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Change in activity of antioxidative enzymes in young leaves of sunflower (*Helianthus annuus* L.) by application of super absorbent synthetic polymers under drought stress condition

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Abstract

Drought stress causes molecular damage to plants, either directly or indirectly through formation of reactive oxygen species (ROS). Alteration of antioxidant enzymes activities is an element in the defense process. Changes in antioxidant enzymes activities and seed yield in sunflower (*Helianthus annuus* L. cv. master) were investigated under drought stress and super absorbent synthetic polymer application. The experiment was carried out in a split plot based on randomized complete block design with three replications. Three irrigation levels after 6 (I_1), 10 (I_2) and 14 (I_3) days and five amounts of super absorbent polymer (0, 75, 150, 225, 300 kg/ha) were set as main and sub factors, respectively. Drought stress was applied at fourteen-leave-stage of sunflower and polymer was added at the same stage to soil in depth of root development. Our results showed that by increasing irrigation intervals, seed yield was decreased significantly. On the other hand, polymer application reduced the harmful effects of drought stress whereas application of 300 kg/ha polymer in long irrigation intervals a reasonable seed yield obtained. According to the results, drought stress induced increases in catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) activities. The antioxidant enzymes activities in drought-stressed plants might be accompanied with the production of active oxygen species (AOS). Application of a lower dose of polymer (75 Kg/ha) moderated the negative effects of drought stress, while higher doses of the polymer (225 and 300 kg/ha) prohibited the bad effects of drought stress. In total, our results suggest that application of polymers could be advantageous against drought stress, and could protect sunflower plants in drought stress conditions.

Keywords: Catalase activity, guaiacol peroxidase activity, hydro gels, reactive oxygen species, seed yield, watering frequency, water stress conditions.
Abbreviations: CAT-catalase; APX-ascorbate peroxidase; GPX-guaiacol peroxidase; SAP-super absorbent polymer; ROS- reactive oxygen species.

Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops in the world. Because of its moderate cultivation requirements and high oil quality, its cultivation has increased in both developed and developing countries (Skoric, 1992). Although sunflower is known as a drought tolerant crop that can be grown under dry land conditions, substantial yield increase is usually achieved by irrigation and many researchers have reported that its performance decreases under water stressed conditions (Erdem et al., 2006; Nezami et al., 2008). Sunflower may experience water deficit cycles during its life cycle, which induces oxidative stress. Consequently, drought stress causes molecular damage to plants, either direct or indirectly through formation of reactive oxygen species (ROS). The production of ROS is linearly increased by increasing the severity of drought stress, which leads to enhanced peroxidation of membrane lipids and degradation of nucleic acids, and both structural and functional proteins. Various organelles including chloroplasts, mitochondria and peroxisomes are the seats and first targets of reactive oxygen species, produced under drought stress conditions (Farooq et al., 2009). In order to increase the crop production and prevention of harmful effects of drought stress on the crops in dryland environments, a greater percentage of the precipitation must be stored in the soil, and the stored water and growing-season precipitation must be used more efficiently. Super absorbent polymer (SAP) particles may be taken as miniature water reservoirs in soil. Water will be removed from these reservoirs upon the root demand through osmotic pressure difference. The SAP works by absorbing and storing water and nutrients in a gel form, hydrating and dehydrating as the demand for moisture fluctuates (Nazarli et al., 2010). The experience of different companies in using SAP has proved many advantages for its application in agriculture. The most important of which are lower costs of plant production, important improvement of water management, increase of water accessibility for plants (reduces watering frequency by up to 50%), increased amount of soil aggregates, better permeability and soil aeration, easier evacuation of carbon dioxide from the root zone and finally, increased osmotic moisture and possibility of nutrients absorption (Orzeszyna et al., 2006). SAP application plays an important role in increasing the absorption capacity and retention of water in soil, fighting against water shortage and decreasing harmful effects of drought stress (Chatzopoulos et al., 2000). Super absorbent polymers may have great potential in restoration and reclamation of soil and storing water available for plant growth and production. Incorporation of polymers into soil has increased both dry weight of wheat and the time from irrigation stop to wilting (Johnson and Leah, 1990). The SAP can hold 400-1500 g of water per dry gram of hydro gel (Boman and Evans, 1991). Allahdadi et al. (2005a) studied the impact of SAP (A200) on drought stress of soybean (*Glycine max* L.). In their field trial, the different rates of polymer: 0.75, 150 and 225 kg/ha along with irrigation intervals of 6, 8, and 10 days were tested on yield and yield components of soybean. The results showed that the highest yield and yield components could be obtained from 6 day irrigation interval and 225 kg/ha polymer. Moreover, the longest irrigation interval without polymer resulted in the lowest yield and yield components.

