

THE EFFECT OF MICRONUTRIENTS ON ANTIOXIDANT ENZYMES METABOLISM IN SUNFLOWER (*Helianthus annuus L.*) UNDER DROUGHT STRESS

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SUMMARY

Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) are antioxidant enzymes which have important role in the metabolic reactive oxygen species (ROS) and defence against oxidative stress damage. Antioxidant enzymes activity increases 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, 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 was significantly different ($\alpha=5\%$) between control and stress treatments. The antioxidant enzymes concentrations were increased at 11-31% under high stress. Also there were significant differences ($\alpha=5\%$) between control and micronutrient treatments under different enzyme concentrations. 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.

Key words: antioxidant enzymes, sunflower, drought stress, micronutrients

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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 results from attack by ROS. A variety of enzymatic and non-enzymatic mechanisms exist that metabolize ROS into less harmful chemical species (Jaing and Huang, 2001). Antioxidant enzymes activity increases in plant cells as a response to environmental stresses. These enzymes have 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 Cuttidge (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, superoxide dismutase (SOD) constitutes the first line of defense against ROS (Alscher *et al.*, 2002). Here are three distinct types of SOD classified on the basis of the metal cofactor: copper/zinc (Cu, Zn_SOD), manganese (Mn_SOD) and 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 selenocystein 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 Fe, Zn, Cu, Mn and Mg are essential mineral micronutrients and cofactors of most antioxidant enzymes. Marschener (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) 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 micronutrients application on the antioxidant enzymes metabolism of sunflower oil under drought stress.

MATERIAL AND METHOD

This experiment was carried out at the Golmakan Agriculture Research Station (Iran) on a loam soil having: 0.03% total nitrogen content, 8.8 ppm phosphorus

(P₂O₅), 230 ppm potassium (K₂O), 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 replications. 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 sub-plots within the main plots. The irrigation treatments were as follows:

1. Irrigation after 60 mm evaporation of Pan Class A (no stress)
2. Irrigation after 120 mm evaporation of Pan Class A (low stress)
3. Irrigation after 180 mm evaporation of Pan Class A.

Basic fertilizers (N,P,K) and micronutrient treatments were used as required on the basis of the soil test. Iron was used as FeSO₄ at the rate of 120 kg ha⁻¹, zinc as ZnSO₄ at the rate of 70 kg ha⁻¹, copper as CuSO₄ at the rate 40 kg ha⁻¹, manganese as MnSO₄ at the rate of 60 kg ha⁻¹ and boron as HBO₃ at the rate 30 kg 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 promptly transported to the laboratory. 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 0.16 M 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 O₂ 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 NaN₃, 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 the activity of these enzymes (SOD, CAT, GPX) increased under drought stress and there were significant differences ($P < 0.01$) between activity levels of superoxide dismutase, catalase and glutathione peroxidase in the irrigated treatments. SOD, CAT and GPX activities were increased by 31%, 12% and 11%, 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) and Habibi *et al.* (2004). The simultaneous increase in the activity of these enzymes contributes to a decrease of the deleterious effects of H_2O_2 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 activities were increased by 89%, 69% and 48%, respectively, with the Fe+Zn+Cu+Mn treatment. The lowest activity of these enzymes was obtained in the non-fertilizer (control) treatment. Hacısalihoglu *et al.* (2003) reported that under Zn deficiency stress, activity of Cu/Zn SOD decreases 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 enzyme. Similarly, Rahmati *et al.* (2004) found that the activity of SOD, CAT and APX (ascorbate peroxidase) in excess Mn-treated cells increased compared with control treatment. In addition, results of experiments indicated that micronutrient application reduces 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 in the field tended to decrease the effects of drought stress.

