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CHANGES IN ANTIOXIDATIVE SYSTEMS AND MEMBRANE STABILITY INDEX OF CANOLA IN RESPONSE TO SALINE SOIL AND FERTILIZER TREATMENT APPLICATION†

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ABSTRACT

The ability of fertilizer treatments (O (Optimum level), OP (Optimum level + twice amount of K^+), OZ (Optimum level + twice amount of Zn^{2+}) and OZP (Optimum level + twice amount of K^+ + twice amount of Zn^{2+})) to ameliorate salinity stress was studied in canola plants. In addition, fertilizer treatments; O, OZ and OZP couldn't increase the activity of antioxidant enzymes, such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and ascorbate peroxidase (APX; EC 1.11.1.11). When these plants were treated with OP treatment, the inhibitory effects of salinity stress were decreased by increasing the membrane stability index and antioxidant enzyme activities by ameliorating the salinity injury. These results suggested that 500 (mg kg^{-1}) potassium has an important role in the enhancement of plant antioxidant systems and resistance to salinity in canola plants.

Keywords: zinc chelate, ameliorate, potassium, before flowering stage, canola

1. Introduction

High salinity is a major stress factor that affects crop production in arid and semi-arid regions (Ashraf and Q.Ali, 2008; Dolatabadian *et al.*, 2008a; Hashemi *et al.*, 2010). It is estimated that 20% of all cultivated lands in the world are salt-stressed (Sayar *et al.*, 2010). It has been documented that salinity stress causes higher plasma membrane permeability and increases the oxidative stress in plants (Shafi *et al.*, 2009). The deleterious effects of salinity stress on plant growth are (1) low osmotic potential of soil solution, (2) nutritional imbalance, (3) specific ion effects (Ahmad, 2012; Ashraf and Harris, 2004) On the other hand, plants have well-developed the protective system against oxidative stress (Alscher *et al.*, 2002). One of the protective mechanisms is the enzymatic antioxidant system, which involves the sequential and simultaneous action of a number enzyme including superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and ascorbate peroxidase (APX; EC 1.11.1.11) (Heidari, 2010). The present study was conducted to evaluation

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the effect of fertilizer treatment on the salinity stress, antioxidant enzyme activities and membrane stability index of two canola cultivars. This approach may be useful to identify which fertilizer can apply in the salinity stress management.

2. Materials and methods

2.1. Soil sample location, plant material, stress induction and fertilizer application

The soil of this research, from the field of Tehran University, College of Agriculture and Natural Resources in the Karaj, Iran (1.2 (dS m^{-1})) with the longitude and latitude 46˚ 30' 12" and 32˚ 12' 28" respectively was provided. Another soil with 14 (dS.m^{-1}) salinity from the around of Karaj with the longitude and latitude 45˚ 12' 5.015" and 66° 51' 46" respectively were mixed to soil incorporated with 8 (dS m^{-1}) is obtained. With this way, oxidative stress caused by soil salinity. Physical and chemical properties of this soil was determined (Table 1).

Table 1. Physical and chemical properties of soil tested

Value	Characteristics	Value	characteristics
7.69	Mn^{2+} (mg kg^{-1})	Sandy clay loam	Soil texture
1.10	Cu^{2+} (mg kg^{-1})	0.07	N total (%)
0.28	Zn^{2+} (mg kg^{-1})	36.56	SP (%)
8.00	Fe^{2+} (mg kg^{-1})	46.00	ESP (%)
14.24	S-SO_4^{2-} (meq l^{-1})	0.34	OM (%)
8.00	EC (dS m^{-1})	7.60	HCO_3^- (meq l^{-1})
19.80	CEC (meq/100g)	0.00	CO_3^{2-} (meq l^{-1})
22.70	P (mg kg^{-1})	3.51	Na^+ (meq l^{-1})
10.43	$CaCO_3$ (%)	0.55	Mg^{2+} (meq l^{-1})
8.00	pH	0.94	Ca^{2+} (meq l^{-1})
		184.86	K^+ (mg kg^{-1})

Fertilizer treatments during the growth stage, before starting the measurement of cultivation stage, as fertilizer KNO_3 (for potassium), zinc chelate 7% (for zinc) and were applied as soil application. Similarly other nutrients were brought to optimum status. Since the critical levels of potassium and zinc are respectively 250 and 1.2 (mg kg^{-1}), fertilizer treatments consisted of the twice potassium (500 mg kg^{-1}), twice zinc (2.4 mg kg^{-1}), twice potassium plus zinc and control (pots that in the optimum nutrients, 250 mg kg^{-1} potassium and 1.2 mg kg^{-1} zinc) (Table 2).

