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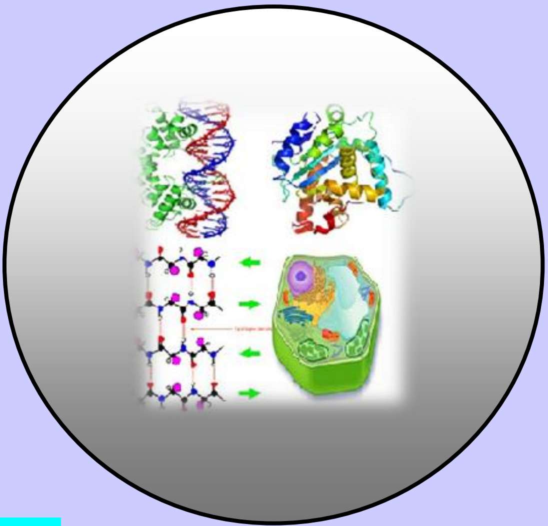

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RESEARCH PAPER

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Effect of Zn on Antioxidant Enzyme Activities of Common Bean under Drought Stress

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ABSTRACT

Superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), polyphenoloxidase (PPO) and Ascorbate peroxidase (APX) 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 Zn application on the antioxidant enzyme metabolism (SOD, CAT, Pox, PPO and APX) in common bean under drought stress. This experiment was carried out at Alazhar garden; Egypt during 2012 and 2013 growth season, using a split plot randomized complete block design with three replications. Irrigation as a main factor at three levels (normal, low stress and high stress) and Zn 50 ppm treatment. Results showed that the time of foliar application on the antioxidant enzyme metabolism (SOD, CAT, Pox, PPO and APX) in common bean ($p < 0.05$) was significant. This was the case under of three levels of irrigation. In general, results showed that under drought stress micronutrient application increase drought resistance in common bean.
Keywords: Drought, antioxidant enzymes, Phaseolus vulgaris, Zinc, nutrition, micronutrients.

INTRODUCTION

Drought is one of the major abiotic stresses that severely affect and reduce the yield and productivity offood crops worldwide up to 70% **(Kaur et al., 2008; Thakur et al., 2010; Akram et al., 2013).** The response of plants to drought stress is complex and involves changes in their morphology, physiology and metabolism. Reduction of plant growth is the most typical symptom of drought stress **(Sairam and Srivastava, 2002).** Drought stress leads to accumulation of reactive oxygen species (ROS), generated mostly in chloroplast and to some extend in mitochondria, causing oxidative stress. Major ROS molecules are singlet oxygen, superoxide anion radicals, hydroxyl radicals and hydrogen peroxide (H2O2). Plants under drought stress display some defense mechanisms to protect themselves from the damaging effect of oxidative stress. Plants with high induced antioxidant levels have better tolerance and resistance to oxidative damage **(Paridaand Das, 2005).** The ROS scavenging mechanism is among the common defense responses against abiotic stresses **(Vranová et al., 2002).** To detoxify ROS, plants can intrinsically develop different types of antioxidants reducing oxidative damage and conferring drought tolerance. The ROS scavengers are antioxidant enzymes containing superoxide dismutase, peroxidase and catalase **(Demiral and Turkan, 2005; Khan and Panda, 2008).**

Common bean (*Phaseolus vulgaris* L.) is grown over a wide range of environments, including sites with low or high soil temperatures at sowing time. Bean plant is sensitive to chilling soil temperatures often encountered during early sowing. Early sown seeds that are subjected to chilling temperatures were smaller, suffered reductions in the rate of emergence and maximal emergence than late sowing **(Rodiño et al., 2006).**

Plant nutrition one of the most important factors that increase plant production. Zinc (Zn) is an essential nutrient required in some fertilizer programs for crop production. While some soils are capable of supplying adequate amounts for crop production, addition of zinc fertilizers is needed for others **Mousavi et al. (2012).**

MATERIALS AND METHODS

Uniform common bean seeds were planted in El-Behaira, Egypt on the date 6.4.2013 in three plots (3 m width and 15 m length for each plot) containing 24 ridges for each plot. The seeds were sown on one side of the ridge, with 20 cm apart between the hills. The developed plants were irrigated whenever required with tap water until the complete germination. Irrigation was done seedlings when the ages of seedlings are 17 days by using three different level of irrigation (10, 15, 20 days).

