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European Journal of Experimental Biology, 2014, 4(4):61-66

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Studying the effect of Fe-foliar and its effect on the biochemical characteristics of sunflower under low irrigation

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ABSTRACT

To study the effect of different concentrations of Fe-foliar on the biochemical characteristics of sunflower under water stress, an experiment was conducted in Varamin in the agricultural year of 2013. The experiment was conducted as split plot in completely randomized block design with three replications. Main plot included three irrigation levels (normal irrigation, irrigation stop at flowering stage (8 leaves) and irrigation stop at grain filling stage (8 leaves and 16 leaves)) and sub-plot included three levels of Fe-foliar (pure water foliar, Fe-foliar once, Fe-foliar twice). stopping irrigation at different growth stages increased the amount of antioxidant enzymes. Also, Fe-foliar increased the antioxidant enzymes.

Key words: sunflower, Fe-foliar, irrigation stop, antioxidant enzymes.

INTRODUCTION

Sunflower *(Helianthus annuns L.)* is one of the most important crops in Iran and in the world so that it is considered as one of four major oilseed crops [1]. The sunflower seed is consumed for extracting oils, nut consumption, and feeding the poultry based on oil level and seed size [2]. During growth and development the plants encounter a variety of environmental stresses that depending on the sensitivity and growth stage of plant species each of these stresses can have different effects on their growth, metabolism and yield. The environmental stresses such as drought can have a bad effect on the percentage and quality of sunflower oil [3]. Any action that can reduce the bad effects of stress can increase the percentage and quality of the oil. The results of recent studies also indicate the role of Fe-foliar in reducing oxidative stress caused by biological and abiotic stresses, especially drought and salinity. Under salinity and drought stresses, the concentration of harmful free radicals and active oxygen species in plant is increased and causes the oxidation of biological membranes. In contrast, some elements such as iron cause the deactivation of radicals and the reduction of oxidative stress in the plants by increasing the activity of antioxidative enzymes such as superoxide dismutase. For this reason, Fe-foliar increases the plant tolerance to the drought and salinity stresses [3].

The role of iron in nitrogen fixation and the activity of some enzymes such as catalase, peroxidase and cytochrome oxidase has well studied [4].

In studying the way of anti-oxidation enzyme activity in several barley cultivars it has been shown that SOD activity increases due to the effect of drought stress [5].

The major cleansing mechanisms in plants include superoxide dismutase, ascorbate peroxidase, peroxidase and catalase. The balance between the activities of superoxide dismutase and ascorbate peroxidase or catalase in the cells is very important to determine the steady-state levels of superoxide radicals and hydrogen peroxide [6].

An increase in SOD activity could be due to the increase of superoxide radical and the defense mechanism against oxidant stress in the plants [7].

[8]reported that the activity of the enzymes of ascorbate peroxidase, peroxidase and catalase is reduced under the condition of iron deficiency.

The spatial distribution of enzymes such as superoxide dismutase in membranes could partly be important in establishing resistance to drought [9].

[10] To study the effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in sesame cultivars of Darab 14 and Yekta, observed that the severe stress increased the activity of dismutase and superoxide, catalase and polyphenol oxidase in leaves and roots, particularly in Yekta cultivar.

MATERIALS AND METHODS

The superoxide dismutase enzyme (SOD): To calculate this factor, three leaves were removed from each sub-leaf from the field in the morning before warming weather. The leaves should be are quite young and broad. We put the leaves into a labeled nylon and put it into the icebox with the bottom covered by ice and transport it to the laboratory. The amount of changes in this enzyme is determined by the method of Misra. First, the tris buffer solution (containing phosphate, D-sodic, pH= 7.2) with 1.3 mM EDTA, and 0.1 mmol monosodic carbonate are prepared, and then the epinephrine with the concentration of 0.25 mM is used as substrate and then we add the prepared solution to it, the changes in the light absorbance resulting from the oxidation of epinephrine is evaluated as the enzyme activity and the standard and purified enzyme is used for standardization of results that its unit can oxidize 0.5 mM epinephrine in one minute.