Table 2. Combination of fertilizer treatments and cultivars

LO	Licord cultivar, optimum level of nutrients	Sarigol cultivar, optimum level of nutrients	SO
LOP	Licord cultivar, optimum level of nutrients + twice amount of potassium	Sarigol cultivar, optimum level of nutrients + twice amount of potassium	SOP
LOZ	Licord cultivar, optimum level of nutrients + twice amount of zinc	Sarigol cultivar, optimum level of nutrients + twice amount of zinc	SOZ
LOZP	Licord cultivar, optimum level of nutrients + twice amount of (zinc plus potassium)	Sarigol cultivar, optimum level of nutrients + twice amount of (zinc plus potassium)	SOZP

To avoid increasing soil salinity, irrigation with distilled water during the plant growth took place. To prevent leaching of salts, the pots had no drainage. Canola seeds (Sarigol and Licord) were obtained from the Seed and Plant Improvement Institute of Karaj, Iran. Canola seeds were surface sterilized in a 5% sodium hypochlorite solution for 5 min and in 96% ethanol for 1 min, and they were then thoroughly washed with

distilled water. In the research greenhouse of College of Agricultural and Natural Resources in the 5 kg pots was sown. Plants were randomly harvested after five months, and the growth parameters, biochemical parameters and antioxidant status were analyzed. The experiment was carried out in a glasshouse with a photoperiod of 12 h, irradiance of 250 $\mu molm^{-2}$ s^{-1} and air humidity between 60% and 65%.

2.2. Determination of antioxidants

For extracting the antioxidant enzymes, fresh leaves (0.5 g) were ground in an ice cooled tissue grinder in 5 ml of 50 mM cooled phosphate buffer (pH 7.8). The homogenate was centrifuged at 15000×g for 20 min at 4 °C. The supernatant was used for determining the activities of superoxide dismutase and catalase. The activity of SOD was determined following (Giannopolitis and Ries, 1977) by measuring its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT). Catalase activity was determined following (Hatch, 1963) by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm of a reaction mixture containing 1.9 ml H_2O, 1.0 ml of 5.9 mM H_2O_2 in potassium phosphate buffer (pH 7.0), and 1.0 ml extract. Ascorbate peroxidase activity was determined by following the decrease of ascorbate and measuring the change in absorbance at 290 nm for 1 min (Nakano and Asada, 1981).

2.3. Determination of membrane stability index

Membrane stability index (MSI) was determined as described by (Sairam *et al.*, 2002). The leaves were excised and put in test tubes containing 10 ml of deionized distilled water. These tubes were kept at 40 °C for 30 min and then assayed for initial electrical conductivity EC_1 (dS m^{-1}). The same samples containing leaves were autoclaved at 100 °C for 15 min to determine EC_2 (dS m^{-1}). Percent MSI was calculated as

$$MSI\ (\%) = [1-(EC_1/EC_2)] \times 100$$

2.4. Statistical analysis

The experiment was carried out with a randomized complete block (RCB) design with three replicates. All data were subjected to analysis, and the significance of the differences among treatment means was tested at a 5% probability level. Due to data analysis and draw graphs, SPSS (version 17.0) and Excel softwares were used.

3. Results and discussion

As shown in Table 3, analysis of variance generally showed a significant effect of the fertilizer treatment and their interaction effect on antioxidant enzymes activity.