Irrigation types:

- First level (after 10 days tap water)
- Second level (after 15 days tap water)
- Third level (after 20 days tap water)

Combined treatments

Zn (10 days irrigation water + 50 ppm zinc sulphat).

Zn (15 days irrigation water + 50 ppm zinc sulphat).

Zn (20 days irrigation water + 50 ppm zinc sulphat).

The plants of common bean were treated twice with the above mentioned treatments (as foliage spraying). The first treatment was made when the age of plants was 33 days, while the second treatment was made when the age of plants was 70 days of sowing. The plant samples were collected for analysis when the plants were 45 (Stage I) and 85 (Stage II) days old. At the end of the growth season (149 days), analysis of the seeds yielded from the different treatments as well as the control was done.

1-The physical and chemical properties of the soil are present in Tables 1 and 2

Table1.Physical properties of the used soil land.

Gravels	Fine gravels	Coarse Sand	Medium sand	Fine sand	silt	Clay	Texture class
1.3	4	5	45	23	7.5	17	Sandy-clay soil

Table 2.Chemical properties of the used soil.

TSS Ppm	pH	E.C. mmhos/cm	Cationsmeq/L				Anion meq/L			
658	7.2	2	Na+	K+	Ca++	Mg++	Cl-	SO4- -	HCO3-	CO3--
			1.85	0.5	2.58	1	2.64	1	1	Zero

1. **Chemical analysis**

Superoxide dismutase (SOD) activities were estimated using the method of **Marklund and Marklund (1974).** Catalase (CAT) (s) activities were estimated according to the methods of **(Aebi 1983).** Peroxidase (POX) (s) activities: were estimated according to the method of **(Bergmeyer, 1974)**, Polyphenoloxidase (PPO) (s) activities were estimated according to the methods of **Kar and Mishra, (1976).** Ascorbate peroxidase (APX) activities were estimated according to the methods of **Koricheva et al (1997).**

2- **Statistical methods**

All statistical calculations were done using computer programs. Microsoft excel version 10 and spss (statistica package for the social science version 20.00) statistical programat 0.05 level of probability **(Snedecor and Cochran, 1989).**

RESULTS AND DISCUSSION

Superoxide dismutase (SOD) activities

Results shown in **Fig. (1)**Clearly reveal that, treating common bean plants with irrigation water at (15 or 20 days) resulted in highly significant increases in the activities of SOD enzymes throughout the two stages of growth. When being compared with plants grown in normal non-stress condition. The obtained results **Fig. (1)**Showed that, activities of SOD were increased in response to the treatment with Zn + irrigation water at (10 days) Statistically, these increases were found to be highly significant. Regarding the interactive effects, the obtained results **Fig. (1)** revealed that, in plants treated with Zn + irrigation water at (15 days) significant decreased the activities of SOD enzymes throughout the first stages of growth, while the second stage they were significantly increased. The obtained results **Fig. (1)** Revealed that, insignificant increases in the SOD activities were recorded due to the treatment with Zn + irrigation water at (20 days). This was the case throughout the first stage of growth, and highly significant decrease at the second stage.