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INFLUENCIA DE MICRONUTRIENTES EN METABOLISMO DE ENZIMAS ANTIOXIDANTES EN GIRASOL (*Helianthus annuus L.*) BAJO LA INFLUENCIA DEL ESTRÉS, CAUSADO POR SEQUÍA

RESUMEN

Superóxido dismutasa (SOD), catalasa (CAT) y glutación peroxidasa (GPX) son enzimas antioxidantes que tienen un papel importante en el metabolismo de ciertas especies vegetales (así llamadas "especies reactivas de oxígeno", ROS) y defensa de deterioros causados por el estrés oxidativo. La actividad de los enzimas antioxidantes se incrementa en la célula vegetal, como reacción a las condiciones estresantes del medio. El objetivo de esta investigación fue evaluar la influencia de aplicación de micronutriente en el metabolismo de los enzimas antioxidantes (SOD, CAT y GPX) en girasol que está bajo la influencia del estrés. El experimento fue llevado a cabo en la estación agrícola Golmakán (Irán) en el año 2005, utilizando el método de bloques separados al azar (split plot randomized complete block design) en cuatro repeticiones. La irrigación fue el factor principal, dividido en tres niveles (normal, estrés débil, estrés fuerte) y seis tratamientos con micronutrientes (controles, Fe, Fe+Zn, Fe+Zn+Cu, Fe+Zn+Cu+Mn, Fe+Zn+Cu+Mn+B) que eran subensayos dentro de la parcelita principal. Los fertilizantes fundamentales (N, P, K) y los micronutrientes, fueron aplicados sobre la base de investigación del terreno. Los resultados demostraron que la actividad de los enzimas investigados se diferenciaba significativamente ($\alpha=5\%$) entre el control y las variantes del estrés. La concentración de los enzimas antioxidantes incrementó por 11-31% en las condiciones del estrés fuerte. La diferencia significativa ($\alpha=5\%$) fue determinada también entre el control y el tratamiento con micronutrientes bajo diferentes concentraciones de enzimas. La concentración de los enzimas antioxidantes incrementó por 48-89% en el tratamiento con Fe+Zn+Cu+Mn. Los resultados indican que, en las condiciones del estrés causado por la sequía, la aplicación de micronutrientes aumentó la resistencia de girasol a sequía.

EFFET DES MICRONUTRIMENTS SUR LES ENZYMES ANTIOXYDANTS DANS LE TOURNESOL (*Helianthus annuus L.*) SOUS LE STRESS DE LA SÉCHERESSE

RÉSUMÉ

Le superoxyde dismutase (SOD), la catalase (CAT) et la glutathione peroxidase (GPX) sont des enzymes antioxydants qui ont un rôle important dans le métabolisme de certaines espèces végétales (appelés "espèces réactives de l'oxygène" (ERO) et dans la défense contre les dommages causés par le stress oxydatif. L'activité des enzymes antioxydants augmente dans les cellules des plantes en tant que réponse aux stress environnementaux. Le but de cette étude était d'évaluer les effets de l'application de micronutriments sur le métabolisme des enzymes antioxydants (SOD, CAT et GPX) dans le tournesol sous le stress de la sécheresse. L'expérience a été faite à la station agricole de Golmakan (Iran) en 2005, elle utilisait la méthode de blocs séparés (conception de bloc complète randomisée) en quatre répétitions. Le facteur principal était l'irrigation, partagée en trois niveaux (normal, stress faible, stress important) et six traitements avec des micronutriments (contrôles, Fe, Fe+Zn,

Fe+Zn+Cu, Fe+Zn+Cu+Mn, Fe+Zn+Cu+Mn+B) qui étaient des sous-blocs à l'intérieur des blocs principaux. Les fertilisants de base (N, P, K) et les traitements aux micronutriments ont été appliqués selon les résultats des examens du sol. Les résultats ont montré que l'activité des enzymes examinées était significativement différente ($\alpha=5\%$) entre les contrôles et les variantes de stress. Les concentrations d'enzymes antioxydantes étaient augmentées de 11-31% dans les conditions de stress important. Une différence importante ($\alpha=5\%$) a été établie entre le contrôle et les traitements aux micronutriments sous différentes concentrations d'enzymes. La concentration d'enzymes antioxydantes a augmenté de 48-49% dans le traitement avec Fe+Zn+Cu+Mn. Les résultats montrent que dans des conditions de stress provoqué par la sécheresse, l'application de micronutriments avait augmenté la résistance du tournesol à la sécheresse.