Table 3. Results of variance analysis (ANOVA) of Cultivar (C), Treatment (T), Time (S) and their interaction for antioxidant activity

SOV	df	Mean squares		
		SOD (U mg^{-1} fw)	CAT (nmol mg^{-1} fw)	APX (µmol mg^{-1} fw)
Cultivar (C)	1	0.013^{*}	0.008^{ns}	0.115^{ns}
Treatment (T)	3	0.011^{**}	0.027^{**}	0.426^{**}
Time (H)	2	0.018^{**}	0.0035^{ns}	0.315^{*}
C×T	7	0.010^{**}	0.006^{**}	0.426^{**}
C×H	5	0.011^{**}	0.007^{ns}	0.195^{*}
T×H	11	0.008^{**}	0.004^{**}	0.285^{**}
C×T×H	23	0.007^{**}	0.003^{**}	0.252^{**}
Error	-	0.001	0.012	0.010
CV (%)	-	1.21	1.32	2.1

Numbers represent F-values at 5% level.

*and **, significant at P<0.05 and P<0.01, respectively; ^{ns}, non-significant.

The activity of antioxidant enzymes such as SOD significantly increased in the before flowering stage as compared to flowering stage and after flowering stage but this increasing were seen in the OP treatment (Figure 1 and 2).

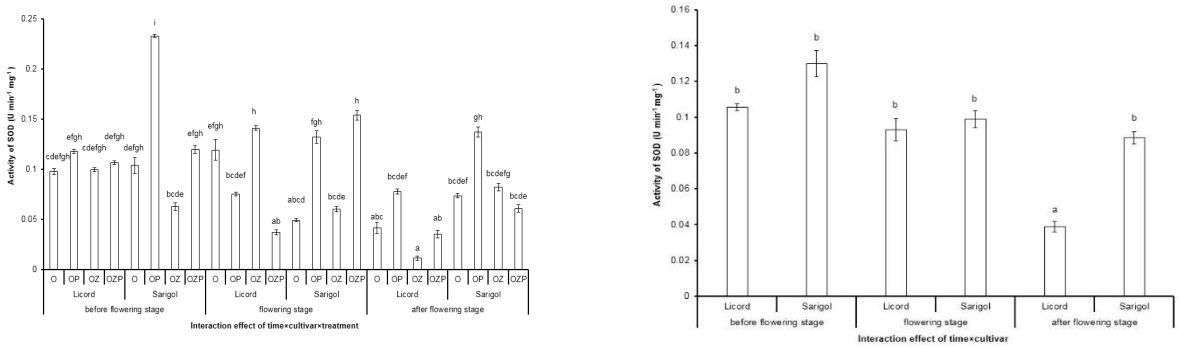

Figure 1. Interaction effect of three sampling times and two cultivars of canola on the activity of SOD (right) and interaction effect of three sampling times, cultivars and fertilizer treatments (left). Different letters indicate significant differences at P<0.05

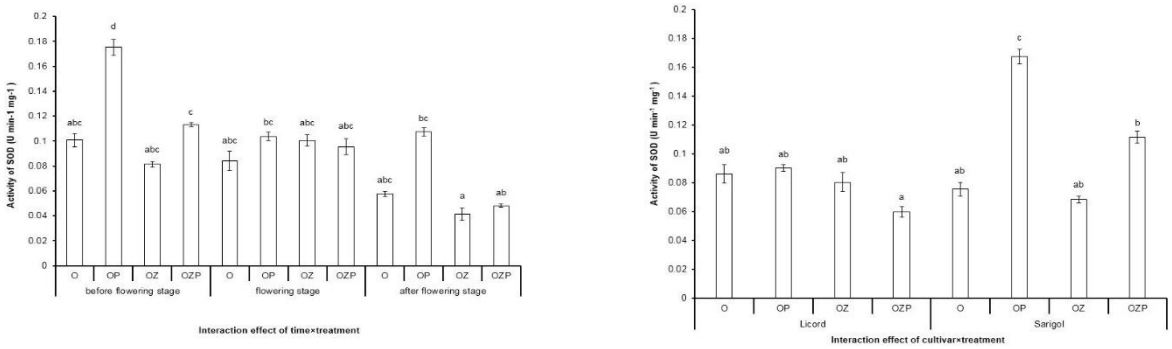

Figure 2. Interaction effect of cultivars and fertilizer treatments (right) and interaction effect of three sampling times and fertilizer treatments on the activity of SOD (left). Different letters indicate significant differences at P<0.05

Fertilizer treatment especially OP treatment significantly increased interaction effect of CAT activity in the before flowering stage when compared to the activity in the flowering stage and after flowering stage except the interaction effect of C×H (Figure 3 and 4). OP treatment significantly increased interaction effect of APX activity in the before flowering stage when compared to the activity in the flowering stage and after flowering stage (Figure 5 and 6). Table 4, shows that fertilizer treatment and cultivar have a significant effect on the MSI but the interaction effect of C×T has not significant. OP treatment and Sarigol cultivar have a significant effect on the MSI (Figure 7).