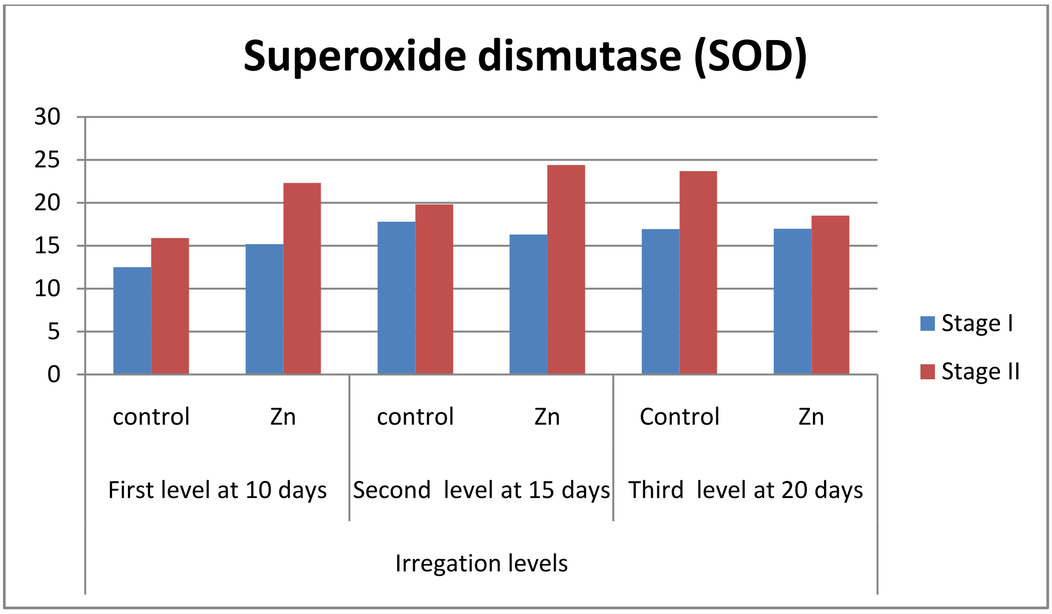

Figure 1.Effect of drought, Zinc and their interactions on the activity ofSuperoxide dismutase (SOD).

Catalases (CAT) activities

Results of the present work **Fig. (2)** revealed that, common bean plants irrigated water at (15, 20 days) resulted in significant decreases in the activities of catalase enzyme. This was the case throughout the first stages of growth. While the irrigation with irrigated water at (10, 15, 20 days caused highly significant increases at second stage.

On the other hand, the obtained results **Fig. (2)** revealed that, generally, highly significant increases in the activities of catalases were recorded in response to the treatment with Zn + irrigated water at (10, 15, 20 days). This was the case throughout the first stages of growth. While treatment of Zn + irrigated water at (10, 20 days) revealed that, generally, highly significant decreases in the activities of catalases were recorded throughout the second stage of growth.

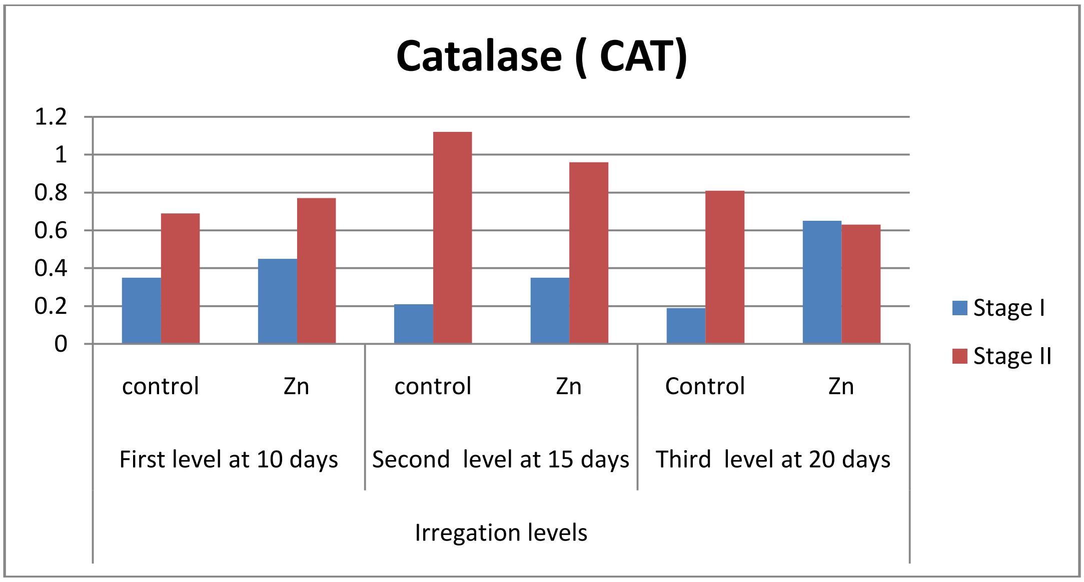

Figure 2.Effect of drought, Zinc and their interactions on the activity of Catalases (CAT).

