Influence of Drought Stress, Vermicompost and N Fertilizer on Safflower Leaves Antioxidant Enzymes Activity

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Abstract: *Superoxide dismutase (SOD), Peroxidase activity (POD), catalase (CAT) and glutathione peroxidase (GPX) are antioxidant enzymes which have important role in the metabolic reactive oxygen species (ROS) and defense 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 vermicompost and N fertilizer application on the antioxidant enzyme metabolism (CAT and POD) in sunflower under drought stress. To investigate the effect of vermicompost and N fertilizer application on antioxidant enzymes activity under water deficit stress of Safflower in 2012 at Khorramabad- Lorestan , Iran. The experiment was laid out Split plot-factorial in Randomized Complete Block Design with 3 replications. Treatments were, Vermicompost rate in 4 levels (V1=0 (control), V2=2, V3=4 and V4=6 ton Vermicompost ha $^{-1}$) and Nitrogen rate, (N1= 0 (control), N2= 84 (30% less from N3), N3= 120 (from the soil analyze lab), N4= 154 (30% more than N3) kg N ha $^{-1}$), and Irrigation as the main factor in two levels, S1= Control and S2=Water Stress (stress from bloom growth stages). And N fertilizer was in the the form of Urea. Results showed that the activity of these enzymes was significantly different (a= 1%) between control and water stress treatments. The water stress increased antioxidant enzymes activities and whit application Vermicompost and Nitrogen the antioxidant enzymes activity decreased then the control treatments.*

Keywords: *Drought Stress, vermicompost, N fertilizer, safflower, antioxidant, enzymes*

1. Introduction

Adequate water and nutrient supply are important factors affecting optimal plant growth and successful crop production. Water stress is one of the severe limitations of crop growth especially in arid and semiarid regions of the world as it has a vital role in plant growth and development at all growth stages (Shamim *et al*., 2009). Nitrogen, phosphorous and potassium are major elements essential for plant growth and development. To date use of chemical fertilizers has been confined mainly to the application of nitrogen and phosphorous and due attention has not been paid to the potassium. Its role is well documented in photosynthesis, increasing enzyme activity, improving synthesis of protein, carbohydrates and fats, translocation of photosynthetic, enabling their ability to resist pests and diseases. Potassium also plays key role in increasing crop yield and improving the quality of produce (Tisdale *et al*., 1985). 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). 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). ROS play an important role in endonuclease activation and consequent DNA damage (Hagar *et al*., 1996). 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 (Blokhina et al., 2003; Habibi et al., 2004). Halliwel and Cutteridge (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), glutatione reductase (GR) and malondialdehyde (MDA) in seeds will increase under drought stress condition.

Safflower (*Carthamus tinctrius* L), Compositae family, is mainly used as oil gains in Iran. Deep roots, Waxy leaves, grains with thick crust make the safflower an ideal option for arid regions. (Carvalho et al; 2006) Iran supplies major portion of its needs for edible oil from other countries.

Fertilizer application represents an important measure to correct nutrient deficiencies and to replace elements removed in the products harvested, and N fertilisation has been shown to be particularly effective with respect to yield formation (Connar, 1992). The results of different studies represent the importance of chemical fertilizer's consumption in the safflower. Hence, it is very important to use the accurate amount of fertilizers to compensate the deficiency of nutrients removed by the previous products in order to prepare sufficient and necessary nutrients demand of new plants to meet acceptable harvest.

2. Materials and methods

Current study was carried out in the Lorestan- Khoram-abad, Iran in 2012 growing season (Longitude=47˚ 40' Latitude=33˚ 36'). Khoram-abad is a moderate climate region and receives average annual rainfall of 530^{mm}. The experimental field was silty clay loam textured soil having a PH value of 7.5 and 0.8% organic carbon. The experiment was laid out Split plot- factorial in Randomized Complete Block Design with 3 replications. Treatments were, Vermicompost rate in 4 levels (V1=0 (control), V2=2, V3=4 and V4=6 ton Vermicompost ha $^{-1}$) and Nitrogen rate, (N1= 0 (control), N2= 84 (30% less from N3), N3= 120 (from the soil analyze lab), N4= 154 (30% more than N3) kg N ha $^{-1}$), and Irrigation as the main factor in two levels, S1= Control and S2=Water Stress (stress from bloom growth stages). And N fertilizer was in the the form of Urea.

2-1: Measurement of Catalase and peroxidase enzymes activity:

Catalase and peroxidase activities were determined from the leaves according to the methods of (Nakano and Asada, 1987) with some modifications. All steps of the extraction were carried out at 4 ℃. Leaf samples (0.1 g fr wt) were homogenized in a cold mortar in 1 ml of 50 mM Na-phosphate buffer (pH 7) containing 2 mM α-dithiothreitol, 2 mM EDTA, 0.2% triton x-100, 50 mM Tris-HCl and 2% polyvinylpyrroli done and mixed for 15 min. The obtained extracts were immediately used to assay enzyme activities. The statically analysis was conducted using MSTAT-c software. Mean comparison was also conducted with Duncan's Multiple Rang Test (DMRT). And for charts was drawn with Excel software.

