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Effect of Microelements and Selenium on Superoxide Dismutase Enzyme, Malondialdehyde Activity and Grain Yield Maize (*Zea mays* L.) under Water Deficit Stress

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Abstract

This study was carried out to investigate effects of microelements under water deficit stress at different growth stages on antioxidant enzyme alteration, chemical biomarker and grain yield of maize in the years 2007 and 2008. The experiment was conducted in a split plot factorial based on a randomized complete block design with four replications. There were three factors, water deficit stress at different stages of growth as main plot and combinations of selenium (with and without using) and microelements (with and without using) as sub plots. The result indicated that the activity of superoxide dismutase and malondialdehyde content under water deficit stress increased, but grain yield was reduced. The highest grain yield was obtained from optimum irrigation, while in the case of with water deficit stress at V_8 stage it was non significant. Selenium spray increased activity of superoxide dismutase enzyme, malondialdehyde content of leaves in V_8, R_2 and R_4 stages and also grain yield. Application of microelements increased the leaves superoxide dismutase enzyme activity and malondialdehyde content. Selenium and microelements spray under water deficit stress conditions during vegetative growth and dough stage increased grain yield in comparison to not spraying elements under water stress conditions. The present results also showed that by using selenium and microelements under water stress can obtain acceptable yield compared to not using these elements.

Keywords: maize, microelements, selenium, superoxide dismutase enzyme, malondialdehyde

Introduction

Maize (*Zea mays* L.) is the world's most widely grown cereal and is the primary staple food in many developing countries (Morris *et al*., 1999). About 26% of the world's total cultivable land falls in arid and semi-arid areas (Paylore and Greenwell, 1979). The exposure of plants to environmental stresses such as drought, heat, chilling, salt and plant diseases can result in the production of reactive oxygen species (ROS) that contributes to diminished plant performance (Cheong *et al*., 2003). These abiotic stresses can result in the accumulation of reactive oxygen species (ROS) and other toxic compounds (Xiong *et al*., 2002). Production of ROS during environmental stress is one of the main causes for decreases in productivity, injury, and death that accompany these stresses in plants. ROS are produced in both unstressed and stressed cells, and in various locations (Upadhyaya and Panda, 2004). In plant cell chloroplasts, mitochondria and peroxisomes, there are important intracellular generators of ROS (Elstner, 1991). ROS play an important role in endonuclease activation and consequent DNA damage (Hagar *et al*., 1996). Oxidative stress can prevent photosynthetic activity, respiration process and plant growth. Plants are naturally provided by enzymatic and non-enzymatic systems to take care of active oxygen (Giang and Huang, 2001). Photosynthetic plants have a strong demand for combating oxidative stress and other abiotic stresses (Xiong *et al*., 2002). Plant cells respond defensively to oxidative stress by removing the ROS and maintaining antioxidant defense compounds at levels that reflect ambient environmental conditions (Scandalios, 1997). The mechanisms that act to adjust antioxidant levels afford the protection and include changes in antioxidant gene expression (Cushman *et al*., 2000). Some well-known antioxidants in plants include glutathione, vitamin C, vitamin E, antioxidant enzymes and carotenoids. Catalases, superoxide dismutase, peroxides, are antioxidant enzymes. Bailly *et al*. (2000) reported that the content of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and malondialdehyde (MDA) in sunflower seeds will increase under drought stress condition. Within a cell, superoxide dismutase (SOD) constitutes the first line of defense against ROS (Alscher *et al*., 2002). Malondialdehyde has been known as the end product of peroxidation of membrane lipids. Water deficit stress by increase of generation ROS is responsible for stress-dependent peroxidation of membrane lipids (Upadhyaya and Panda, 2004).

