

## Differential Changes of Proline Content and Activities of Antioxidant Enzymes Results in Varied Salt-Tolerance in Canola Genotypes

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### Abstract

Saline soils and saline irrigation waters present potential hazards to canola production. Therefore, in this study in order to find indicators for salt tolerance, the changes of proline content and the activities of antioxidant enzymes were investigated in two canola genotypes (H308, H420) under various salt concentrations (0, 50, 100, 150 and 200 mM). Results showed that compared to the control, salinity reduced seed germination and biomass of plants and caused the significant increase in proline content and lipid peroxidation in leaves of both genotypes. On the basis of growth parameters, H420 was more salt sensitive than H308. H308 accumulated higher proline contents and had higher antioxidant enzyme activities (SOD, POX, APX, and CAT) than H420 especially at higher salinity levels. The activities of POX, APX, and CAT in H420 slightly increased in low salinity levels but in high salinity, their activities decreased and return to less or same level of the control. The differences in proline accumulation ability and the activities of antioxidative enzymes in leaves, at least in part, explained greater tolerance of H308 to salt stress than H420.

**Key words:** Antioxidative enzymes; Canola; Genotypes; Lipid peroxidation; Proline; Salinity

### Introduction

Salinity has long been identified as one of the most pervasive environmental hazards limiting crop production mostly in arid regions of the world. Excess of salts in root medium exerts effects like osmotic stress, ion specificity/toxicity, nutritional imbalances, and changes in the levels of cell metabolites, diminished growth and the decrease of yield (Roy *et al.*, 2014).

Water deficit originating from saline environment causes that plant cells accumulate different kinds of osmolytes like proline (Parvaiz and Satyawati, 2008). *De novo* synthesis and reduction in proline oxidation seemed to be the most predominant mechanism of proline accumulation. Proline acts as a compatible solute, an osmoprotectant and a protective agent for cytosolic enzymes and cellular organelles. Proline has also been considered as a carbon and nitrogen source for rapid recovery from stress and growth, a stabilizer for membranes and some macromolecules and also a free radical

scavenger (Parvaiz and Satyawati, 2008; Rejeb *et al.*, 2014). A significantly high accumulation of proline that was probably associated with osmotic adjustment and protection of membrane stability has been reported in salt stressed alfalfa (Amooaghaie, 2011), soybean (Weisany *et al.*, 2011), sesame (Hulusi *et al.*, 2007) and barley (Turkyilmaz *et al.*, 2014) plants.

In addition to ionic and osmotic components, salt stress, like other abiotic stresses, also leads to oxidative stress through an increase in reactive oxygen species (ROS), such as superoxide ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radicals ( $OH^\cdot$ ). It is already known that these cytotoxic reactive oxygen species (ROS), which are also generated during metabolic processes in the mitochondria, chloroplast and peroxisomes, under stress condition, can destroy normal metabolism through oxidative damage of lipids, proteins, and nucleic acids (Bahmani *et al.*, 2015).

One of the prominent effects of salinity is the peroxidation of lipids and loss of membrane

integrity due to ROS production (Amooaghaie and Tabatabaie, 2017). Thus, cell membrane stability and malondialdehyde (MDA) content, as a product of lipid peroxidation has widely been utilized to differentiate salt-tolerant and salt-sensitive cultivars (Amooaghaie, 2011; Ashraf and Ali, 2008; Bhutta, (2011); Khodabakhsh *et al.*, 2014; Sekmen *et al.*, 2007). To mitigate the oxidative damage initiated by ROS, plants have developed a complex defense antioxidative system, including low-molecular mass antioxidants as well as antioxidative enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POX) and glutathione reductase (GR) (Abogadallah, 2010; Amooaghaie and Tabatabaie, 2017).

SOD is the major  $O_2^-$  scavenger and its enzymatic action results in  $H_2O_2$  formation (Tuna *et al.*, 2008). The  $H_2O_2$  produced is then scavenged by CAT and several classes of peroxidases. CAT, which is found in peroxisomes, cytosol and mitochondria, dismutates  $H_2O_2$  into  $H_2O$  and  $O_2$ . Ascorbate peroxidase (APX) and peroxidase (POX) are distributed throughout the cell and catalyze the reduction of  $H_2O_2$  to  $H_2O$ . APX uses ascorbate as electron donor in the first step of the ascorbate–glutathione cycle and is considered the most important plant peroxidase in  $H_2O_2$  detoxification. POX, which is less specific to electron donor substrate, decomposes  $H_2O_2$  by oxidation of co-substrates such as phenolic compounds and/or ascorbate. GR catalyzes the last and rate-limiting step of the Halli well–Asada enzymatic pathway (Gill and Tuteja, 2010). The capacity to scavenge reactive oxygen species (ROS) and to reduce their damaging effects on macromolecules appears to represent an important stress tolerance trait in higher plants (Roy *et al.*, 2014). A close correlation between the antioxidant capacity and NaCl tolerance has been demonstrated in numerous crops such as: sesame (Bazrafshan and Ehsanzadeh, 2016), soybean (Weisany *et al.*, 2011), barley (Turkyilmaz *et al.*, 2014), alfalfa (Amooaghaie, 2011) and chickpea (Khodabakhsh *et al.*, 2014).

Unfortunately the salinization process in agricultural fields will decrease the suitable land for cultivation by 30% within the next 25 years, and up to 50% by the year 2050. Therefore, developments of salt tolerant crops can be the best and most practical way to

produce enough food (Bahmani *et al.*, 2015). Understanding the cellular basis of salt stress tolerance mechanisms is necessary for breeding and genetic engineering of salt tolerance in crops. *Brassica* oilseed species now hold the third position among the oilseed crops and are an important source of vegetable oil. The crop has considerable potential to grow in salt-affected areas (Rameeh, 2013). Therefore, the aim of this study was to evaluate the effects of salt stress on the activity of antioxidative enzymes and lipid peroxidation level and proline accumulation in leaves of two canola genotypes differing in salt tolerance, in order to better understand of the physiological and biochemical mechanisms of salt tolerance in canola genotypes.