The aim of present study was to identify the effects of drought stress and super absorbent synthetic polymers (SAP) on the seed yield and antioxidant enzymes catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) activities in sunflower.

Results and discussion

Analysis of variance on seed yield data revealed significant effect of polymer and irrigation intervals on this trait (Table 1). Severe drought stress caused 77% reduction of seed yield compared to control (normal irrigation; I_1). The results showed that the highest level of seed yield was achieved by applying the highest amounts of polymer (300 kg/ha) under the condition of no drought stress, whereas the lowest yield was related to the treatment of severe drought stress without using polymer (Fig.1, D). Nezami et al. (2008) reported that the dry matter of sunflower was increased with the increase in water supply. The maximum dry matter was obtained in well watered condition. Our results are in agreement with those obtained by Allahdadi et al. (2005b). They studied the impact of SAP (A200) on *Zea mayes* yield and yield components and reported that there was no significant difference between polymer application of 200 kg/ha along with the irrigation intervals of 7 days and untreated control with the irrigation intervals of 3 days. Analysis of variance showed that there were significant differences (P<0.01) between activity levels of ascorbate peroxidase, catalase and guaiacol peroxidase enzymes under drought stress (Table 1) and their activities were increased under drought stress. Activities of APX, CAT, and GPX enzymes were also highly affected (P< 0.01) by application of super absorbent polymer. Interaction between the irrigation regimes and super absorbent polymer was only significant (P<0.01) on CAT, but no significant effect observed on APX, and GPX activities (Table 1), indicating that the expression pattern of genes coding for CAT varies from an irrigation condition to another one depending on applied amount of super absorbent polymer. The highest catalase activity (163.33 µmol min^{-1} g^{-1} FW) was related to irrigation after 14 days without polymer application, whereas the lowest one (37 µmol min^{-1} g^{-1} FW) related to 300 kg/ha polymer application and normal irrigation (Fig 1. A). APX and GPX activities increased in irrigation regimes I_2 and I_3 compared to control (I_1). The highest APX activity (44.33 µmol min^{-1} g^{-1} FW) was observed for irrigation after 14 days (I_3), whereas the lowest one (18.33 µmol min^{-1} g^{-1} FW) was related to the normal irrigation (Fig 1. B).The highest GPX activity (41 µmol min^{-1} g^{-1} FW) is related to irrigation after 14 days (I_3), whereas the lowest one (17.66 µmol min^{-1} g^{-1} FW) achieved by normal irrigation (Fig 1. C). According to the results mentioned above, drought stress induced an increase in catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) activities. Application of minimum 75 Kg/ha polymer in long irrigation intervals reduced activity level of the antioxidant enzymes and resulted in yield increase in the field conditions. An important finding of this work was the harmonic behavior of CAT, APX and GPX activities in leaves during water stress (Fig 1). The increase in CAT, APX and GPX activities in leaves is probably a response to the enhanced production of ROS, and particularly H_2O_2, under water stress (Smirnoff, 1993).