Searching for suitable ameliorants or stress alleviant is one of the tasks of plant biologists. Recent researches have identified several beneficial effects of selenium (Se) in plants although Se is not considered to be required by higher plants. Positive effects of Se on plants mainly exhibited: promoting plant growth, alleviating UV-induced (Yao *et al*., 2009). Selenium is an element whose deficiency causes the decrease in defense mechanisms of living organisms. Earlier studies have indicated that selenium (Se) maintains antioxidative defence systems and enhances sugar and starch accumulation. Among naturally existing elements seven micro elements including i.e., Fe, Zn, Cu, Mn, B, Mo and chlorine are needed for plant growth. Ionic proms of Fe, Zn, Cu, Mn and Mg as co-factors exist in many antioxidant enzymes. Under deficiency of microelementss antioxidant enzymes activity is reduced, therefore plants sensitivity to environmental stresses increases (Cakmak, 2000). Application of microelement fertilizers can enhance plants resistance to environmental stresses such as drought and salinity (Maleckuti and Sepehri, 2001; Movahed Dehnaviet *et al*., 2002). Malan *et al* (1990) reported that drought-tolerant and intolerant maize inbred correlated with Cu/Zn SOD and glutathione reductase activities although higher levels of one enzyme alone apparently did not confer drought tolerance. The relative tolerance of a genotype to water stress as reflected by its comparatively lower lipid peroxidation and higher membrane stability index, chlorophyll and carotenoid contents was closely associated with its antioxidant enzyme system (SOD, APO, GR, CAT). Results of experiments indicated that micronutrient application reduces the effects of environmental stresses such as drought stress and salt stress (Wang *et al*., 2004).

The objective of this research was to investigate the effects of selenium and microelements spraying under deficit stress on maize yield and biochemical characteristics at different growth stages.

Materials and methods

This study was conducted under water deficit stress with maize (*Zea mays* L. 'S.C 704'). These experiments were carried out in the Agricultural Research Station of Islamic Azad University, Arak Branch, Iran, during the growing season of 2007-2008 and 2008-2009. This site is located at 34˚30'N latitude, 40˚41'E longitude, with the altitude of 1779 m above sea level in Markazi Province in the center of Iran. This region has a semi-arid climate on the base of metrological data in Arak, Iran in 2007-2008 and 2008-2009 (Tab. 1).

Experimental treatments were irrigation levels in the main plots including full watering as control, water stress in vegetative stage (V_8), seed formation or blister stage (R_2) and grain pre maturity or dough stage (R_4). The sub plots included mix levels of selenium and microelements fertilizer. Water stress was executed by temporary stopping of irrigation at each mentioned stage. Full irrigation (control) was arranged by crop water requirement according to

Tab. 1. Metrological data in Arak, Iran

Month	Monthly total of precipitation (mm)		Average of temperature (˚C)	
	2007-2008	2008-2009	2007-2008	2008-2009
May	17.1	17.9	16.4	19.5
June	23.7	23.4	5.4	0
July	27.1	27.7	36.5	0.1
Aug	26.2	27.1	7.3	0
Sep	23.7	23.8	0.1	0
Oct	17.6	16.9	1.5	15
Mean	22.5	22.8	18.7	5.8

daily evaporation from basin pan. Daily evaporation from basin pan was calculated by equation of

V = S× H

V: Daily evaporation from basin pan

S: Area of basin pan

H: Rate of evaporated water

Also from multiple coefficients of basin pan and evaporated water, the potential of evapotranspiration was obtained. Rate of entered water to every plot was calculated by the following equation (Alizadeh, 2002).

Water volume (m^3) = Plot area × Irrigation water efficiency× Maize coefficient × Potential of evapotranspiration

Maize coefficient is 0.36 up to 0.58 at growth initiation, 0.71 up to 1.13 at growth meddle and 0.98 up to 0.68 at growth final (Farshi *et al*., 1997). Irrigation water efficiency of 80% was considered. Irrigation type was a siphoning system with polyethylene tubes that was controlled by tube regulator tap. Total of consumptive water during season of corn for every treatment was calculated (Tab. 2).

Microelements fertilizer (Biomin 212) treatments were in two levels without and with application. Foliar application at early vegetative stage (V_6) and a week before tassling stage at the rate of 2 liter ha^{-1}. Biomin 212 fertilizer content (%wt/wt) was 2.0 Fe, 2.0 Zn, 0.5 Cu, 1.0 Mn, 0.025 B and 0.5 Mg.

Selenium treatments were at two levels, as well. The first one was selenium application with 20 g ha^{-1} sodium selenite at early vegetative stage (V_6) and second one was control.