## Materials and Methods

### Plant material, growth and treatment conditions

Seeds of two canola genotypes, Hyolla308 and Hyolla420 (H308, H420) from oil seeds research center (Karaj, Iran), were used in this experiment. Seeds were washed with water, dipped in 10% (v/v) sodium hypochlorite for 5 min and again washed thoroughly with sterilized water. Seeds were germinated on filter paper at 25°C. Three replicates of 50 seeds for each treatment were used. Seeds were considered germinated when a 1 mm length radicle protruded through the seed coat. Final germination percentage was calculated at the tenth day after sowing.

The uniform seedlings were transferred to plastic pots and roots immersed in 270 ml of aerated half-strength Hoagland nutrient solution. Plantlets were hydroponically grown with half strength Hogland's solution for 20 days at  $25 \pm 2^\circ\text{C}$  with a photo-cycle 16 h light (an illuminance of  $1.5 \times 10^4$  lux) and 8 h dark in growth chamber. Salt treatments were applied as half strength Hogland's nutrient solution containing NaCl concentration at final concentrations of 50, 100, 150 and 200 mM after 6 days of establishment, while control plants were grown with a half strength Hogland's nutrient solution without NaCl. Each set of experiment was repeated three times and each pot had six seedlings. The plant dry weights were measured immediately after drying the plants at 75°C.

### Lipid peroxidation

The level of lipid peroxidation in leaf samples was determined in terms of malondialdehyde content according to the method of Heath and Packer (1968). Content of MDA, was determined using the thiobarbituric acid reaction. MDA concentration was measured at 532 nm and measurements were corrected for nonspecific turbidity by subtracting the absorbance at 600 nm. The concentration of MDA was calculated using an extinction coefficient of  $155 \text{ mM}^{-1} \text{ cm}^{-1}$ .

### Proline content

Determination of free proline content was done according to Bates (1973) method. Leaf samples (0.5 g) were homogenized in 3% sulphosalicylic acid and homogenate was filtered through filter paper. After addition of ninhydrin and glacial acetic acid, resulting mixture was heated at  $100^\circ\text{C}$  for 1 h in water bath. Reaction was then stopped by using ice bath. The mixture was extracted with toluene, and toluene phase aspired from liquid phase and its absorbance was read at 520 nm. Proline concentration was determined using calibration curve as  $\mu\text{mole proline/g F.W.}$

### Enzyme extractions and assays

For protein and enzyme extractions, 0.5 g of freeze leaf samples were homogenized with 50 mM sodium phosphate buffer (pH 6.8) containing 1 mM EDTA and 2% (w/v) polyvinyl pyrrolidone (PVP). Homogenates were then centrifuged at  $4^\circ\text{C}$  for 40 min at  $13,000 \times g$  and supernatants were used for determination of enzyme activity.

Total superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed by its ability to inhibit photochemical reduction of NBT at 560 nm (Giannopolitis and Ries, 1977). The reaction mixture (1.5 ml) contained 50 mM phosphate buffer (pH 7.8), 0.1  $\mu\text{M}$  EDTA, 13 mM methionine, 75  $\mu\text{M}$  NBT, 2  $\mu\text{M}$  riboflavin and 50  $\mu\text{L}$  enzyme extract. Riboflavin was added last and the test tubes containing reaction mixture stood for 10 min under  $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$  irradiance at  $25^\circ\text{C}$ . The reaction mixture developed maximum color due to maximum rate of reduction of NBT. A non-irradiated reaction mixture did not develop color and was used as the control. One unit of

SOD was defined as the amount of enzyme that inhibits 50% NBT photoreduction.

Catalase (CAT; EC1.11.1.6) activity was estimated according to the method of Aebi (1984), which measures the initial rate of disappearance of  $\text{H}_2\text{O}_2$  at 240 nm. The reaction mixture contained 0.05 M potassium phosphate buffer (pH 7.0) with 15 mM  $\text{H}_2\text{O}_2$  and 100  $\mu\text{L}$  of enzyme extract. The decrease in the absorption was followed for 3 min and enzyme activity was calculated using an extinction coefficient of  $39.4 \text{ mM}^{-1} \text{ cm}^{-1}$ . One unit of CAT activity was defined as  $\mu\text{mol H}_2\text{O}_2$  eliminated per minute.

APX (EC 1.11.1.1) activity was assayed according to Nakano and Asada (1981). The reaction mixture (1.5 ml) contained 50 mM phosphate buffer (pH 6.0), 0.1  $\mu\text{M}$  EDTA, 0.5 mM ascorbate, 1.0 mM  $\text{H}_2\text{O}_2$  and 50  $\mu\text{L}$  enzyme extract. The reaction was started by the addition of  $\text{H}_2\text{O}_2$  and ascorbate oxidation measured at 290 nm for 1 min. The concentration of oxidized ascorbate was calculated by using extinction coefficient of  $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ . One unit of APX activity was defined as 1  $\mu\text{mol}$  ascorbate oxidized per minute.

POX (EC 1.11.1.7) activity was determined as described in the study of Tuna *et al.* (2008) The reaction mixture (2.0 ml) containing 100 mM phosphate buffer (pH 7.0), 0.1  $\mu\text{M}$  EDTA, 5.0 mM guaiacol, 15 mM  $\text{H}_2\text{O}_2$  and 50  $\mu\text{L}$  enzyme extract. The addition of enzyme extract started the reaction and the increase in absorbance was recorded at 470 nm for 1 min. Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar extinction coefficient ( $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ ). One unit of POX activity was defined as the amount of enzyme that caused the formation of  $1 \mu\text{M}$  of tetraguaiacol per minute.