Under oxidative stress, plants produce active oxygen species, which are harmful to plant growth due to their detrimental effects on the subcelluar components and metabolism. In particular, superoxide (O_2^-) of the active oxygen species is generated if the dark reaction of photosynthesis is hindered by environmental stresses and the excessive light energy cannot be used for the reduction of $NADP^+$ in the ferredoxin of the chloroplast (Asada and Takahashi, 1987). It is suggested that the higher concentrations of catalase and ascorbate peroxidase might have removed the O_2 radicals and its products such as H_2O_2 induced by water stress conditions. The increase of this antioxidant may be triggered by excess production of reactive oxygen species in the photosynthetic apparatus under water stress conditions. In general, antioxidant enzyme content of leaves is increased with the decline in irrigation, suggesting that the production of antioxidant enzymes is probably a common response of plants to drought stress conditions. Activity of antioxidant enzymes is generally increased during abiotic stress that correlates with enhancing cellular protection. The increased activity of antioxidant enzymes including CAT, APX and GPX may be responsible for the delay of leaf senescence. Moreover, the active oxygen-induced damage to the cell may be minimized or prevented by increased antioxidant activities, but this protection is not enough, especially under severe drought stress. Scarcity of water is a severe environmental constraint to plant productivity. Drought-induced loss in crops yield probably exceeds losses from all other causes, since both the severity and duration of the stress are critical (Farooq et al., 2009). All types of hydrogels when used correctly and in ideal situation will have at least 95% of their stored water available for plant absorption (Johnson and Veltkamp, 1985). Therefore, application of super absorbent polymer can be considered as a useful strategy for steady-state irrigation of crops in drought stress condition. Reagrding the global scarcity of water resources and increased salinization of soil and water, abiotic stress is a limiting factor for plant growth, so super absorbent polymer may have great potential in restoration and reclamation of soil and storing water for plant growth and production. However, our results need to be validated by conducting some more experiments in several different environmental conditions. However, Darvishzadeh et al. (2011) evaluated twenty-one genotypes of sunflower (*Helianthus annuus* L.) derived from a half diallel cross between six inbred lines in stress and non-stress environments at Urmia University. In their experiment significant difference was observed among genotypes for grain yield. In combined analysis of variance, a significant environment and genotype effect was observed for grain yield while no significant genotype × environment interaction effect detected, suggesting that response to environment by a given genotype, in relation to other genotypes, does not vary among different environments.

Table1. Analysis of variance for effects of drought stress and polymer on catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) activities and seed yield in sunflower.

Source of variation	df	MS			
		CAT	GPX	APX	Yield
Replication	2	20.24^{**}	0.17^{**}	53.21^{**}	4093150.5^{**}
Irrigation	2	81885.26^{**}	1516^{**}	2365.75^{**}	20901995.3^{**}
Linear (L)	1	163098.13^{**}	3010^{**}	4720.05^{**}	41757841.2^{**}
Quadratic (Q)	1	672.40^{ns}	22^{ns}	11.44^{ns}	46149.4^{ns}
Polymer	4	9139.88^{**}	191^{**}	182.11^{**}	31417578.8^{**}
Linear (L)	1	33369.87^{**}	751.11^{**}	723.63^{ns}	121977890.8^{**}
Quadratic (Q)	1	748^{ns}	1.14^{ns}	0.02^{ns}	59758.2^{ns}
Cubic (C)	1	1831.51^{**}	11.73^{**}	4.62^{ns}	3211866.7^{**}
Quadratic (Qt)	1	610.15^{ns}	0.03^{ns}	0.19^{ns}	420799.2^{ns}
Irrigation × Polymer	8	1375.32^{**}	5.71^{ns}	5.23^{ns}	420400^{ns}
LI×LP	1	5900.41^{**}	26.66^{ns}	3.26^{ns}	483842.4^{ns}
LI×QP	1	953.44^{ns}	0.42^{*}	2.82^{ns}	1336609.7^{ns}
LI×CP	1	881.66^{ns}	2.60^{ns}	5.40^{ns}	356664.6^{ns}
LI×QtP	1	715^{ns}	0.5^{ns}	2.46^{ns}	64728.1^{ns}
QI×LP	1	2156.17^{ns}	0.55^{*}	16.44^{ns}	709388.9^{ns}
QI×QP	1	32.86^{ns}	0.57^{ns}	0.24^{ns}	2365^{ns}
QI×CP	1	213.42^{ns}	0.86^{ns}	4.60^{ns}	147347.2^{ns}
QI×QtP	1	149.48^{ns}	1.51^{ns}	6.63^{ns}	262253.7^{ns}

df: degree of freedom; MS: mean of square; *,** significant at 0.05 and 0.01 probability levels, respectively. ns; non significant.

In an experiment, the relative influence of water irrigation in improving productivity of some barley genotypes was investigated under low rainfall condition of Saudi Arabia (Refay, 2010). The experiments included the combination of four barely genotypes and five water irrigation regimes. Results indicated that drought stress condition at a high level has significant effects on all of the studied characters. However, interaction effects for most of the parameters remained non-significant.