Tab. 2. Volume of consumptive water

Year	Experiments of irrigation	Irrigation water(m^3/ha)
2007-2008	optimum condition	8552
	water deficit stress in V_8	7205
	water deficit stress in R_2	7295
	water deficit stress in R_4	7278
2008-2009	optimum condition	7939
	water deficit stress inV_8	7871
	water deficit stress in R_2	7904
	water deficit stress in R_4	7847

Tab. 3. Chemical and physical properties of farm soil

Year	Depth	EC	pH	OC	N	P	K	Zn	Fe	Mn	Cu	Se	Sand	Silt	Clay
	(cm)	(dsm^{-1})		(%)					(ppm)					(%)	
2007	0-30	1.20	7.5	0.82	0.08	5	150	0.8	4.6	10.6	1.14	0.27	29	35	36
2008	0-30	1.70	7.7	0.78	0.06	8.5	130	9.2	3.9	9.2	1.1	0.23	26	38	36

Before seed sowing, multiple soil samples were collected for determination of their physical and chemical properties (Tab. 3). According to soil testing the amounts of fertilization were applied including 375 kgha^{-1} urea, 150 kgha^{-1} triple super phosphates and 150 kgha^{-1} potassium sulphate fertilizers. 30 percentages of nitrogen (N) and all of phosphorous (P) and potassium (K) fertilizers were applied at planting time. The remaining N fertilizer was broadcasted twice during the vegetative growth as top-dress fertilizer at six-leaf stage and two weeks before tassling stage.

Each experimental plot included four 60 cm distanced rows with 20 cm spacing between plants in rows. Land preparation, including ploughing, was conducted in fall and perpendicular disking in May The length of each row was 6 m and two rows were left uncultivated between the adjacent plots. The hybrid maize was 'S.C 704'. The seeds were sown at 50-60 mm depth on 18th May, 2007(first year) and 14th May, 2008 (second year).

Biochemical analysis was based on plant sampling 24 h before irrigation times from five leaves of each plant. The harvested leaves were frizzed and kept at -80°C for further biochemical analysis. Leaf samples (0.2 g) were homogenized in a mortar and pestle with 3 ml ice-cold extraction buffer (25 mM sodium phosphate, pH 7.8). The homogenate plant material was centrifuged by18000 rm^{-1} for 30 minutes, and then the supernatant was filtered through filter paper. The supernatant fraction was used as a crude extract for the assay of enzyme activity. The biochemical activity was measured based on Misra and Fridorich (1979). The ability to inhibit free radical chain-propagating radical and the auto oxidation of epinephrine (0.25 mM) were determined. Furthermore, SOD standard was used for calibration of activity and lipid peroxidation was determined by estimating the malondialdehyde (MDA) content in 1g fresh leaf material according to Madhava Rao and Sresty (2000). Determination of MDA that is a product of lipid peroxidation was determined by thiobarbituric acid reaction. The concentration of MDA was calculated from the spectroscopy absorbance at 532 nm (correction was done by subtracting the absorbance at 600 nm for unspecific turbidity).

Two years data were combined and the analysis of variance was performed using SAS (SAS Institute Inc, 1997). Each treatment was analyzed in four replications. The means comparisons were estimated by Tukey's multiple tests at %5 probability level.

Results and discussion

According to the combined mean comparison results, the years significantly affected superoxide dismutase enzyme activity and malondialdehyde content. The superoxide dismutase enzyme activity and malondialdehyde leaves content in the second year in all three plant growth and development stages were higher than in the first year (Tab. 4, Fig. 1 and 2).

In this study, the highest and lowest superoxide dismutase enzyme activity and malondialdehyde content of leaves was observed in water deficit stress for V_8 and R_4 stage respectively. Under water deficit condition, the ROS generation and antioxidant enzymes activity increased at V_8 and R_4 growth and development stages. In other words, water deficit condition in V_8, R_2 and R_4 in maize growth and development stages could be increase malondialdehyde level by 21, 23 and 26% respectively compared to control (Tab. 4). The simultaneous increase in these enzymes activity contributes to a decrease of injurious effects of H_2O_2 under drought stress. As plants produce new organs and have high metabolism at V_8 stage, there upon ROS generation will increased under water deficit stress condition. Upadhyaya and Panda (2004) reported that