The specific enzyme activities were expressed as  $\text{U mg}^{-1}$  protein for all enzymes assayed. Protein concentration was determined according to Bradford (1976), using bovine serum albumin as a standard. All operations were performed at  $4^\circ\text{C}$ .

### Statistical analysis

Research was carried out as factorial with complete randomized design in 3 replications. Data were subjected to analysis of variance using computer software of SPSS, version 13.

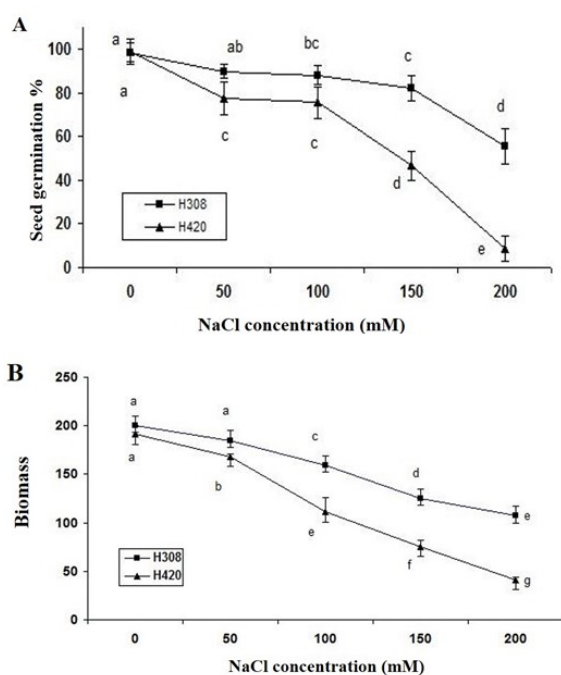
Duncan's multiple range tests were applied to compare the means of treatments at  $P < 0.05$ .

## Results

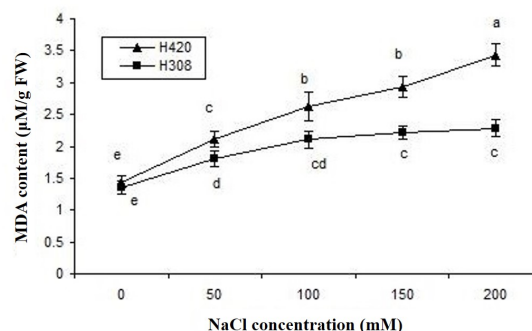
Analysis of variance (ANOVA) for seed germination, biomass, proline content, lipid peroxidation (MDA), SOD, CAT, POX and APX activities showed that effects of cultivar and salinity and their interactions were significant for all traits (data not shown).

### Seed germination and biomass

According to the analysis of variance, seed germination and biomass of plants were significantly ( $P < 0.001$ ) affected by salinity and cultivars. At control conditions seed germination and biomass of both genotypes were similar (Fig. 1). In contrast, a well-defined difference trend occurred when the plants were exposed to high salt concentration. The difference at biomass between two genotypes reached to 52% at high salt (200 mM) concentration. Seed germination percentage in H420 cultivar also was very less than H308 cultivar at 200 mM NaCl.



**Fig. 1.** The effect of various salt concentrations on the seed germination (A) and biomass (B) of two canola genotypes (H420 and H308). Values are mean  $\pm$  SE (n=3). Means followed by the same letter did not significantly differ at  $P \leq 0.05$  according to Duncan's multiple range tests.



**Fig. 2.** The effect of various salt concentrations on lipid peroxidation (MDA content) in leaves of two canola genotypes (H420 and H308). Values are mean  $\pm$  SE (n=3). Means followed by the same letter did not significantly differ at  $P \leq 0.05$  according to Duncan's multiple range tests.

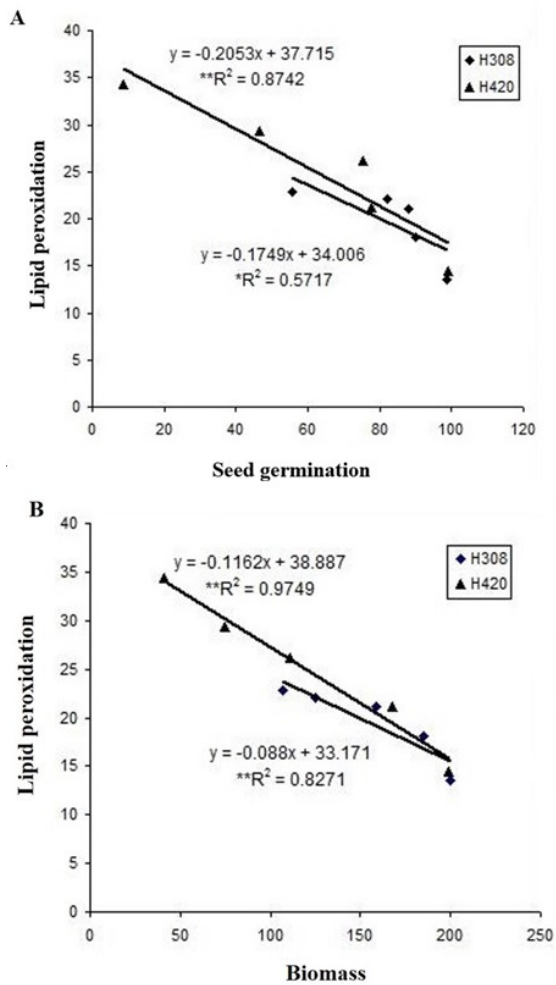
### Lipid peroxidation

To investigating the oxidative damage caused by salt stress in canola genotypes, lipid peroxidation was determined by evaluating MDA content of the tissue. Under normal conditions (0 mM NaCl), MDA contents of H308 and H420 were similar. However, the levels of MDA were significantly ( $P > 0.05$ ) increased in leaves of both genotypes at 50 mM NaCl. The level of MDA in leaves subjected to salt stress was higher in H420 than H308. MDA content increased linearly in H420 but changed slightly in H308 (Fig. 2). Our results showed significant negative correlation between lipid peroxidation to seed germination and biomass in both genotypes. These correlations were higher in H420 than H308 (Fig. 3).