Catalase enzyme (CAT): to measure the catalase enzyme in leaves, the first and fifth lines of each plot will be considered as margin and the first and last half meter of each line in each plot will also be considered as margin. Then sampling is conducted randomly from three midlines and also from upper part of the plant with removing three leaves from three lines of each plot. The leaves are placed into nylon bags and the ice and immediately are moved to the laboratory. [11] ,measuring CAT activity was conducted by reducing the light absorbance of H_2O_2 at 240 nm and by using a standard curve. The test solution included the potassium phosphate buffer (25 mM, pH= 6.8) and hydrogen peroxide with the concentration of 20 mM (H_2O_2) [12]. By adding 50 micro liter of enzyme extract to a final volume of 1 ml of the mixture, the reaction is started, the change in absorbance at 240 nm after one minute is calculated and evaluated by the standard curve of enzyme activity and is expressed in terms of mg protein.

Glutathione peroxidase enzyme (GPX): at first the leaves moved to laboratory will be washed with distilled water, immediately entered the tris-phosphate buffer of 0.16 M with pH= 7.5 and then crushed and homogenized. Then it will be allowed that the cell wall and membrane digestion process is carried out in presence of the same volume of the same buffer containing digitonin and digestive enzyme. At the end, 0.5 ml of homogenized solution will be removed to measure the amount of protein and the amount of its protein was determined based on mg per ml. Then according to the method of [12] ,the amount of glutathione enzyme will be measured in the remaining extracted solution. The extract enter a buffer solution containing monopotassic phosphate of 0.56 M (pH=7.5) with 1.2 mM of EDTA and 1 mM of $NaNO_3$ and 0.2 mM of NADPH. Then 0.2 ml of restoration glutathione with 0.1 mM of hydrogen peroxide will be added to it. Immediately the amount of NADPH oxidation will be determined by the amount of absorbance change at 340 nm at 30 °C by Spectrophotometer (model u- shimadzu- u- z100) and simultaneously a blank solution containing all above materials without the extract will be used to correct and eliminate the possible errors. One unit of glutathione peroxidase activity, equivalent to an amount of enzyme that can catalyze to 1 micromole of substrate NADPH within one minute, was considered. For standardization the standard sample of glutathione peroxidase enzyme will be used.

Experimental design

This experiment was studied as split plot (once split plots) in a completely randomized block design on sunflower, Azargol cultivar, with three replications.

The main plot includes three levels of irrigation:
I1: Lack of drought stress

I2: stopping irrigation at flowering stage
I3: stopping irrigation at grain filling stage
The sub-plot includes three levels of Fe-foliar:
F1: purified water-foliar application
F2: foliar application once (8 leaves)
F3: foliar application twice (8 leaves and 16 leaves)

RESULTS AND DISCUSSION

As identified from Table 1 of variance analysis, the simple effect of irrigation at the level of 0.01 and the simple effect of foliar application at the level of 0.01 on the superoxide dismutase activity were significant. But the interaction of irrigation on Fe-foliar was not significant. The simple effect of irrigation at the level of 0.01 and the simple effect of foliar application at the level of 0.01 on the catalase activity were significant. But the interaction of irrigation on Fe-foliar application was not significant. Also, the simple effect of irrigation at the level of 0.01 on the activity of glutathione peroxidase was significant but the simple effect of foliar application and the interaction of irrigation on Fe-foliar application were not significant.

Table 1 - variance analysis of the effect of Fe-foliar application on morphological characteristics

Change source	Degree of freedom	superoxide dismutase enzyme (SOD)	catalase	glutathione peroxidase
Replication	2	5.31^{ns}	5.56^{ns}	1.81^{ns}
Irrigation	2	326676.92**	34205.87**	19508.1**
A error	4	8.27	1.08	4.54
Foliar application	2	15514.11**	4348.38**	1334.96^{ns}
Irrigation * foliar application	4	957.12^{ns}	193.75^{ns}	2.18^{ns}
B error	12	1254.58	502.83	21.438
Change coefficient		7.74	14.85	15.42

NS,, ** insignificant, significant at the levels of 1% and 5%, respectively*

Superoxide dismutase enzyme

In the mean comparison of simple effect of irrigation on the activity of superoxide dismutase enzyme (SOD) it is clear that the highest amount of SOD enzyme activity is related to stopping irrigation at the beginning of grain filling stage and with the amount of 922.78 units of international activity per mg protein and its difference with other levels of irrigation was statistically significant and the lowest amount of SOD enzyme activity is related to the lack of drought stress and with the amount of 544.33 units of international activity per mg protein.