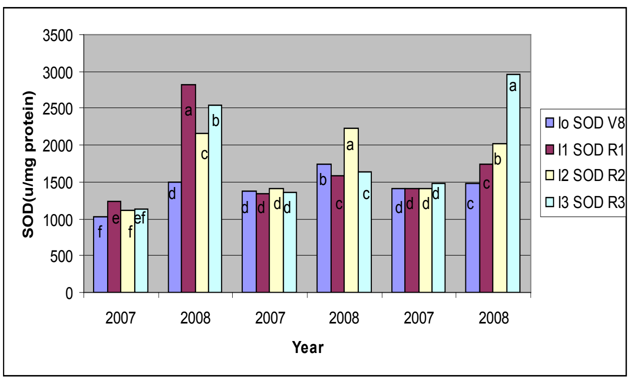

Fig. 1. Effect year on SOD enzyme activity

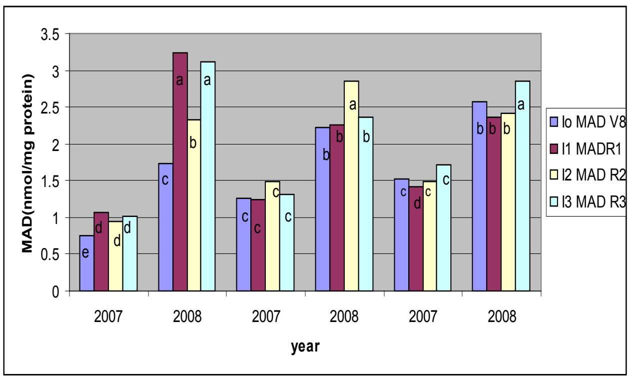

Fig. 2. Effect year on MAD content

Tab. 4. Mean comparisons for traits at different treatment levels

Treatment	SOD V_8	SOD R_2	SOD R_4	MAD V_8	MAD R_2	MAD R_4	Grain yield
	(u/mg.protein)			(nm/mg.protein)			Kg ha^{-1}
Year							
2007	1132.97 b	1373.01b	1434.25 b	0.95b	1.33b	1.54b	6781.55a
2008	2257.42 a	1802.57a	2136.39a	2.61a	2.43a	2.55a	6118.14a
Water Limitation							
L_1	1263.93 d	1560.50b	1575.96 c	1.25c	1.75b	2.05b	7076.44a
L_2	2031.25a	1466.59c	1627.37 bc	2.15a	1.75b	1.89b	6603.75ab
L_3	1642.46c	1825.75a	1716.93b	1.63b	2.18a	1.95b	4241.05c
L_4	1841.78b	1498.34c	2221.00a	2.07a	1.845b	2.29a	5878.14bc
Selenium							
Se0	1505.12 b	1447.28 b	1600.40 b	1.43 b	1.59b	1.77b	6419.87a
Se1	1884.59 a	1728.31a	1970.23 a	2.12a	2.16a	2.32a	6479.82a
Microelements							
M0	1540.51b	1421.64b	1714.60b	1.53b	1.59b	1.94b	6618.21a
M1	1849.20 a	1753.95a	1856.03 a	2.02a	2.17a	2.15a	6281.47b

Means followed by similar letters in each column are not significantly different at the 5% level of probability according to Tukey's Test. L_1: Optimum condition (control) L_2: Water limitation in V_8 stage. L_3: Water limitation in blister stage. L_4: Water limitation in dough stage. Se0: Without selenium. Se1: With selenium. M0: Without microelements. M1: With microelements. SOD: Superoxid dismutase. MAD: Malondialdehyde