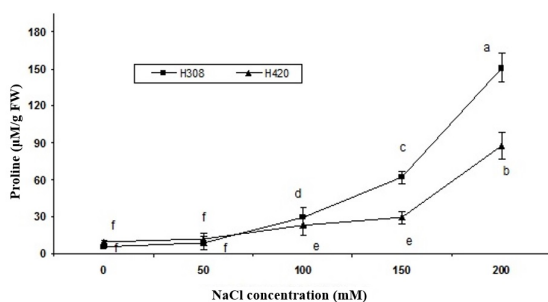
### Proline content

NaCl treatments significantly increased the proline contents in both canola genotypes. In 0 and 50 mM of NaCl, proline contents in leaves of both genotypes were similar ( $P > 0.05$ ). The proline content in leaves subjected to 100, 150 and 200 mM was higher in cultivar H308 than H420. Free proline content reached to highest level in leaves of both genotypes when exposed to 200 mM NaCl and was 15 and 5 times more than control plants of H308 and H420 genotypes respectively (Fig. 4). Under salt stress, there was significant positive correlation between proline accumulation and lipid peroxidation and negative correlation between biomass and proline content in both genotypes (Fig. 5). H308, which is a high

accumulator of proline showed less lipid peroxidation and more biomass than H420, which is low accumulator of proline.



**Fig 3.** The correlations between lipid peroxidation with seed germination (A) or biomass (B) in two canola genotypes (H308, H420). \*\*, \* means significant at  $P < 0.01$  and  $P < 0.05$ , respectively.



**Fig. 4.** The effect of various salt concentrations on the proline content in leaves of two canola genotypes (H420 and H308). Values are mean  $\pm$  SE (n=3). Means followed by the same letter did not significantly differ at  $P \leq 0.05$  according to Duncan's multiple range tests.

### Antioxidant enzyme activities

In order to determine the nature of the antioxidant responses of genotypes to salt stress, we measured the enzymatic activities of SOD, APX, POD and CAT in leaves of both genotypes. There were striking differences in antioxidant enzyme activities of two genotypes with increasing NaCl concentration (Fig. 6).

As shown in Fig. 6A, NaCl treatment increased the activity of SOD in leaves of both genotypes. More precisely, the SOD activity in leaves of H308 was higher than H420 under all salinity levels, while both genotypes exhibited a similar SOD activity in the control conditions. Maximum SOD activity obtained at 100 mM NaCl and stayed consist up to 200 mM in H308 but, SOD activity increased from 50 to 150 mM NaCl in H420 slight linearly and the highest activity was observed at 150 and 200 mM.

The CAT activity of leaves was higher in H308 than H420 in saline or non-saline condition. Salinity increased slightly CAT activity in leaves of H308 genotype and this response was significant relative to control only at 150 and 200 mM NaCl (Fig. 6B). In contrast, salt stress increased CAT activity in leaves of H420 in 50 mM NaCl but this activity decreased in higher salinity levels and return to its level in control at 100 mM and CAT activity in leaves of H420 at 150 and 200 mM NaCl was significantly less than control.

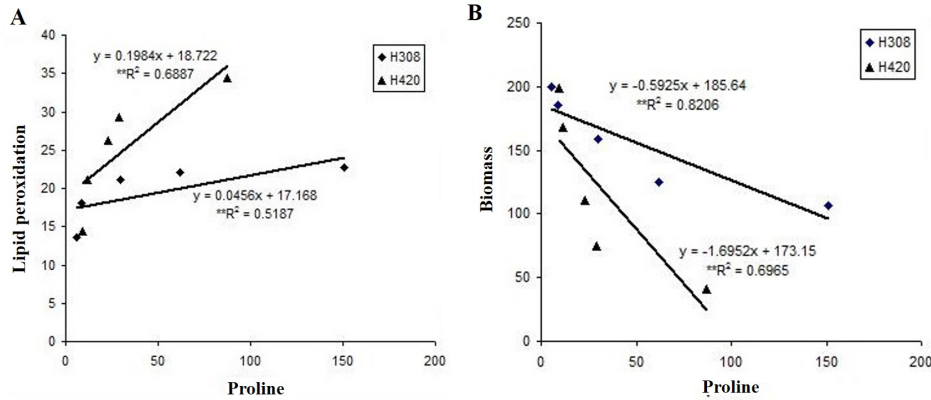
In normal condition (0 mM of NaCl) POX activity was significantly higher in H308 than in H420. POX activity sharply increased at 50 and 100 mM NaCl in leaves of H308 and at 50 mM NaCl in H420 cultivar. Under more salinity levels, the activity of POX in leaves of H420 genotype significantly decreased but fluctuations of POX activity in H308 at 150 and 200 mM showed no significant difference statistically than its values at 100 mM (Fig. 6C).