According to the Table 3, the effect of fertilizer treatments on the antioxidants activity (SOD, CAT and GPX) and also interaction effects of cultivar×time and time×cultivar and cultivar×time×treatment at P<0.01 were significant. The effect of time and cultivar×time on activity of catalase was not significant.

Table 4 shows the effect of cultivar and treatment on the membrane stability was significant but their interaction effect was not significant.

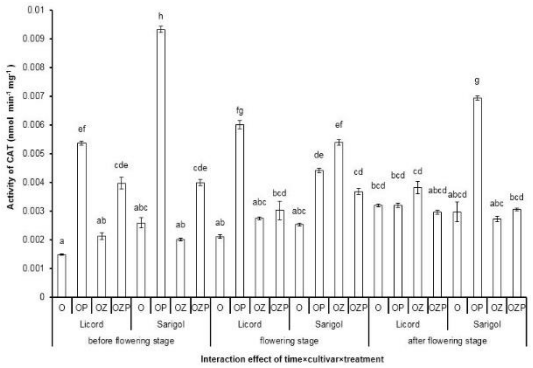

Figure 3. Interaction effect of three sampling times, two cultivars of canola and fertilizer treatments on the activity of CAT. Different letters indicate significant differences at P<0.05

Reactive oxygen species (ROS) are produced in both unstressed and stressed cells and SODs constitute the first line of defense against ROS (Alscher *et al.*, 2002). In fact, SODs converted ROS to the hydrogen peroxide (H_2O_2) and oxygen. However, hydrogen peroxide is also toxic to cells and has to be further detoxified by CAT and peroxidases such as APX to water and oxygen (Tavallali *et al.*, 2010). APX enzymes are distributed in almost every compartment of plant cells and participate in the removal of H_2O_2 as part of the ascorbate-glutathione (Bonifacio *et al.*, 2011). Hydrogen peroxide is also scavenged by CAT (Dolatabadian *et al.*, 2008b) this enzyme converts H_2O_2 to the H_2O.

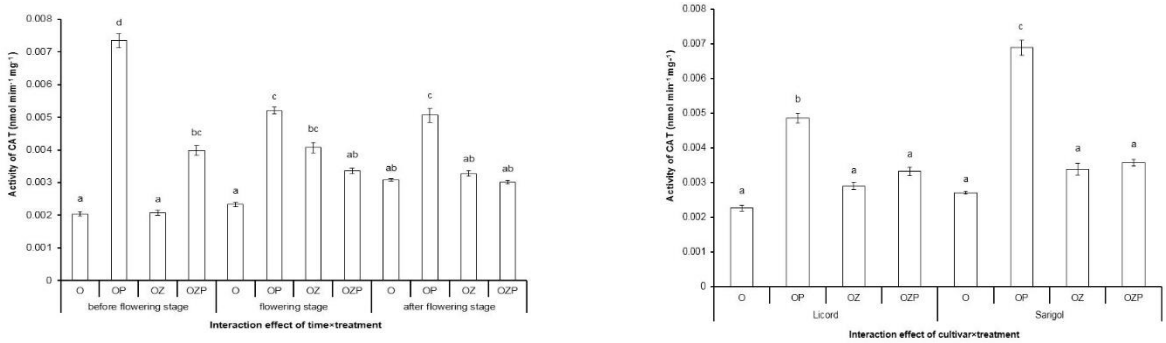

Figure 4. Interaction effect of cultivars and fertilizer treatments (right) and interaction effect of three sampling times and cultivars of canola on the activity of CAT (left). Different letters indicate significant differences at P<0.05

Table 4. Results of variance analysis (ANOVA) of Cultivar (C), Treatment (T) and their interaction for membrane stability index (MSI)

SOV	df	Mean square
		MSI (%)
Cultivar (C)	1	220.39*
Treatment (T)	3	198.67**
C×T	7	66.88^{ns}
Error	-	36.59
CV (%)	-	2.1

Numbers represent F-values at 5% level.
*and **, significant at P<0.05 and P<0.01, respectively; ^{ns}, non-significant.

