban A, Sabet AM (2007) The effect of micronutrients on antioxidant enzymes metabolism in sunflower (*Helianthus annuus* L.) under drought stress. *Helia* 30:167–174
- Rhoades JD (1982) Soluble salts. In: Page AL, Miller RH, Keeney DR (eds) *Methods of soil analysis*, part 2, 2nd edn. American Society of Agronomy, Madison, pp 167–178
- Sajedi NA, Ardakani MR, Rejali F, Mohabbati F, Miransari M (2010) Yield and yield components of hybrid corn (*Zea mays* L.) as affected by mycorrhizal symbiosis and zinc sulfate under drought stress. *Physiol Mol Biol Plant* 16:343–351
- Sas Institute Inc (1988) SAS/STAT user's guide. Version 6, 4th edn. Statistical Analysis Institute Inc, Cary
- Shen Q-Y, Turakainen M, Seppänen M, Mäkelä P (2008) Effects of selenium on maize ovary development at pollination stage under water deficits *Agricul Sci China* 7:1298–1307
- Singh M, Singh N (1978) Selenium toxicity in plants and its detoxication by phosphorous. *Soil Sci* 126:255–262
- Steel RGD, Torrie JH (1980) *Principles and procedures of statistics*, 2nd edn. McGraw-Hill Publishing Company, USA
- Tamaoki M, Freeman JL, Pilon-Smits EAH (2008) Cooperative ethylene and jasmonic acid signaling regulates selenite resistance in *Arabidopsis thaliana*. *Plant Physiol* 146:1219–1230
- Tan Y, O'Toole N, Taylor N, Millar A (2010) Divalent metal ions in plant mitochondria and their role in interactions with proteins and oxidative stress-induced damage to respiratory function. *Plant Physiol* 152:747–761
- Wang SH, Yang ZM, Yang H, Lu B, Li SQ, Lu YP (2004) Copper-induced stress and antioxidative responses in roots of *Brassica juncea*. *Bot Bull Acad Sinica* 45:203–212
- Xue TL, Hartikainen H, Pironen V (2001) Antioxidative and growth-promoting effect of selenium on senescing lettuce. *Plant Soil* 237:55–61
- Yang X, Chen W, Feng Y (2007) improving human nutrition through biofortification in the soil-plant system: China as a case study. *Environ Geochem Health* 29:413–428
- Yao X, Chu J, Wang G (2009) Effects of drought stress and selenium supply on growth and physiological characteristics of wheat seedlings. *Acta Physiol Plant* 31:1031–1036
- Yu Q, Rengel Z (1999) Micronutrient deficiency influences plant growth and activities of superoxide dismutases in narrow-leaved lupins. *Ann Bot* 83:175–182