The APX activity was significantly higher in H308 than in H420 in non-saline condition. Salinity increased APX activity at 50 mM in both genotypes. In higher salinity levels, APX activity significantly decreased in H420 genotype but APX activity fluctuations in H308 showed no significant difference statistically than its values at 50 mM (Fig. 6D). There was positive correlation between lipid peroxidation to all antioxidant enzymes activity in H308. But there was no significant

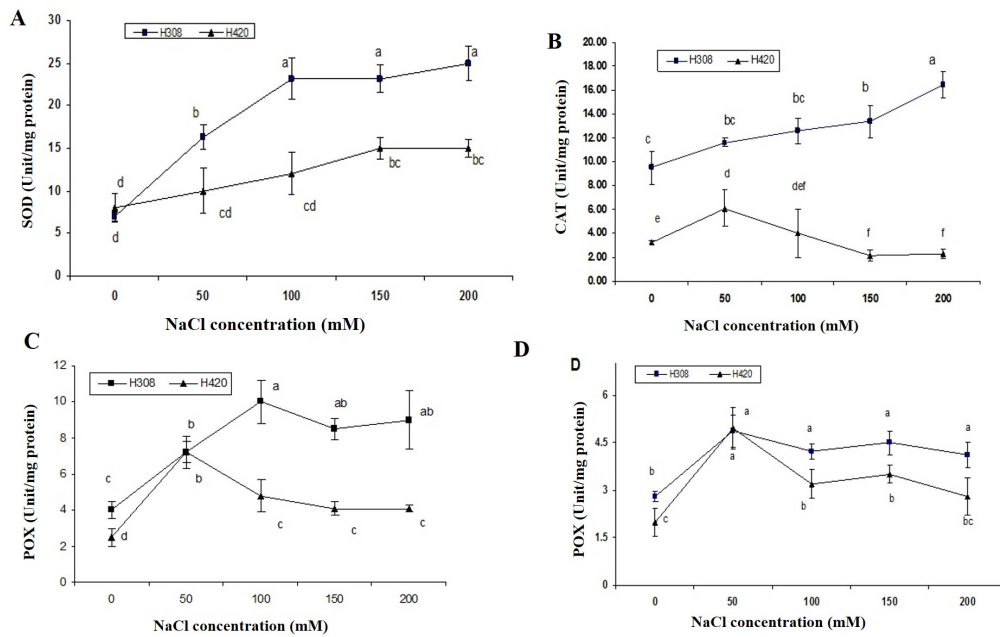
correlation between activities of H<sub>2</sub>O<sub>2</sub> scavenging enzymes (POX, CAT and APX) and lipid peroxidation in H420 under NaCl treatment. Only correlation between SOD activity and lipid peroxidation was significant in this genotype (Fig 7).

In addition, there was significant correlation between biomass to activity of SOD, CAT and

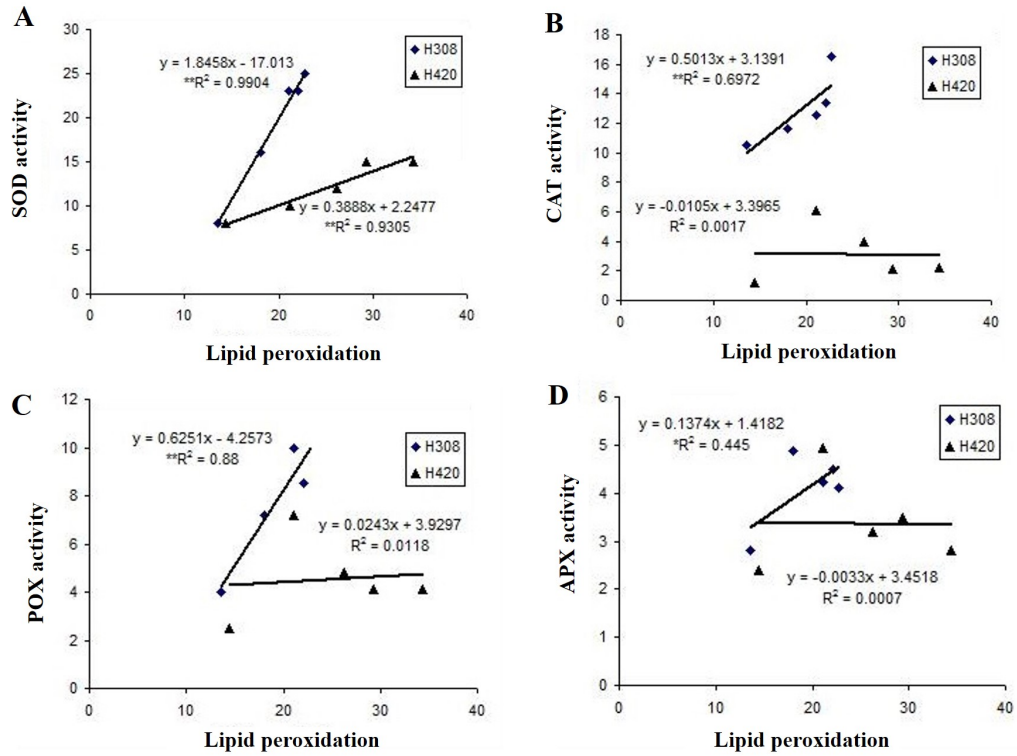
POX in H308 genotype. But there was no significant correlation between activities of H<sub>2</sub>O<sub>2</sub> scavenging enzymes (POX, CAT and APX) and biomass in H420 under NaCl treatment. Only correlation between SOD activity and biomass was significant in this genotype (Fig. 8).



