

The effect of micronutrients on antioxidant enzymes metabolism in Sunflower (*Helianthus annuus L.*) under drought stress

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Abstract:

Superoxide dismutase(SOD), Catalase (CAT) and Glutathione peroxidase (GPX) are antioxidant enzymes which have important role in the metabolize reactive oxygen species(ROS) and defense against oxidative stress damage. Antioxidant enzymes activity increase in plant cells as a response to environmental stresses. The objective of this study was to evaluate the effects of micronutrients application on the antioxidant enzyme metabolism (SOD,CAT and GPX) in sunflower under drought stress. This experiment was carried out at Golmakan agriculture research station (Iran) in 2005,that using a split plot randomized complete block design with four replications. Irrigation as a main factor at three levels (normal, low stress and high stress) and six micronutrient treatments (control, Fe, Fe+Zn, Fe+Zn+Cu, Fe+Zn+Cu+Mn Fe+Zn+Cu+Mn+B) as sub plots within the main plots. Base fertilizers (N,P,K)and micronutrient treatments also used as required on the basis of the soil test. Results showed that the activity of these enzymes were significant different ($\alpha = \%5$) between control and stress treatments. The antioxidant enzymes concentrations were increased at %11-31 under high stress. Also there were significant different ($\alpha = \%5$) between control and micronutrient treatments under enzyme concentration. The antioxidant enzymes concentrations were increased at %48-89 level with Fe+Zn+ Cu+Mn treatment. The results showed that under drought stress micronutrients application increase drought resistance in sunflower.

Keywords: Antioxidant enzymes, Sunflower, Drought stress, Micronutrients.

Introduction

Environmental stress adversely affects plant performance and often results in significant reductions in crop yield and quality world wide (Boyer, 1982). The exposure of plants to environmental stresses such as drought stress, heat stress, chilling stress, salt stress and plant diseases can result in the production of reactive oxygen species (ROS) that contributes to diminished plant performance (Grill et al., 2001). Increasing evidence indicates that oxidative damage to critical cell compounds resulting from attack by ROS. A variety of enzymatic and non enzymatic mechanisms exist to metabolize ROS into less harmful chemical species (Jaing and Huang, 2001). Antioxidant enzymes activity increased in plant cells as a response to environmental stresses. These enzymes have an important role in the defense against oxidative stress (Cakmak, 2000; Foyer, 2001; Jiang and Huang, 2001; Blokhina et al., 2003; Habibi et al., 2004). Halliwell and Cutler (1990) reported that in oilseed crops such as sunflower, the content of free radicals such as superoxide and peroxide in tissue will increase under stress conditions. Bailly et al. (2000) reported that in sunflower, the content of Superoxide dismutase (SOD), Catalase (CAT), Glutathione reductase (GR) and Malondialdehyde (MDA) in seeds will increase under drought stress condition. Within a cell, the Superoxide dismutase (SOD) constitute the first line of defense against ROS (Alscher et al., 2002). There are three distinct types of SOD classified on the basis of the metal cofactor: the copper / zinc (Cu,Zn_SOD), the manganese (Mn_SOD) and the iron (Fe_SOD) isozyme (Bannister et al., 1987). Catalase (CAT) is a heme-containing enzyme that catalyzes the dismutation of hydrogen peroxide into water and oxygen. Glutathione peroxidase (GPX) has a residue of selenium of selenocysteine on four unit branches that is very important for enzyme activity. GPX catalyzes the reduction of hydrogen peroxide by GSH (reduced glutathione), thereby protecting the cells from oxidative damage (Esterbauer et al., 1992). Metal ions such as Fe, Zn, Cu, Mn and Mg are essential mineral micronutrients and cofactors of most antioxidant enzymes. Marschner (1986) and Cakmak et al. (1999) indicated Zn is a cofactor of over 300 enzymes and proteins involved in cell division, nucleic acid metabolism and protein synthesis. Also crop yields are often limited by low soil levels of mineral micronutrients in calcareous soils of arid and semiarid regions (Graham et al., 1992; Cakmak et al., 1999). Cakmak (2000) has speculated that Zn deficiency stress may inhibit the activities of a number of antioxidant enzymes. The objective of this study was to investigate the effect of micronutrient application on the antioxidant enzyme metabolism of sunflower oil under drought stress.

Material and method

This experiment was carried out at the Golmakan Agriculture Research Station (Iran) in 23 May 2005 on a loam soil having: 0.03% total nitrogen content, 8.8 ppm phosphorus (P_2O_5), 230 ppm potassium (K_2O), 0.4% organic matter, 3.1 ppm Fe, 0.52 ppm Cu, 0.3 ppm Zn, 6.74 ppm Mn, 0.5 ppm B and a pH = 7.7. The hybrid cultivar Record was used as plant material. In this study using a split plot completely randomized block design with four replication Irrigation as a main factor at three levels (normal, low stress and high stress) and six fertilizer treatments (control, Fe, Fe+Zn, Fe+Zn+Cu, Fe+Zn+Cu+Mn, Fe+Zn+Cu+Mn+B) as subplots within the main plots. The irrigation treatments were as follows:

- 1- Irrigation after 60mm evaporation of Pan Class A (no stress)

2-Irrigation after 120mm evaporation of Pan Class A (low stress)

3- Irrigation after 180mm evaporation of Pan Class A.