Tab. 5 . Mean comparison of twofold interaction effects of traits

Treatment		SOD V_8	SOD R_2	SOD R_4	MAD V_8	MAD R_2	MAD R_4	Grain yield
		(u/mg.protein)			(nm/mg.protein)			Kg ha^{-1}
Water Limitation	Selenium							
L_1	Se0	1165.00f	1404.62c	1449.37d	1.05e	1.49 d	1.79e	7656.73 a
	Se1	1362.87e	1716.37b	1702.56c	1.45d	2.00 bc	2.31b	6496.14 b
L_2	Se0	1716.68c	1368.93c	1451.87d	1.70c	1.49 d	1.71e	6616.50 b
	Se1	2345.81a	1564.25b	1802.87 c	2.61a	2.00bc	2.08 cd	6591.00 b
L_3	Se0	1549.50d	1672.68b	1515.37d	1.34d	1.86c	1.69e	6019.26b
	Se1	1735.43c	1978.81a	1918.50b	1.93b	2.49a	2.22bc	6462.83b
L_4	Se0	1589.31b	1342.8c	1985.0b	1.65c	1.53d	1.89de	5386.99c
	Se1	2094.25d	1653.81b	2457.00a	2.48a	2.15b	2.69a	6369.29b
Water Limitation	Microelement							
L_1	Mo	1144.12e	1408.12c	1494.62e	0.97d	1.48e	1.95 bc	6850.14 ab
	M1	1383.75d	1712.87b	1657.31 cd	1.53c	2.01c	2.15b	7302.73 a
L_2	Mo	1754.37c	1311.06c	1574.75 de	1.84b	1.56e	1.82c	6768.25 ab
	M1	2308.12a	1622.12 b	1680.00cd	2.47a	1.93 cd	1.97 bc	6439.25 bc
L_3	Mo	1468.12c	1580.37b	1715.62c	1.55c	1.81d	1.92bc	6422.27 bc
	M1	1636.81c	2071.12a	1718.25c	1.72b	2.54a	1.99 bc	6059.82c
L_4	Mo	1626.75c	1387.00c	2073.43b	1.78b	1.49e	2.09b	6432.19 bc
	M1	2056.81b	1609. 68b	2368.56a	2.35a	2.19b	2.49a	5324.09d
Selenium	Microelement							
Se0	Mo	1920.46a	1851.62 a	2041.59a	2.22a	2.35ab	2.44a	6664.41a
	M1	1848.71 ab	1605.00 b	1898.87b	2.00b	1.98bc	2.20b	6175.33b
Se1	Mo	1777.93b	1656.28 b	1670.46c	1.80c	1.99bc	1.86c	6572.01 ab
	M1	1232.31c	1238.28 c	1530.34d	1.07d	1.20e	1.68d	6387.62 ab

Means followed by similar letters in each column are not significantly different at the 5% level of probability according to Tukey's Test

malondialdehyde has been known as the end product of peroxidation of membrane lipids. Water deficit stress by the increase of generation ROS is responsible for stress-dependent peroxidation of membrane lipids. It seems that the enhancement in produce ROS can increase substrate for superoxide dismutase reaction. Increased superoxide dismutase and catalase activities in response to water deficit stress have been reported (Halliwell and Gutteridge, 1989).

Our results showed that drought stress in maize farms could make the significant changes in antioxidant enzymes activity in leave, similar to those reported by Ghorbanli *et*

Tab. 6. Mean comparison of threefold interaction effects of traits

Treatment			SOD V_8	SOD R_2	SOD R_4	MAD V_8	MAD R_2	MAD R_4	GY
			(u/mg protein)			(nm/mg.protein)			(Kg ha^{-1})
Water Limitation	Selenium	Microelements							
L_1	Se0	M0	1540.37 e	1725.37 b	1781.75 ef	1.782e	2.06 bcd	2.41 b	7336.55 ab
L_1	Se0	M1	1185.37 g	1707.37 b	1623.37 fg	1.134g	1.95 cd	2.20 bcd	7976.91a
L_1	Se1	M0	1227.12 g	1700.37 b	1532.87 gh	1.28fg	1.97 cd	1.88d-g	6790.73 bcd
L_1	Se1	M1	1102.87 g	1108.87 c	1365.87 h	0.81h	1.01 f	1.69efg	6628.55 bc
L_2	Se0	M0	2458.62 a	1625.00 b	1834.50 de	2.73 a	2.02 cd	2.18 bcd	6799.52 bc
L_2	Se0	M1	2233.00 b	1505.50 b	1771.25 ef	2.48ab	1.98 cd	1.97 c-f	6433.48 bcd
L_2	Se1	M0	2157.62 bc	1621.25 b	1525.50 gh	2.21cd	1.84 de	1.76 efg	6736.97 bc
L_2	Se1	M1	1275.75 fg	1116.62 c	1387.52 h	1.20g	1.14 f	1.67 fg	6445.03 bcd
L_3	Se0	M0	1453.25 ef	2398.25 a	1884.00 de	1.93e	3.03 a	2.22 bc	6448.16 bcd
L_3	Se0	M1	2017.62 cd	1559.37 b	1953.00 cd	1.93e	1.96 cd	2.21 bc	5590.36 d
L_3	Se1	M0	1843.00 d	1744.00 b	1552.50 g	1.36f	2.06 bcd	2.76 efg	6396.38 bcd
L_3	Se1	M1	1256.00 g	1601.37 b	1478.25 gh	1.17g	1.67 e	1.62 g	6529.28 bcd
L_4	Se0	M0	2229.62 b	1659.87 b	2666.12 a	2.52ab	2.27 b	2.96 a	6073.43 cd
L_4	Se0	M1	1958.87 d	1647.75 b	2247.87 b	2.44bc	2.02 cd	2.42 b	4700.55e
L_4	Se1	M0	1884.00 d	1559.50 b	2071.00 c	2.19d	2.10 bc	2.02cde	6363.96bcd
L_4	Se1	M1	1294.62 fg	1126.25 c	1899.00 de	1.11g	0.97 f	1.76 fg	5947.62cd