Peroxidases (POX) activities

Results of the present investigation **Fig. (3)** Revealed that, common bean plants irrigated at (20 days), showed significant increases in the activities of peroxidases throughout the duration at the first stage of the experiment. Also, highly significant increases in the activities of peroxidases were observed in response to irrigation at (15 days). This was the case throughout the second stages of growth when compared with non-stress conditions. On the other hand, significant increases, regarding the activities of peroxidases were resulted due to the treatment with Zn + irrigation at (10, or 15 days). This was the case throughout the two stages, On the contrary, application of Zn + irrigation at (10, or 15 days) caused insignificant changes decrease in peroxidases activities at the first stage of growth. While at the second stage it caused significant increases when being compared with plants grown in 20 days irrigated.

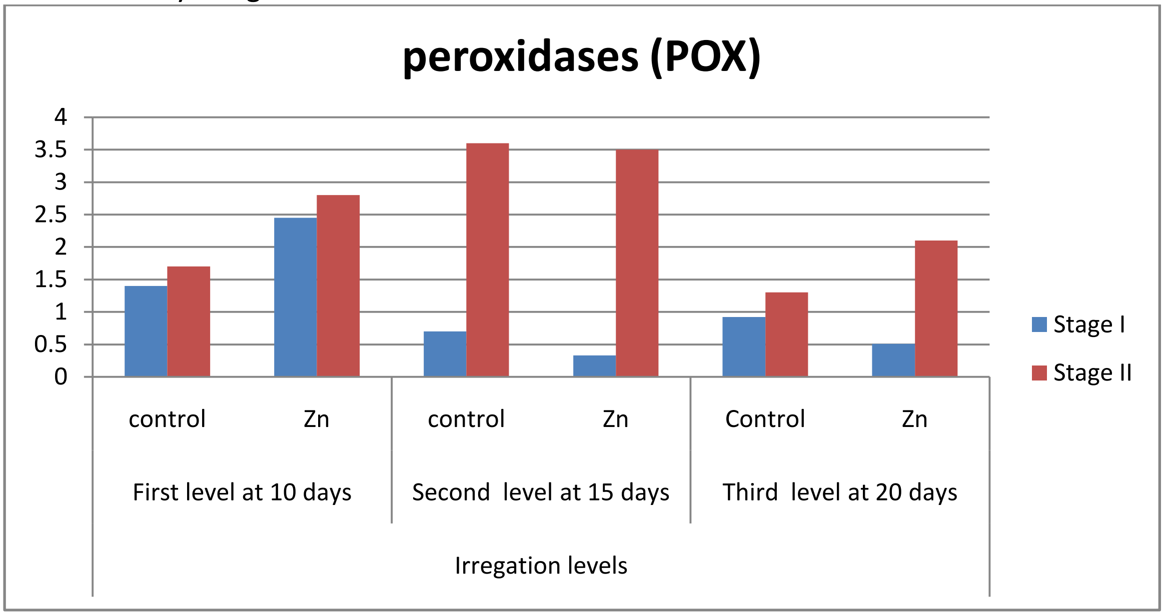

Figure 3. Effect of drought, Zinc and their interactions on the activity of peroxidases (POX).

Polyphenoloxidase (PPO) activities

Results shown in **Fig. (4)** Clearly reveal that, plants grown under irrigation water at (15, 20 days) resulted in highly significant decreases in the activities of PPO enzymes throughout the two stages of growth.

The obtained results **Fig. (4)** showed that, activities of PPO were decreased in response to the treatment with Zn + irrigation water at (10, 15 days). Statistically, these increases were found to be significant at the two stage of the experiment.

Regarding the interactive effects, the obtained results **Fig. (4)** Revealed that, in plants irrigated water at (20 days) & Zn markedly highly significant increased the activities of PPO enzymes throughout the two stages of growth.