3. Result

Thy leaves antioxidant enzymes activates increased by effects of water deficit stress and decreased 0f application Vermicompost and chemical fertilizer. Analysis of variance (ANOVA) revealed that the effect of variance components (V, N, S) were significant at ($P<0.01$) and their interactions didn't have significant effect on leaves Antioxidant enzymes activity (Table: 1). The water deficit stress imposed in this experiment induced a significant increase leaves Antioxidant enzymes activity.

Duncan's Multiple Range test showed significant differences between normal irrigation and draught stress treatments. Based on Figure 2, maximum leaves peroxidase and catalase activity were obtained from water stress with 0.68 and 0.58 (OD min $^{-1}$. g $^{-1}$. Fw) respectively and minimum leaves peroxidase and catalase activity were obtained from irrigation treatments. Whit application Vermicompost and N fertilizer the leaves antioxidant activity were decreased. As the highest and lowest leaves peroxidase activity were obtained from V1 and V4 whit 0.64 and 0.35 (OD min $^{-1}$. g $^{-1}$. Fw) respectively and the highest and lowest leaves catalase activity were obtained from V1 and V4 whit 0.54 and 0.28 (OD min $^{-1}$. g $^{-1}$. Fw) (Fig. 2). Moreover, the rate of leaves peroxidase and catalase activity was decreased whit application of N fertilizer, as the highest and lowest leaves peroxidase activity were obtained from N1 and N4 whit 0.61 and 0.35 (OD min $^{-1}$. g $^{-1}$. Fw) respectively and catalase activity was obtained from N1 and N4 with 0.54 and 0.27 (OD min $^{-1}$. g $^{-1}$. Fw) respectively (Fig.3).

4. Discussion

When plants are subjected to different stresses, antioxidant activity increases. To alleviate stress, by degrading the byproducts of that stress, plants produce free radicals such as superoxide and peroxide, which are not favorable to plant growth (Rahimizadeh et al. 2007). The enzymatic antioxidant system is one of these protective mechanisms that includes activity of superoxide dismutase, which can be found in various cell compartments, it catalyses the isproportion of the O_2 radicals H_2O_2 and O_2 (Hegedus et al. 2001).The development of more drought-resistant crops is necessary to alleviate future threats to food availability in the world (Plucknett et al. 1987). However, this requires comprehensive studies of the many potential genetic resources and understanding of the adaptive mechanisms and responses to water deficit stress that allows survival in arid and semiarid environments (Rampino et al. 2006). The present study was done for the investigation of antioxidant effect of levees of safflower. And the research showed that the water deficit stress caused a reduction in antioxidant enzyme activity. It was proved that the drought stress increases the production of reactive oxygen species (ROS) (Mittler 2002). To scavenge these ROS, plants either synthesize different antioxidant compounds or activate antioxidant enzymes. Among them, non-enzymatic antioxidants such as AA, a-Toc and GSH play an important role to scavenge these radicals (Noctor and Foyer, 1998). Plants can detoxify ROS by up-regulating antioxidant enzymes, such as Catalase and peroxidase as well as some non-enzymatic antioxidant compounds. It is evident that high levels of antioxidants are related to plant water deficit tolerance (Tahi et al. 2008). Antioxidant enzymes activity increases in plant cells as a response to environmental stresses. Environmental stresses can result in the production of Reactive Oxygen Species (ROS), including O , H O and OH; these ROS adversely affect crops yield and quality (Baby, and Jini, 2011). Hojati et al., (2010) reported that Water deficit treatments increased antioxidant compounds such as ascorbic acid (AA), superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and peroxidase (POX, EC 1.11.1.7) activities. Water deficit stressed plants maintained higher levels of compounds and scavenging enzymes. Mohammadi et al, (2011) indicated that Drought stress treatment (103.84 µ/g protein) and normal irrigation treatment (59.95 u/g protein) had the highest and the lowest CAT enzyme activity, respectively in Some Chickpea Cultivars.

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TABLE I: Analysis of variance components Water stress (S) Vermicompost (V), Nitrogen (N), and their interaction for assessed traits.

S.O.V	df	Mean square	
		leaves peroxidase activity	Catalase activity
Replication	2	0.011	0.050
S	1	0.455^{**}	0.316^{**}
Error S	2	0.032	0.012
V	3	0.087^{*}	0.045^{**}
SV	3	0.013^{ns}	0.001^{ns}
N	3	0.060^{**}	0.055^{**}
SN	3	0.006^{ns}	0.008^{ns}
VN	9	0.007^{ns}	0.008^{ns}
SVN	9	0.005^{ns}	0.003^{ns}
Total Error	60	0.005	0.004
CV(%)		13.5	11.5

*, **: Significantly different at 5 and 1% levels of probability, respectively; ns: non-significant.