Means followed by similar letters in each column are not significantly different at the 5% level of probability according to Tukey's Test

al. (2004), Malan *et al.* (1990), Bailly *et al.* (2000), Giang and Huang (2001) and Habibi *et al.* (2004) in sunflower.

Selenium and microelement fertilizers could also increase superoxide dismutase enzyme activity and malondialdehyde content in leaves in V_8, R_2 and R_4 stages. Selenium role in plants under water stress could increase antioxidant enzymes activities by reducing oxidative conditions and free radicals which have a determinate effect on plant cells.

According to means comparison microelements application was increased antioxidant activities of superoxide dismotase in V_8, R_2 and R_4 plant growth and development stages by 16.7%, 18.9% and 7.6%. The malondialdehyde content of leaves was increased by 24%, 26.7% and 9.7% respectively compared to control (Tab. 4). Rahimizade *et al.* (2007) reported that SOD activity was increased by 31%, under drought stress in sunflower. Hacisalihoglu *et al.* (2003) reported that under Zn deficiency stress, activity of Cu/Zn SOD decreases as Zn is directly involved in both gene expression and protein synthesis. Cakmak (2000) reported that Zn deficiency stress may inhibit the activities of a number of antioxidant enzyme. Similarly, Rahmati *et al.* (2004) found that the activity of SOD, CAT and APX (ascorbate peroxidase) in excess Mn treated cells increased compared to control treatment.

Using selenium in water deficit stress condition increased superoxide dismutase enzyme activity and malondialdehyde content as compared to treatments of not using selenium. The highest amounts of measured traits under water deficit were found in V_8 and R_4 stages (Tab. 5).

The effects of three way factors interactions on superoxide dismutase enzyme activity and malondialdehyde V_8 and R_2 stages were significant but, in R_4 stages not significant. In water deficit stress condition in each three stage, the highest malondialdehyde content were observed from treatments of without microelements and without selenium. This might indicate plant sensitivity due to no protection factor under water stress.

It was found in this study that superoxide dismutase enzyme had positive and significant correlation with malondialdehyde content under water deficit stress in vegetative stage (V_8), in blister stage (R_2) and water deficit stress in dough stage (R_4) (Tab. 7).

Tab. 7. Matrix of simple correlation coefficient among different traits

	SODV$_8$	MADV$_8$	SODR$_2$	MADR$_2$	SODR$_4$	MADR$_4$	Grain yield
SODV$_8$	1						
MADV$_8$	0.92**	1					
SODR$_2$	0.34*	0.46**	1				
MADR$_2$	0.61**	0.71**	0.83**	1			
SODR$_4$	0.65**	0.69**	0.36**	0.56**	1		
Grain yield	-0.12^{ns}	-0.18*	-0.17*	-0.18*	-0.06^{ns}	-0.10^{ns}	1