Materials and methods

The experiment was carried out at Urmia University in 2009 under filed conditions. The latitude and longitude of region is 37° and 32' north and 45° and 5' east and its altitude is 1313 m above the sea level. Climate of the region is cold and semidry and the average rainfall and the area temperature are 184 mm and 12 °C, respectively, according to 16 years statistics. The experiment was carried out as a split plot based on randomized complete block design with three replications. Three irrigation intervals (after 6 (I_1), 10 (I_2) and 14 (I_3) days) and five levels of super absorbent polymer (0, 75, 150, 225, 300 kg/ha) were designated as the main and sub plots, respectively. Experimental units in each block were composed of five lines of 5 m length. Row to row and plant to plant spacing was kept 0.60 and 0.25 m, respectively. Cultural practices and control of insects and weeds were made as needed during the growth season according to the local recommendations. Drought stress was applied at fourteen-leave-stage of sunflower and polymer was added at the same stage to soil in depth of root development by making ten centimeter holes in soil. The holes were filled with mixture of soil and polymer. Soil compaction around the roots was done manually. The super absorbent polymer A200 was provided from Rahab Rezin Company, Institute of Polymer Research, Karaj, Iran. According to the manufacturer's protocol, this substance had seven years durability in soil and its practical capacity of water absorption was equal to 220 g. Some properties of super absorbent polymer A200 are: water content (%) = 5-7; Grain size (µm) = 50-150; Density (g/cm^3) = 1.4 - 1.5 and pH = 6-7. In this study 5 bushes were randomly selected from the middle part of each plot and seed yield was recorded.

Enzyme assay

One-tenth g of fresh foliar tissue (uppermost leaves taken at the end of two growth stages) was analyzed for enzymatic assays. Catalase activity (µmol H_2O_2 min^{-1} g^{-1} FW) was assayed by measuring the initial rate of hydrogen peroxide disappearance (Chance and Maehly, 1959). The reaction mixture contained 2.5 ml of 50 mM potassium phosphate buffer (pH 7.4), 0.1 ml of 1% hydrogen peroxide and 50 µl of enzyme extract. The homogenate was centrifuged at 15000 g for 15 min at 4 ºC and the supernatant was immediately used for the enzyme assay. Decrease in hydrogen peroxide was measured as the decline in optical density at 240 nm and the activity was calculated using the extinction coefficient of 36 mM cm^{-1} for hydrogen peroxide. For the measurement of guaiacol-dependent peroxidase (EC 1.11.1.7) activity, the reaction mixture contained 25 mmol L^{-1} phosphate buffer (pH 7.0), 0.05 % guaiacol, 10 mmol/L H_2O_2 and enzyme. Activity was determined by the increase of absorbance at 470 nm due to guaiacol oxidation (E = 26.6 mM^{-1} cm^{-1}) (Hemeda and Klein, 1990). Ascorbate peroxidase (µmol min^{-1} g^{-1} FW) activity was determined as described by Asada (2001). The reaction mixture contained 2.5 ml of 50 mM potassium