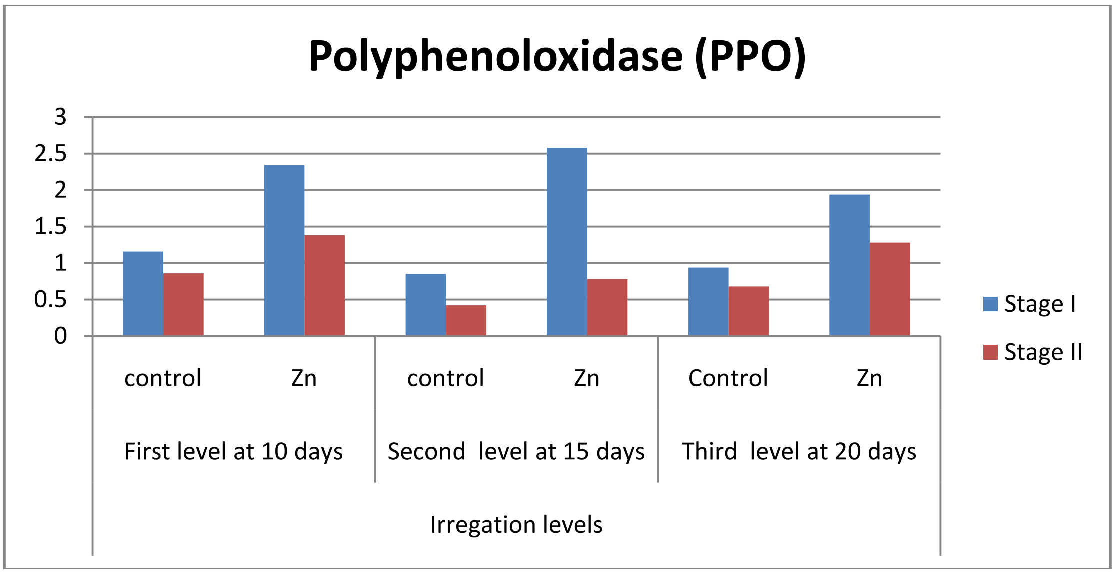

Figure 4. Effect of drought, Zinc and their interactions on the activity of Polyphenoloxidase (PPO).

Ascorbate peroxidase (APX) activities

Results shown in **Fig. (5)**Clearly reveal that, plants grown under irrigation water at (15 or 20 days) resulted in highly significant decreases in the activities of APX enzymes throughout the two stages of growth.

The obtained results **Fig. (5)**Showed that, activities of APX were decreased in response to the treatment with Zn + irrigation water at (10 or15 or 20 days) statistically, these increases were found to be insignificant at the two stage of the experiment.

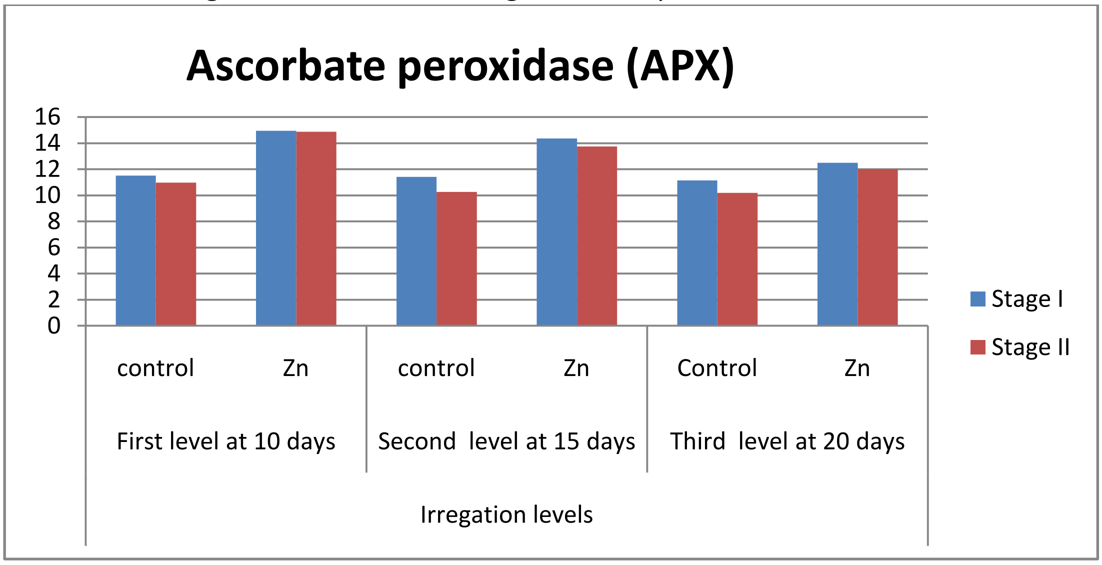

Figure 5.Effect of drought, Zinc and their interactions on the activity of Ascorbate peroxidase (APX).

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 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.POX, PPO and APX activities were increased with Zn treatment. The lows activity of these enzymes was obtained in the (control) treatment. **Hacisalihoglu et al. (2003)** reported that under Zn deficiency stress, 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).**

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