In the means of the simple effect of foliar application it was determined that the highest amount of SOD enzyme activity is related to the level of Fe-foliar application twice (8 leaves and 16 leaves) and with the amount of 783.64 units of international activity per mg protein while its differences with the level of foliar application once is not statistically significant, but its differences with the level of without foliar application is statistically significant and the lowest amount of SOD enzyme activity is related to irrigation stage in accordance with the region convention and with the amount of 701.61 units of international activity per mg protein.

Also, as identified from Figure 1 of mean comparisons of interaction on superoxide dismutase enzyme (SOD), the highest amount of SOD is related to the stage of stopping irrigation at the beginning of grain filling stage and when Fe-foliar is conducted twice with the amount of 961.65 units of international activity per mg protein and the lowest amount of SOD enzyme is related to the normal irrigation stage when Fe-foliar was not conducted and with the amount of 521.65 units of international activity per mg protein.

Also, considering Figure 1 of mean comparisons of interaction under normal irrigation, the highest amount of SOD enzyme activity is at the level of Fe-foliar twice (8 leaves and 16 leaves) and with the amount of 566.6 units of international activity per mg protein that there is no difference between the numbers of Fe-foliar applications and all are at one level. Under stopping irrigation at the beginning of flowering stage the highest amount of SOD enzyme activity is related to the level of Fe-foliar twice in the vegetative and reproductive stages and with the amount of 822.66 units of international activity per mg protein; however, its differences with the level in which sunflower is at the beginning of flowering stage when foliar application is conducted once were not statistically significant but its differences with the level with no foliar application was statistically significant. Also, under stopping irrigation at the beginning of grain filling stage the highest amount of SOD enzyme activity is related to the level of Fe-foliar twice in the vegetative and reproductive stages and with the amount of 961.65 units of international activity per mg protein; however, its differences with the level in which sunflower is at the beginning of flowering stage when foliar application is conducted once were not statistically significant but its differences with the level with no foliar application was statistically significant. Also, under stopping irrigation at the beginning of grain filling stage the superoxide dismutase increased 26.58% compared with normal irrigation and also under Fe-foliar application twice

the superoxide dismutase increased 136.62% compared with the condition without foliar application under stopping irrigation at the beginning of flowering stage and also under Fe-foliar application twice the superoxide dismutase increased 9.26% compared with the condition without foliar application under stopping irrigation at the beginning of grain filling stage.

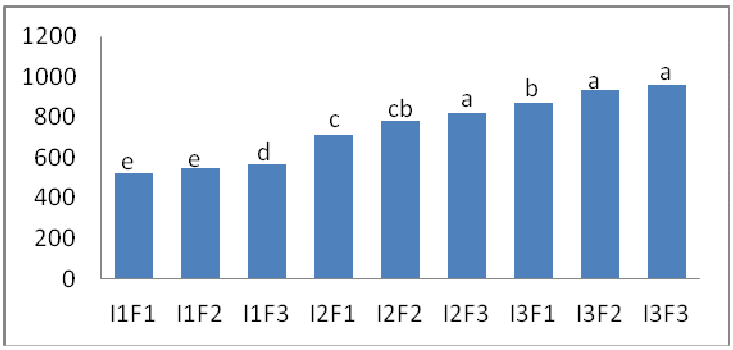

Figure 1: mean comparison of interaction of irrigation and Fe-foliar application on superoxide dismutase

Catalase

In the mean comparisons of simple effect of irrigation on the activity of catalase enzyme it is clear that the highest amount of catalase activity is related to stopping irrigation stage at the beginning of grain filling stage and with the amount of 213.37 units of international activity per mg protein and its difference with other levels of irrigation was statistically significant and the lowest amount of catalase enzyme is related to the lack of drought stress and with the amount of 90.1 units of international activity per mg protein.

In the mean comparisons of the simple effect of foliar application it was determined that the highest amount of catalase enzyme activity is related to the level of Fe-foliar application twice (8 leaves and 16 leaves) and with the amount of 172.13 units of international activity per mg protein while its differences with the level of foliar application once was not statistically significant, but its differences with the level of without foliar application was statistically significant and the lowest amount of catalase enzyme activity is related to irrigation stage in accordance with the region convention and with the amount of 128.25 units of international activity per mg protein.

Also, as identified in Figure 1 of mean comparisons of interaction on catalase enzyme, the highest amount of catalase is related to the stage of stopping irrigation at the beginning of grain filling stage and when Fe-foliar is conducted twice with the amount of 242.86 units of international activity per mg protein and the lowest amount of catalase enzyme was related to the normal irrigation stage when Fe-foliar was not conducted and with the amount of 74.36 units of international activity per mg protein.