Base fertilizers (N,P,K) and micronutrient treatments also used as required on the basis of the soil test. Iron was used as FeSO_4 at the rate of $120 \text{ kg}\cdot\text{ha}^{-1}$, Zinc as ZnSO_4 at the rate of $70 \text{ kg}\cdot\text{ha}^{-1}$, Copper as CuSO_4 at the rate $40 \text{ kg}\cdot\text{ha}^{-1}$, Manganese as MnSO_4 at the rate of $60 \text{ kg}\cdot\text{ha}^{-1}$ and Boron as HBO_3 at the rate $30 \text{ kg}\cdot\text{ha}^{-1}$ each mixed with soil before planting. Twenty leaves were randomly selected from each plot (at flowering period) for enzyme assay and protein measurement. All samples were transported to the laboratory at least time. All data were subjected to analysis of variance for each character using MSTAT-C software.

Sample preparation for enzyme assay and protein measurement: Leaves from each plant were washed with distilled water and homogenized in 0.16M Tris buffer (pH=7.5) at 4°C . Then, 0.5 ml of total homogenized solution was used for protein determination by the Lowry et al. (1957) method. Based on the amount of protein per volume of homogenized solution, the following enzymes were assayed in the volume containing a known protein concentration in order to calculate the specific activities of the enzymes.

Superoxide dismutase (SOD) activity: The activity was measured based on Misra and Fridovich (1972), in which the activity was measured on the basis of its ability to inhibit free radical chain oxidation in which O_2 was a chain propagating radical and the auto oxidation of epinephrine (0.25mM) was induced. SOD standard was used for calibration of activity.

Catalase (CAT) activity: Catalase activity was measured at 25°C as previously described by Paglia and Valentine (1987), that used hydrogen peroxide as substrate and 1 k of catalase activity was defined as the rate constant of the first order reaction.

Glutathion peroxidase (GPX) activity: The activity was measured by the Paglia and valentine (1987) method in which 0.56M (pH=7) phosphate buffer, 0.5 M EDTA, 1mM NaN_3 , 0.2mM NADPH were added to the extracted solution. GPX catalyses the oxidation of glutathion (GSH) by cumene hydroperoxide. In the presence of Glutathion reductase and NADPH, the oxidized glutathion is immediately converted to the reduced form with the concomitant oxidation of NADPH to NADP. The decrease in absorbance at 340 nm was measured with a spectrophotometer.

Results and discussion

The results showed that activity of these enzymes (SOD,CAT,GPX) increased under drought stress and were significant differences ($P<0.01$) between activity levels of superoxide dismutase, Catalase and Glutathione peroxidase in the irrigated treatments. SOD,CAT and GPX activity were increased at %31,%12 and %11 level respectively, under high drought stress(table .1).

Table 1. Effect of irrigation and fertilizer treatment on antioxidant enzyme activity.

Irrigation treatment	SOD(U/g.protein)	CAT(U/g.protein)	GPX(U/g.protein)
No stress(control)	959.0 C	97.07 B	8.371 B
Low stress	1133.0 B	95.73 B	8.750 AB
High stress	1257.0 A	108.40 A	9.299 A
Fertilizer treatment			
No fertilizer(control)	727.1 C	71.18 C	6.783 C
Fe	793.6 C	80.36 C	7.075 C
Fe+Zn	1220.0 B	107.10 B	9.093 B
Fe+Zn+Cu	1333.0 AB	111.10 AB	9.626 AB
Fe+Zn+Cu+Mn	1378.0 A	120.40 A	10.040 A
Fe+Zn+Cu+Mn+B	1245.0 AB	112.10 AB	10.220 A
% CV	13.9	13.3	11.8

Our findings were in agreement with the results reported by Malan et al.(1990), Bailly et al.(2000), Jiang and Huang(2001), Habibi et al.(2004). The simultaneous increase in the activity of these enzymes contributes to decrease the deleterious effects of H₂O₂ under drought stress. Also, analysis of variance indicated that there were significant differences ($P < 0.01$) between activity levels of these enzymes in the fertilizer treatments. SOD, CAT and GPX activity were increased at 89%, 69% and 48% level respectively, with Fe+Zn+Cu+Mn treatment. The lowest activity of these enzymes was obtained from the non-fertilizer (control) treatment. Hacisalihoglu et al. (2003) reported that under Zn deficiency stress, the activity of Cu/Zn SOD; because Zn is directly involved in both gene expression and protein synthesis. Also, Cakmak (2000) reported that Zn deficiency stress may inhibit the activities of a number of antioxidant enzymes. Similarly, Rahmati et al.(2004) found that activity of SOD, CAT and APX (Ascorbate peroxidase) of excess Mn-treated cells increased compared with control treatment. In addition, results of experiments indicated that micronutrient application decreases the effects of environmental stresses such as drought stress and salt stress (Wang et al.2004).

It is known that the amount and distribution of precipitation and differentiation in temperature are the major factors affecting seed yield and some yield components of sunflower in arid and semi-arid regions. Therefore, micronutrient application on field involves the decrease of drought stress effects in these regions.

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