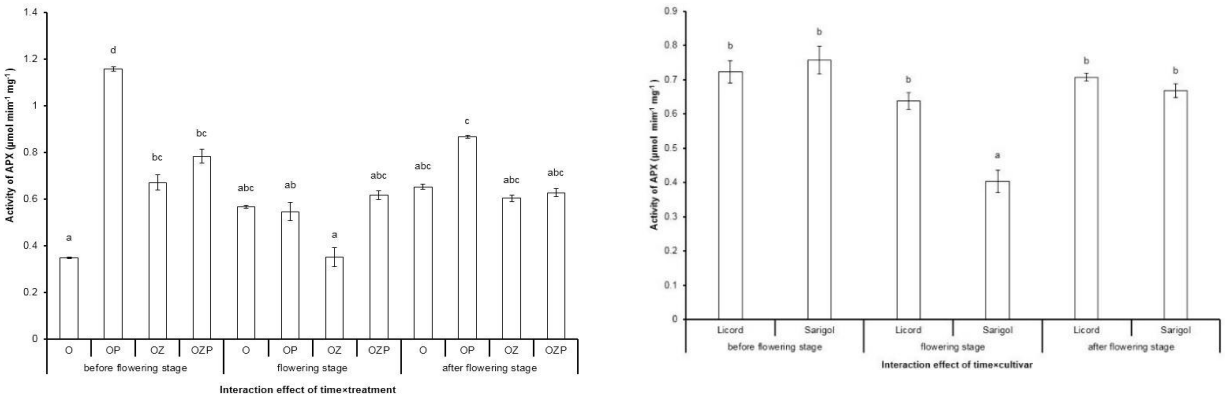

Figure 5. Interaction effect of three sampling times and two cultivars of canola (right) and interaction effect of three sampling times and fertilizer treatments on the activity of APX (left). Different letters indicate significant differences at P<0.05

Maintain a high cytosolic K^+/Na^+ ratio is one of the crucial mechanisms to salt tolerance (Sergey and Tracey, 2007). Soil salinity is known to adversely affect potassium uptake and it can often be ameliorated by increased potassium supply (Szczerbab *et al.*, 2009; Wang *et al.*, 2013). Potassium plays important roles in metabolisms like enzyme activities and protein synthesis (Y. Wen *et al.*, 2011). To enhance agricultural production in saline soils, it is necessary to ensure high crop production against environmental stresses. The present work demonstrated that OP treatment enhanced salinity tolerance in canola cultivars, which led to higher antioxidant potentials under salinity stress conditions when compared to O, OZ and OZP treatment. OP treatments caused activity SOD, CAT and APX enzymes when compared to the O, OZ and OZP, at least 18.7%, 25.65% and 9.63% higher than others, respectively. In the study that were carried out on the rapeseed seedling for investigation response of antioxidant enzymes activity with excess zinc, this research showed that NADH oxidase and peroxidase activity increased in leaves and roots of plants under high zinc, but SOD, CAT and APX activities decreased (Wang *et al.*, 2009).