Also, considering Figure 2 of mean comparisons of interaction under normal irrigation, the highest amount of catalase enzyme activity is the level of Fe-foliar twice (8 leaves and 16 leaves) and with the amount of 102.46 units of international activity per mg protein and its difference with other levels of irrigation was statistically significant. Under stopping irrigation at the beginning of flowering stage the highest amount of catalase enzyme activity is related to the level of Fe-foliar twice in the vegetative and reproductive stages and with the amount of 171.07 units of international activity per mg protein; however, its differences with the level in which sunflower is at the beginning of flowering stage when foliar application is conducted once were not statistically significant but its differences with the level with no foliar application was statistically significant. Also, under stopping irrigation at the beginning of grain filling stage the highest amount of catalase enzyme activity is related to the level of Fe-foliar twice in the vegetative and reproductive stages and with the amount of 242.86 units of international activity per mg protein; however, its differences with the level in which sunflower is at the beginning of flowering stage when foliar application is conducted once were not statistically significant but its differences with the level with no foliar application was statistically significant. Also, under stopping irrigation at the beginning of flowering stage the catalase increased 59.39% compared with normal irrigation. Under stopping irrigation at the beginning of grain filling stage the catalase increased 41.57% compared with normal irrigation. Under Fe-foliar application twice the catalase increased 25.59% compared with the condition without foliar application under stopping irrigation at the beginning of flowering stage and also under Fe-foliar application twice the catalase increased 24.60% compared with the condition without foliar application under stopping irrigation at the beginning of grain filling stage.

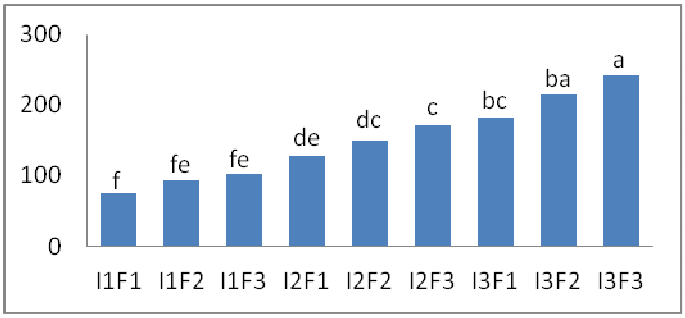

Figure 2: mean comparison of interaction of irrigation and Fe-foliar application on catalase

Glutathione peroxidase

In the mean comparisons of simple effect of irrigation on the activity of glutathione peroxidase enzyme it is clear that the highest amount of catalase activity is related to stopping irrigation stage at the beginning of grain filling stage and with the amount of 177.59 units of international activity per mg protein and its difference with other levels of irrigation was statistically significant and the lowest amount of glutathione peroxidase enzyme is related to the lack of drought stress and with the amount of 85.56 units of international activity per mg protein.

In the mean comparisons of the simple effect of foliar application it was determined that the highest amount of glutathione peroxidase enzyme activity is related to the level of Fe-foliar application twice (8 leaves and 16 leaves) and with the amount of 145.95 units of international activity per mg protein while its differences with the level of foliar application once was not statistically significant, but its differences with the level of without foliar application was statistically significant and the lowest amount of glutathione peroxidase enzyme activity is related to irrigation stage in accordance with the region convention and with the amount of 122.20 units of international activity per mg protein.

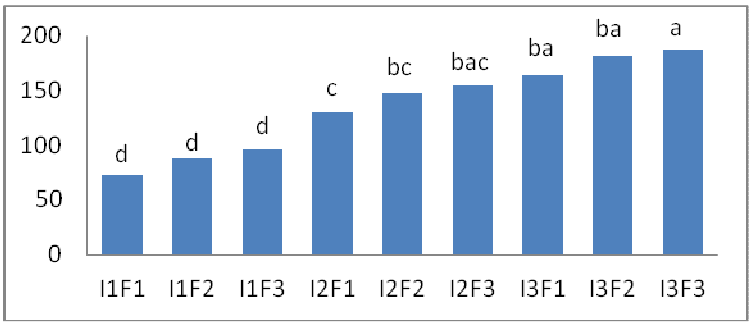

Figure 3: mean comparison of interaction of irrigation and Fe-foliar application on glutathione peroxidase