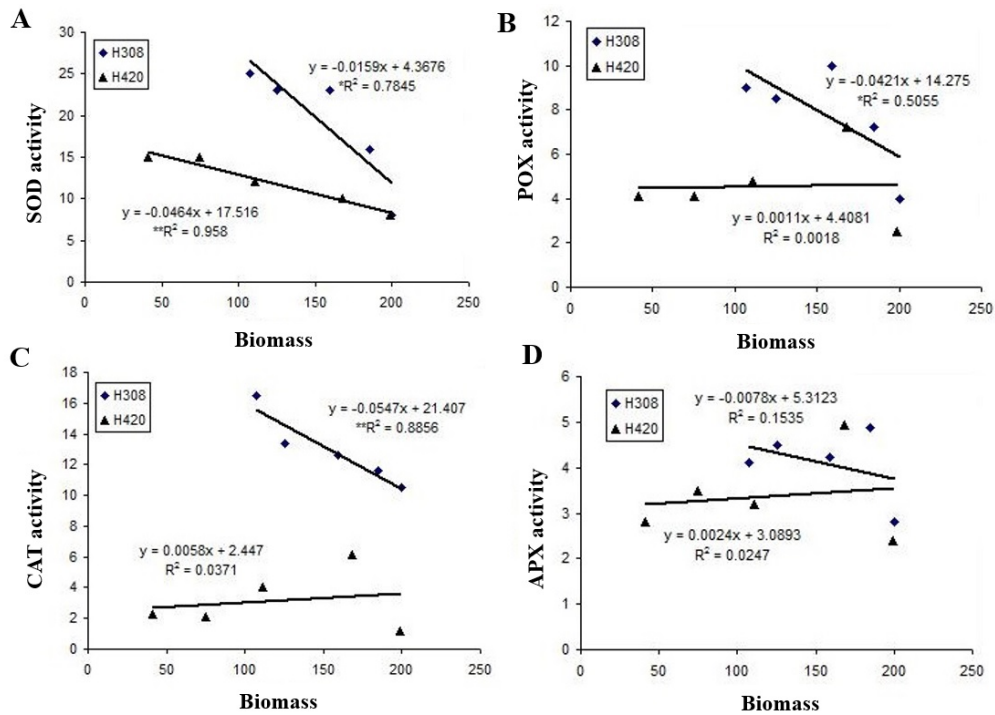
**Fig. 5.** The correlations between proline and lipid peroxidation (A) or biomass (B) in two canola genotypes (H308, H420). \*\*, \* means significant at  $P < 0.01$  and  $P < 0.05$ , respectively.



**Fig. 6.** Effect of various salt concentrations on the activities of SOD (A), CAT (B) POX (C) and APX (D) in leaves of two canola genotypes (H420 and H308). Values are mean ± SE (n=3). Means followed by the same letter did not significantly differ at  $P \leq 0.05$  according to Duncan's multiple range tests.



**Fig. 7.** The correlations between lipid peroxidation and antioxidant enzymes activity in two canola genotypes (H308, H420). \*\*, \* mean significant at  $P < 0.01$  and  $P < 0.05$ , respectively.



**Fig. 8.** The correlations between biomass and free radical scavenger enzymes activity in two canola genotypes (H308, H420). \*\*, \* mean significant at  $P < 0.01$  and  $P < 0.05$ , respectively.

## Discussion

Our data showed that seed germination and plant biomass were affected negatively by the salt stress (Fig. 1) that was in accordance with observations of Ashraf and Ali (2008) on canola and Amooaghaie, (2011) on alfalfa. This growth slowing may be an adaptive response to stress and it could be due to an osmotic effect, ion toxicity or salt-driven oxidative stress (Munns and Tester, 2008). Overall, increasing salt concentrations inhibited growth of both canola genotypes, but the inhibition was genotype-dependent and H420 showed seed germination and biomass less than H308 under high salinity.

Results revealed salinity increased lipid peroxidation in both genotypes (Fig. 2). AL Hassan *et al.* (2015) also reported both stress salinity and drought reduced plant growth and caused oxidative stress in tomato plants, as indicated by a significant increase of malondialdehyde content. In this study MDA content was affected slightly by salinity in leaves of H308, but elevated linearly in leaves of H420 with increasing salinity (50 to 200 mM NaCl). The lower level of lipid peroxidation in H308 (Fig. 3) suggests that this cultivar is better protected from oxidative damage under salt stress. Similar to our finding, Li *et al.*, (2010) also have been emphasized that there are negative correlation between MDA content and level of tolerance to salt stress in castor bean.

Our data showed proline accumulation increased in both canola genotypes under salinity conditions (Fig. 4). The proline is an osmoprotectant and this response might be an adaptive feature to maintain water balance against salinity-induced osmotic stress (Amooaghaie and Tabatabaie, 2017; Rejeb *et al.*, 2014). In addition, proline can scavenge OH<sup>•</sup> and stabilize biomembrane (Cuin and Shabala, 2007; Rejeb *et al.*, 2014). Since proline accumulation in both canola genotypes had positive correlation to lipid peroxidation (Fig. 5) and production of this free amino acid in the salt-tolerant genotype (H308), was more notable (Fig. 4), it seems that proline plays some protective roles against salt-induced oxidative stress in both genotypes. The role of increasing proline accumulation in enhancement of salinity tolerance was also found in tomato (AL Hassan *et al.*, 2015), alfalfa (Amooaghaie, 2011), wheat (Esfandiari

*et al.*, 2011) and chickpea (Khodabakhsh *et al.*, 2014).