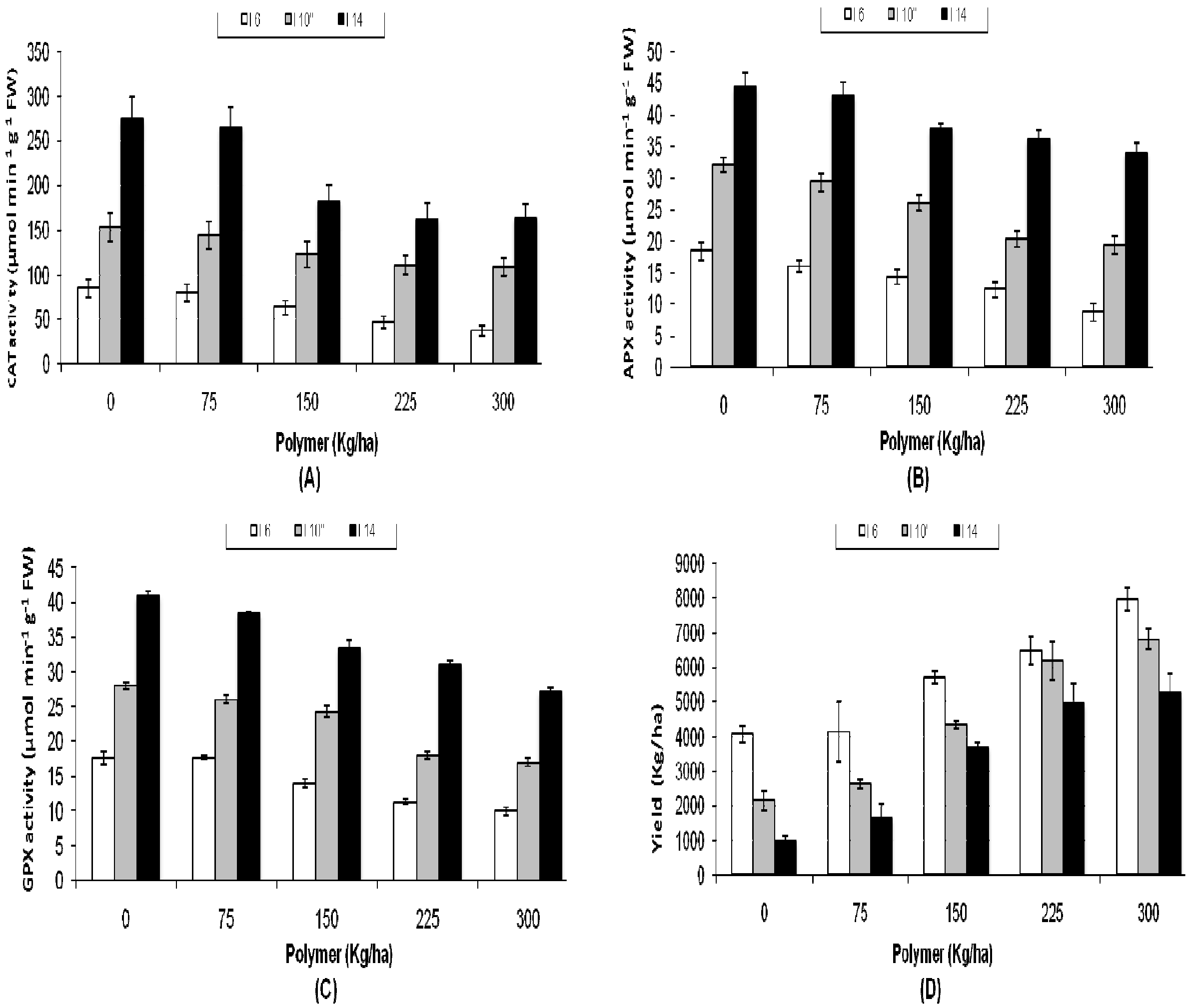

Fig 1. Effect of polymer and drought stress [irrigation after 6 (I_1), 10 (I_2) and 14 (I_3) days] on catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) activity as well as on seed yield in sunflower. Values are the means ± SE of three replicates. Means with different letters are significantly different (P<0.05).

phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5mM ascorbate, 0.2 ml of 1% hydrogen peroxide and 0.1 ml enzyme extract. The homogenate was centrifuged at 15000 g for 15 min at 4 ºC and the supernatant was used to measure enzyme activity. The hydrogen peroxide-dependent oxidation of ascorbate was followed by monitoring the decrease in absorbance at 290 nm, using the extinction coefficient of 2.8 mM cm^{-1}.

Statistical analysis

Statistical analysis was carried out using SAS software version 9.0 (SAS Institute Inc., Cary, NC, USA) and the graphs were drawn using Microsoft Office Excel 2007 software.

Conclusion

Our findings showed that by increase in irrigation intervals, the activity of APX, CAT, GPX enzymes is significantly enhanced, whereas super absorbent polymer can increase osmotic moisture and decrease the harmful effects of drought stress by absorbing and storing water. Therefore, application of polymer can be an important tool in sunflower cultivation to overcome drought stress conditions. Considering the irrigation period, one can select an appropriate amount of super absorbent polymer in sunflower cultivation. Based on the results we found that alleviation of oxidative damage by the use of polymer can protect sunflower plants against drought stress conditions.

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