Also, as identified in Figure 3 of mean comparisons of interaction on glutathione peroxidase enzyme, the highest amount of glutathione peroxidase is related to the stage of stopping irrigation at the beginning of grain filling stage and when Fe-foliar is conducted twice (8 leaves and 16 leaves) with the amount of 187.52 units of international activity per mg protein and the lowest amount of glutathione peroxidase enzyme was related to the normal irrigation stage when Fe-foliar was not conducted and with the amount of 72.73 units of international activity per mg protein.
Also, considering Figure 3 of mean comparisons of interaction under normal irrigation, the highest amount of glutathione peroxidase enzyme activity is the level of Fe-foliar twice (8 leaves and 16 leaves) and with the amount of 96.21 units of international activity per mg protein but there was no significant difference between the numbers of Fe-foliar application and all are at the same level. Under stopping irrigation at the beginning of flowering stage the highest amount of glutathione peroxidase enzyme activity is related to the level of Fe-foliar twice in the vegetative and reproductive stages and with the amount of 154.01 units of international activity per mg protein but its differences with the other level was not statistically significant. Also, under stopping irrigation at the beginning of grain filling stage the highest amount of glutathione peroxidase enzyme activity is related to the level of Fe-foliar twice in the vegetative and reproductive stages and with the amount of 187.52 units of international activity per mg protein but its differences with the other level was not statistically significant. Also, under stopping irrigation at the beginning of flowering stage the glutathione peroxidase increased 55.70% compared with normal irrigation. Under stopping irrigation at the beginning of grain filling stage the glutathione peroxidase increased 43.92% compared with normal irrigation. Under Fe-foliar application twice the glutathione peroxidase increased 15.79% compared with the condition without foliar application under stopping irrigation at the beginning of flowering stage and also

under Fe-foliar application twice the glutathione peroxidase increased 12.43% compared with the condition without foliar application under stopping irrigation at the beginning of grain filling stage.

Iron is an essential element for the production of superoxide dismutase and in a condition that we have an irrigation stop in reproductive stage we have the highest amount of producing oxygen free radicals and plant use its antioxidant system to deal with them and we see an increase in the activity of this enzyme and when we have Fe-foliar application twice, the amount of superoxide dismutase enzyme increases because Fe is a necessary element and increases the activity of superoxide dismutase and superoxide is considered as a defense mechanism of the plant. The catalase in chloroplasts in cooperation with superoxide dismutase plays a key role; iron is an essential element for making catalase and in a condition that we have an irrigation stop at reproductive stage we have the highest amount of producing oxygen free radical and the plant use its antioxidant system to deal with them and we see an increase in the activity of this enzyme and when we have Fe-foliar application twice, the amount of catalase enzyme increases because Fe is a necessary element and increases the activity of catalase and catalase is considered as a defense mechanism of the plant. Also, with Fe-foliar application the amount of glutathione activity is increased that is consistent with the results of (Balakrishnan *et al*., 2000) and (San *et al.*, 2006). Superoxide dismutase (SOD) is considered as a vital factor for being alive and activity of living organisms. The metalloenzyme collects the toxic radicals which are constantly formed as aerobic products. Under drought stress the superoxide concentration increased compared with the normal irrigation because in the drought condition the plant increases the amount of superoxide enzyme in their cells to cope with the damage biomarkers that the superoxide enzyme fights the free radicals. Under drought stress by increasing oxygen free radicals on glutathione peroxidase as an antioxidant enzyme compared with irrigation, the level of its activity increased and increases in the plant to fight free radicals. When oxygen free radicals increase, a chain of reactions begins. In this regard, superoxide dismutase convert O_2^- to H_2O_2 and O_2 and glutathione peroxidase (GPX) catalases the reduction of hydrogen peroxide by using restored glutathione and therefore protects the cells against damage caused by oxidation. In fact, the glutathione metabolism is one of the necessary and oxidative defense mechanisms against. The results of this experiment are consistent with the results of experiments conducted by (Johnson *et al*,. 2003), (Acar *et al*., 2001), (Sharma and Dubey, 2005), (Thipyapong, 2007) and (Fazeli *et al.*, 2007).

CONCLUSION

The stopping irrigation at different growth stages increased the amount of antioxidant enzymes. Also, Fe-foliar increased the antioxidant enzymes.

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