In salt stressed plants, the balance between free radical generation and scavenging reactions might be disturbed in favor of the former (Roy *et al.* 2014). SOD is one of the ubiquitous enzymes in aerobic organisms and plays a key role in regulating cellular defense mechanisms. It is worth to nothing that H<sub>2</sub>O<sub>2</sub> as the product of SOD activity plays role of a signal molecule in triggering plant responses to salinity as shown that H<sub>2</sub>O<sub>2</sub> pretreatment of seeds increases the tolerance of plants to abiotic stresses (Amooaghaie and Tabatabaie, 2017). It is possible that increasing SOD activity (Fig. 6) and its significant correlations with lipid peroxidation (Fig. 7) or biomass (Fig. 8) in both genotypes may be related to this matter. It has been demonstrated that expression and activities of some antioxidant enzymes are stimulated by H<sub>2</sub>O<sub>2</sub> accumulation. Polidoros and Scandalios (1999) demonstrated low concentrations of H<sub>2</sub>O<sub>2</sub> induced the expression of *Cat1* and *Cat3* genes in maize, while higher doses inhibited them. On the other hand, the high dose of H<sub>2</sub>O<sub>2</sub> which is generated in adverse environmental conditions in tissues is deleterious and ROS-induced oxidative damage inhibits or delays plant growth. SOD activity modulates the relative amounts of O<sub>2</sub><sup>•-</sup> and H<sub>2</sub>O<sub>2</sub>, the two Haber–Weiss reaction substrates, and decreases the risk of OH<sup>•</sup> radical formation, which is highly reactive and may cause severe damage to membranes protein and DNA (Abogadallah, 2010; Najafi *et al.*, 2016). In the present study, a higher SOD activity was observed in H308 compared with H420 under salt stress (Fig. 6), which suggests H308 scavenged O<sub>2</sub><sup>•-</sup> more effectively than H420 that evidenced as higher correlations SOD activity with lipid peroxidation (Fig. 7) or biomass (Fig. 8) in H308 than H420 under NaCl treatment. The less ability of H420 to scavenge O<sub>2</sub><sup>•-</sup> radicals, could lead to further cellular damage and more suppression of plant growth.

However, H<sub>2</sub>O<sub>2</sub> as a byproduct of the activity of SOD is a toxic species and for preventing of cellular damage, H<sub>2</sub>O<sub>2</sub> must be eliminated by conversion to H<sub>2</sub>O in subsequent reactions by involving APX, POX, and CAT. CAT eliminates H<sub>2</sub>O<sub>2</sub> by breaking it down directly to form water and oxygen. This enzyme does not require a reducing power and has a high reaction rate but a low affinity for H<sub>2</sub>O<sub>2</sub>,



thereby only removing the high concentration of H<sub>2</sub>O<sub>2</sub> (Gill and Tuteja, 2010). Our data demonstrated that the H308 cultivar increased sharply levels of CAT activity only at 200 mM and lipid peroxidation at 200 mM in this genotype was considerably less than H420, thereby suggesting that CAT is important for the removal of high concentrations of produced H<sub>2</sub>O<sub>2</sub> at 200 mM particularly in the leaves of H308. Unlike CAT, which has a low affinity for H<sub>2</sub>O<sub>2</sub> (being active only at high peroxide concentrations), APX has high substrate affinities and can detoxify H<sub>2</sub>O<sub>2</sub> at low concentrations. It is possible that non-significant correlations between lipid peroxidation (Fig. 7) in H420 or biomass (Fig. 8) in both genotypes may be related to this matter. POX is involved not only in scavenging H<sub>2</sub>O<sub>2</sub> but also in plant growth, development, lignifications, suberization, and cross-linking of cell wall compounds (Gill and Tuteja, 2010). Higher activities of POX in leaves of H308, at least in part, explained greater tolerance of H308 to salt stress than H420.

However, results revealed that these two genotypes have developed different mechanisms to cope salinity effects. Under high concentration of NaCl, the activities of APX, CAT and POX decreased or remained unchanged in the salt-sensitive genotype H420 (Fig. 6) that was coincided with more damage to membranes and a sharp decrease in growth parameters. However, correlations APX, CAT and POX activities with lipid peroxidation (Fig.7) or biomass (Fig.8) were not significant in H420. It is possible that when salt-driven oxidative stress exceeded the tolerance limits of H420, the antioxidant systems were inactivated. In contrast, the activity of SOD, APX, CAT and POX in leaves of the salt-tolerant genotype H308 significantly was enhanced with increasing NaCl concentrations that it is confirming an effective mechanism of ROS scavenging exists in this genotype. High correlation between lipid peroxidation and the activity of antioxidant enzymes in H308 (Fig. 7), further supports the above conclusion. Such a correlation between lipid peroxidation and activity of antioxidative enzymes were also reported by Turkyilmaz *et al.* (2014) in barley. Bhutta (2011) also reported with the increase of salt stress, the activities of SOD, POX and CAT elevated in S-24 genotype as more tolerant wheat cultivar, while in DN-27 as

susceptible cultivar all the enzymes showed constant activity at all the stress levels. Several studies have demonstrated that salt-tolerant species increase their antioxidant enzyme activities and antioxidant contents in response to salt stress whereas salt-sensitive species failed to do so (Khodabakhsh *et al.*, 2014; Mhadhibi *et al.*, 2011; Ashraf and Ali, 2008). Under salinity stress, higher activities of antioxidant enzymes in H308, by comparison with H420, was well-correlated with the maintenance of further biomass in H308 (Fig. 8) that indicating the salt-induced elevation of antioxidant enzyme activities in H308 was sufficient to alleviate negative effects of ROS on H308 growth.

In conclusion, the outcomes of the study indicate that salinity caused oxidative stress and led to growth inhibition in both genotypes. The sensitivity of both genotypes to salinity was linked to the effectiveness of their ROS scavenging systems. On the contrary to the MDA content, proline accumulation was more notable in H308 genotype whose growth attributes was less affected by the salt stress. Therefore, we found clear indications of physiological differences between canola genotypes in their response to salt stress; in comparison with H420, H308 exhibited a more efficient strategy to cope with salinity stress through higher cytosolute accumulation and the enhancement of antioxidant enzyme activities, which maintain the redox homeostasis and the integrity of cellular components. Further in-depth research is needed to explore the salt impact on the regulation of gene expression in canola genotypes. In addition, it remains to be seen whether the probable differences in grain yield and agronomic performance of the latter canola genotypes under field conditions, in response to NaCl, will be consistent with the present findings or not.