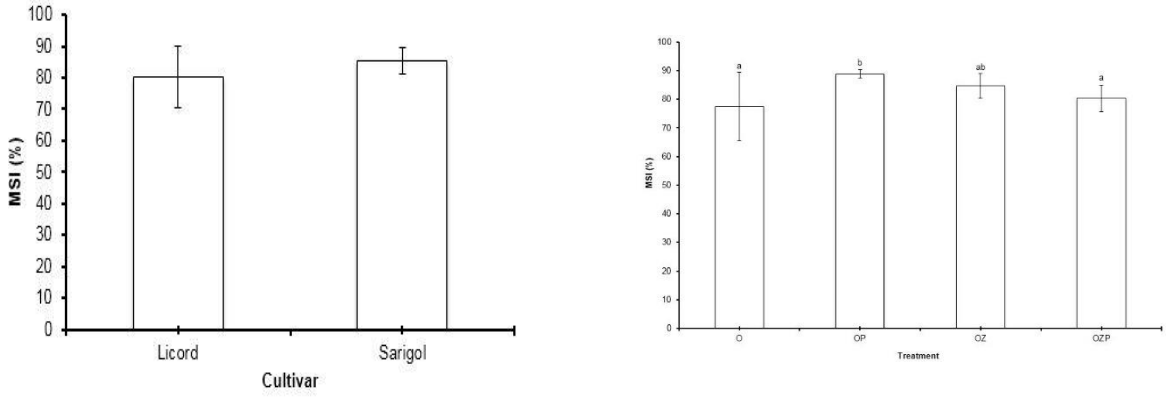

Figure 6. Interaction effect of cultivars and fertilizer treatments (right) and interaction effect of fertilizer treatments, two cultivars and three sampling times on the activity of APX (left). Different letters indicate significant differences at P<0.05

According to the results of Heidari and Jamshidi, (2011), potassium treatment increased activity of CAT and GPX in the pearl millet. Shahbazi *et al.*, (2011) resulted leaf antioxidant activities of SOD, APX and glutathione

reductase (GR) were increased by salinity increase up to 150 mM NaCl concentration. Heidari (2010) resulted by increasing NaCl levels to 300 mmol l^{-1}, the activities of CAT and APX in the canola cultivars increased but guaiacol peroxidase (GPX) in all cultivars decreased. Interaction effects of fertilizer treatments and canola cultivars showed OP treatment and Sarigol cultivar have a higher MSI percentage (0.89%) rather than others. Research indicated that application of K fertilizer generally increased crop yield as well as nutritional quality (Wang *et al.*, 2008). Tavallali *et al.*, (2010) reported that Zn supplement efficiently reduced oxidative stress, electrolyte leakage and increased activity of antioxidant enzymes (SOD, CAT and APX) in the salinity stress conditions in (*Pistacia vera* L.) seedlings. Therefore, the results of this study suggested that OP treatment enhanced antioxidant enzymes (SOD, CAT and APX) in the before flowering stage and increased MSI percentage in *Brassica napus* plants, thus, improving plant tolerance to salinity. Potassium is an essential nutrient that affects most of the biochemical and physiological processes that influence plants metabolisms and it also contributes to the survival of plants exposed to various biotic and abiotic stresses.

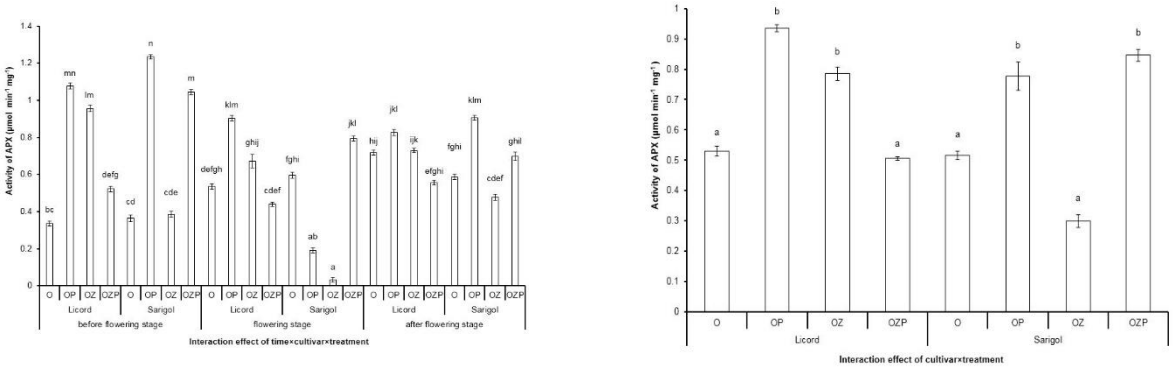

Figure 7. Effect of fertilizer treatments (right) and the effect of cultivars on MSI (%) (left). Different letters indicate significant differences at P<0.05

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