**, *, ^{ns} significant at the 1%, 5% probability levels and non significant respectively

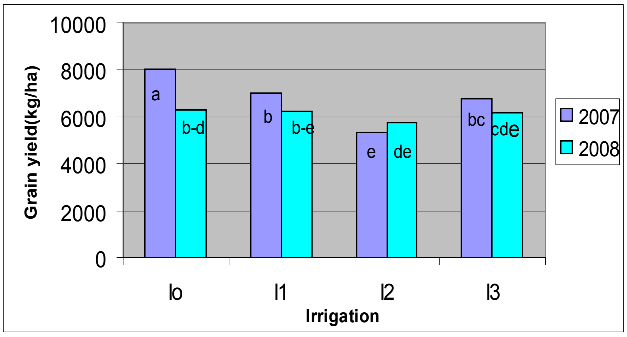

Fig. 3. Effect irrigation and year on grain yield

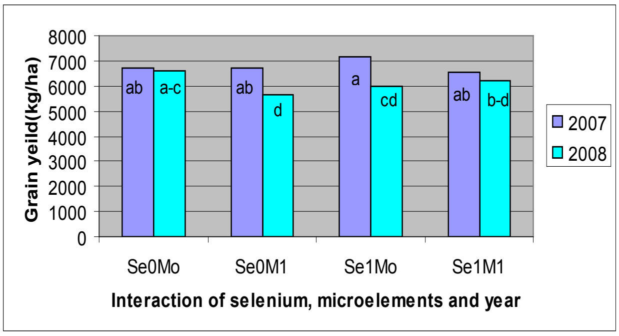

Fig. 4. Effect irrigation, selenium and year on grain yield

The result showed that grain yield was higher in the first year than the second year (Tab. 4 and Fig. 3). This seems to be related to better climate condition in the first year (Tab. 1). The effect of water deficit stress on grain yield was significant. The highest grain yield was obtained from normal irrigation and water deficit at V_8 stage without significant difference (Tab. 4). It seems that under drought stress in maize the antioxidative defence system enhances sugar and starch accumulation in cells.

Grain yield of selenium sprayed-plants was increased as compared with control (Tab. 4). It may be due to enhancement of photosynthesis and decrease in leaf senescence, increases assimilate production and transport towards seeds and as a result seed yield (Xue and Hartikaine 2001).

Grain yield of microelements sprayed-plants was decreased by 5% as compared to no microelements application. This may be related to antagonistic interaction of microelements with each other. These results are similar to those reported by Himayatullah and Khan (1998). They reported that in maize, copper application alone and with iron and manganese decreased kernel number per ear, 1000 grain weight and grain yield.

Results of combined analysis two way interactions of water deficit stress and selenium on grain yield was significant. The highest grain yield was obtained from control treatment (without stress and without selenium) which showed significant differences compared to other treatments. Least grain yield under water deficit was obtained from treatment of water deficit stress in grain filling stage and without selenium application (Tab. 5). This might indicate plant sensitivity due to the lack of a protection factor under water stress. Xue *et al.* (2001) reported that selenium has antioxidant properties and under conditions of environmental stress, especially water stress, it can scavenge reactive oxygen. Seppanen *et al.* (2003) reported that selenium prevents chlorophyll degradation under water stress.

Microelement applications under optimum irrigation increased grain yield compared to non microelement application. But in water deficit stress conditions, grain yield was decreased in all growth and development stages. It seems that, in water deficit conditions due to disorder transmission and increase concentration of microelements plant toxicity was created.

An antagonistic interaction was found for selenium and microelements application for grain yield. This may be due to antagonistic interaction between microelements and selenium (Tab. 5 and Fig. 4). Interactions effect had also a significant result on grain yield. The highest grain yield up to 9519 $kgha^{-1}$ was obtained from optimum irrigation conditions, no selenium and microelements application treatment (Tab. 6). Selenium and microelements application in vegetative growth stage and dough grain development stages in water deficit conditions could increase maize grain yield significantly (Tab. 6). It seems that in the end of the growth and development stage in increased water stress conditions, selenium and microelements find biological activity roles in plant cells. Plant tissue health and protection of cell membranes were formed as a permanent function. In general, by selenium and microelements application under water stress, higher grain yield was obtained. These study results also showed that there is a negative and significant correlation between grain yield with superoxide dismutase enzyme and malondialdehyde content under water deficit in the vegetative stage, blister stage and water deficit in dough grain development stages (Tab. 7). This was observed as the decrease of grain yield.

Conclusions

Microelements and selenium application on maize farms could not only increased superoxide dismutase enzyme activity and malondialdehyde content in leaf tissues under normal irrigation but also, under water deficit in V_8, R_2 and R_4 stages. Grain yield by selenium application was increased. The role of selenium in plants under stress was to increase antioxidant enzyme activities to reduce oxidative conditions or free radical injures which have a determinate effect on plant cells. The effect of microelements acts as a component of superoxide dismutase, catalase, peroxidase and nitrate reductase. Therefore, when plants are deficient in these elements, activities of antioxidant enzymes decrease, thus imposing and increased sensitivity to environmental stresses. Results showed that with microelements in water optimum conditions, and selenium spray under water deficit stress condition an optimum grain yield can be obtained.

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