## References

- Abogadallah GM. 2010. Antioxidative defense under salt stress. *Plant Signal Behav* 5: 369-374.
- Aebi H. (1984). Catalase *in vitro*. *Meth Enz* 105: 121-126.
- AL Hassan M, Fuertes MM, Sanchez FJ R, Vicente O, Boscaiu M. 2015. Effects of salt and water stress on plant growth and on accumulation of osmolytes and antioxidant

- compounds in cherry tomato. *Not Bot Horti Agrobo* 43(1): 1-11.
- Amooaghaie R, Tabatabaei, F. 2017. Osmopriming-induced salt tolerance during seed germination of alfalfa most likely mediates through H<sub>2</sub>O<sub>2</sub> signaling and upregulation of heme oxygenase. *Protoplasma* 254(4): 1791-1803.
- Amooaghaie R. 2011. The Effect of hydro and osmopriming on alfalfa seed germination and antioxidant defenses under high salt concentration stress. *Afr J Biotech* 10: 6269-6275.
- Ashraf M, Ali Q. 2008. Relative membrane permeability and activities of some antioxidant enzymes as the key determination of salt tolerance in canola (*Brassica napus* L.). *Environ Exp Bot* 63: 266- 273.
- Bahmani K, Sadat Noori SA, Izadi Darbandi A, Akbari A. 2015. Molecular mechanisms of plant salinity tolerance: a review. *Aust J Crop Sci* 9(4):321-336
- Bates LS. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil* 39: 205-207.
- Bazrafshan AH, Ehsanzadeh P. 2016. Evidence for differential lipid peroxidation and antioxidant enzyme activities in *Sesamum indicum* L. genotypes under NaCl salinity. *J Agr Sci Tech* 18: 207-222
- Bhutta WM. 2011. Antioxidant activity of enzymatic system of two different wheat (*Triticum aestivum* L.) cultivars growing under salt stress. *Plant Soil Environ* 57 (3): 101-107.
- Bradford MM. 1976. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein- dye binding. *Plant Physiol* 72: 248-254.
- Cuin TA, Shabala S. 2007. Compatible solutes reduce ROS-induced potassium efflux in *Arabidopsis* roots. *Plant Cell Environ* 30: 875- 885.
- Esfandiari E, Enayati V, Abbasi A. 2011. Biochemical and physiological changes in response to salinity in two durum wheat (*Triticum turgidum* L.) genotypes. *Not Bot Hort Agrobot Cluj* 39(1):165-170
- Giannopolitis N, Ries SK. 1977. Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiol* 59:309-314.
- Gill SS, Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* 48: 909-930
- Heath RL, Packer L. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 125: 189-198.
- Hulusi K, Melike B, Filiz Ö, Dsmaıl T. 2007. The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environ Exp Bot* 60: 344-351.
- Khodabakhsh F, Amooaghaie R, Mostajeran A. 2014. Effect of hydro and osmopriming on membrane deterioration, proline accumulation and H<sub>2</sub>O<sub>2</sub> scavenging enzymes in two salt stressed chickpea cultivars. *Environ Eng Manag J* 13(3): 619-626
- Li G, Wan S, Zhou J, Yang Z, Qin P. 2010. Leaf chlorophyll fluorescence, hyperspectral reflectance, pigments content, malondialdehyde and proline accumulation responses of castor bean (*Ricinus communis* L.) seedlings to salt stress levels. *Indust Crop Product* 31: 13-19.
- Mhadhibi H, Fotopoulos V, Mylona PV, Jebera M, Aouani ME, Polidoros AN. 2011. Antioxidant gene enzyme responses in *Medicago truncatula* genotypes with different degree of sensitivity to salinity. *Physiol Plant* 141: 201-214.
- Munns R, Tester M. 2008. Mechanisms of salinity tolerance. *Ann Rev Plant Biol* 59: 651-681.
- Najafi S; Mir N; Shafeghat M. 2016. Antioxidant and Antibacterial Activities of Six Medicinally Important Species of the Genus *Salvia* from North East of Iran. *J Genet Resour* 2(1):41-47.
- Nakano Y, Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* 22: 867-880.
- Parvaiz A, Satyawati S. 2008. Salt stress and phyto-biochemical responses of plants- a review. *Plant Soil Environ* 54 (3): 89-99
- Polidoros AN, Scandalios JG. 1999. Role of hydrogen peroxide and different classes of antioxidants in the regulation of catalase and glutathione S-transferase gene expression in maize (*Zea mays* L.). *Physiol Plant* 106: 112-120.
- Rameeh V. 2013. Effect of salinity stress on yield, component characters and nutrient compositions in rapeseed (*Brassica napus*

- L.) genotypes. *Agri Trop Subtrop* 46 (2): 58-63.
- Rejeb KB, Abdelly C, Savoure A. 2014. How reactive oxygen species and proline face stress together. *Plant Physiol Biochem* 80: 278-284.
- Roy SJ, Negrao S, Tester M. 2014. Salt resistant crop plants. *Curr Opin Biotech* 26: 115-124
- Sekmen AK, Turkan I, Takio S. 2007. Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt tolerant *Plantago maritima* and salt-sensitive *Plantago media*. *Physiol Plant* 131: 399-411.
- Tuna AL, Kaya C, Dikilitas M, Higgs D. 2008. The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. *Environ Exp Bot* 62: 1-9.
- Turkyilmaz UB, Akats LY, Guven A. 2014. Effects of salinity on antioxidant enzymes and proline in leaves of barley seedlings in different growth stages. *Bulg J Agric Sci* 20: 883-887
- Weisany W, Sohrabi Y, Heidari Gh, Siosemardeh A, Ghassemi-Golezani K. 2012. Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). *Plant Omic J* 5(2): 60-67.