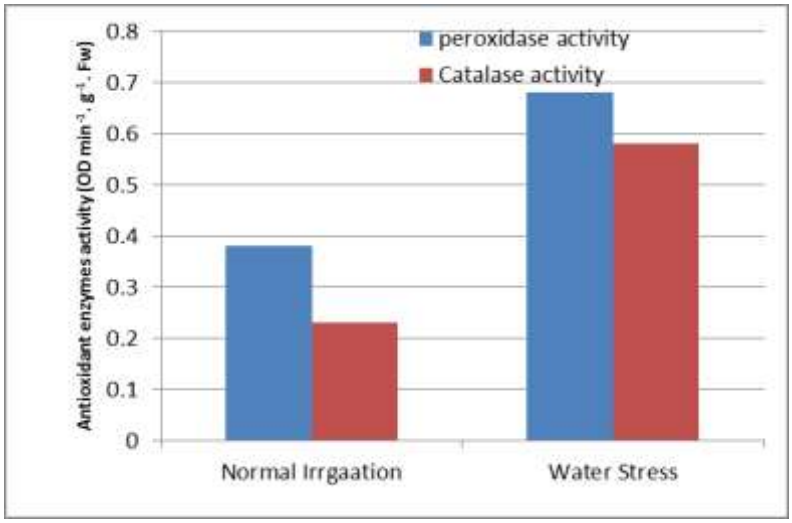

Fig. 1: Effect of different irrigation on leaves Antioxidant enzymes activity

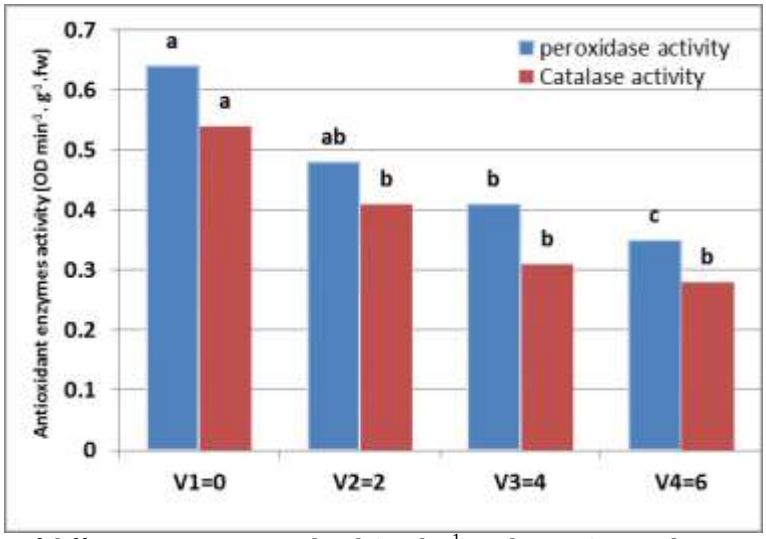

Fig. 2: Effect of different Vermicompost level (ton/ha^{-1}) on leaves Antioxidant enzymes activity

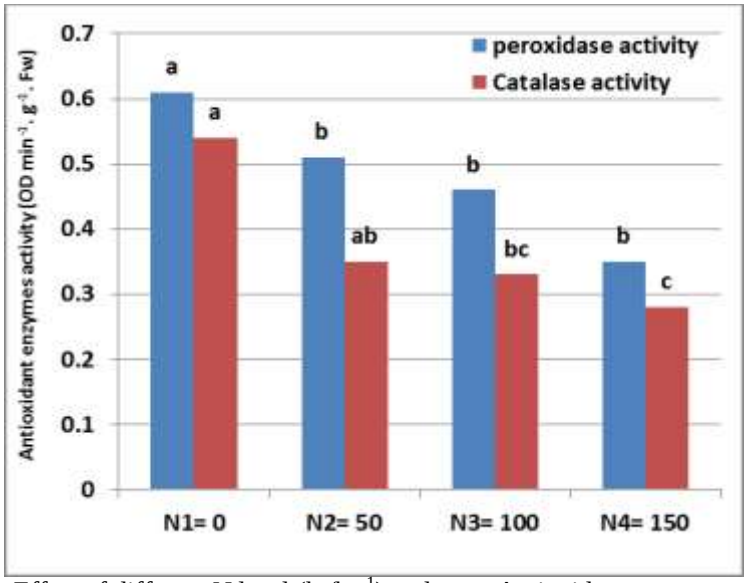

Fig. 3: Effect of different N level (kr/ha^{-1}) on leaves Antioxidant enzymes activity

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