Effect of Different NaCl Salinity on Antioxidant Enzyme Activity and Relative Water in Winter Canola (Brassica napus)

SEDGHALI ZAMANI1, MOHAMMAD TAHER NEZAMI2, AHMAD BYBORDI1, MARYAM BEHDAD3, MOHAMMAD BAGHER KHORSHIDI1
1-East Azarbaijan Agricultural and Natural Resources Research Center, East Azarbaijan, Iran
2-Karaj Branch, Islamic Azad University, Karaj, Iran
3-Department of Horticulture, Khorasgan (Isfahan) Branch, Islamic Azad University, Isfahan, Iran

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*Corresponding author: Email: sedgali@al-yahoo.com

ABSTRACT
To evaluate the effect of different levels of salinity on antioxidant enzymes activity of leaves and plant physiological characteristics of canola as a marker of resistance to salinity, an experiment was conducted in greenhouse of Research Center of Agriculture and Natural resources in East Azarbaijan in 2009. Treatments included combinations of five different levels of salinity (0, 50, 100, 150, and 200 mM) and four cultivars of brassica included (Elite, Licord, SLM046 and Okapi). With increasing salinity, stomatal conductivity, transpiration and leaf relative water decreased in all cultivars. Effect of salt stress on antioxidant enzymes of leaves was significant, and changes in the amount of super oxided dismutase enzymes, catalase and glutathion reductase was observed. Enzyme activity in the period of growth increased and in plant maturity decreased. Comparison between canola cultivars showed the strong correlation between leaf relative water and enzyme super oxide dismutase activity. SLM046 showed the least changes in leaf relative water level and the highest enzyme activity and was resistant cultivar and Elite were the most sensitive cultivar to salinity.

Keywords: Antioxidant enzymes, Salinity, Relative water, Canola

INTRODUCTION
Ghasemi et al. (2002) reported that more than 400 million hectares worldwide affected by the influence of either salt or sodium which was about 6% of the world's lands. From 230 million ha of irrigated land area in the world, 45 million hectares (19.5%) and from 1,500 million ha of rain fed lands under cultivation, 32 million ha (2.1%) affected with different degrees of salinity. From 15 million ha of cultivated lands in Iran, 6 million ha is irrigated land (30%) equivalent to 1.7 million ha affected by salinity. This showed importance of salinity as a serious factor in most parts of the country. Oilseeds are the second world food stocks. Additionally, these seeds have a rich reserve of protein and fatty acids (Sairam and Srivastava, 2001).

Ehsanfar et al. (2006) reported that in poor and saline soils which other plants cannot have good growth, Canola grew well and produced seed. Bahizire and Francois (2007) reported that canola production levels with the variability amount of salt because of the effect of salinity, soil, mineral elements and type on the establishment of canola seedlings is different. According to Corwin et al. (1996), salinity is a worldwide problem in all regions especially in irrigated lands of
arid and semiarid regions. Salinity limits soil fertility in irrigated regions of the world. Due to low rainfall in these areas, soil leaching does not occur. Therefore, crops encountered with salinity problems. Bohnet (1979) believes that salinity increased concentration of sodium anions and other toxic as $\text{HCO}_3^-$, $\text{CO}_3^{2-}$ and $\text{SO}_4^{2-}$ in the soil. For obtained optimal performance under salt stress, resistant plant is required. On the other hand, high diversity in plant species, each with different hereditary traits and specific mechanisms to maintain survival, it seems that identification, modification and selection of salinity resistant species can be useful (Karimi, 1996). Generally, plants understand a wide range of environmental stresses that ultimately cause oxidative stress in plants. Mechanism of resistance in some internal tensions was a result of a plant communication and coordinate complex. In Stress condition, lack of balance between absorb the energy and consumption process caused active oxygen (ROS: Reactive Oxygen Species) production by photosynthetic organs and its inability to inhibit will eventually lead to tension in the cell membrane and symptoms caused by oxidative damage (Blokhina and Virolainen, 2003). Increasing in active oxygen radicals in plants causes reducing of toxic effects of oxidative stresses induced by salt stress, and activated a variety of mechanisms in plant. In these conditions, increased levels of Antioxidants and ROS inhibitor enzymes reduced toxic effects of oxidative stress. Sensitivity of enzymes extracted from cultivars exposed to NaCl salinity is similar to enzymes of salinity sensitive cultivars (Kafi et al., 2003). Antioxidant enzymes are the fastest units checking against attack of active oxygen (Dirk and Montago, 2002).

Physiological characteristics of plants, including changes in stomata closing pattern growth regulators and accumulation of metabolites are important illustration of compatibility to stress conditions (Khavari, 1996; Singh et al., 2004). Therefore, salinity effects studying by help of enzymes, can identify resistant rootstocks more quickly, because there is a strong correlation between tolerance to environmental stresses and changes in the concentration of antioxidant enzymes in photosynthetic plants and provided any substance in favor of synthesis in cells subject by genes, responsible genes can identify for synthesis of this material and its transfer to other plants to produce salinity resistant rootstocks. For optimal performance in salt stress conditions, an appropriate resistant plant is needed. Therefore, to achieve new methods and applications to increase production per area in dry and semi dry climates, for using salt water is necessary (Karimi, 1996; Weiss, 2000).

The main objectives of this study was investigate the response of canola cultivars to saline stress by assessment their antioxidant enzymes in vegetative growth stage to determine the most tolerant canola cultivars.

**MATERIALS AND METHODS**

This study was conducted in greenhouse of Agriculture and Natural Resources Research Center of East Azarbaijan as a factorial experiment based on randomized complete blocks design with three replications in 2009. Five salinity levels combination (0, 50, 100, 150, and 200 mM) and four cultivars of canola (Elite, Licord, SLM046 and Okapi) were arranged as main and subplot, respectively. Disinfectant after sodium hypochlorite with 10 percent 3 to 5 times with distilled water and were washed into plastic pots (30 cm diameter mouth and 35 cm height) containing a mixture of perlite and vermiculite 1:1 ratio to the number five seeds in each pot depth of 1 – 1.5 cm were planted. To prevent salt accumulation in pots, two number of 1 cm
diameter holes were placed at the bottom of the pots as drainage.

During the growth period pots placed among 50 cm in greenhouse with daily temperature 25 ± 3 °C, and 18 ± 3 °C night in normal light and after establishment of seedlings in complete medium, two plants per pot maintained and the rest were removed. Salinity with Hoagland nutrient solution gradually imposed through irrigation in the four-leaf stage. Sampling for Determine antioxidants, at three vegetative growth stages, flowering, early maturity and pod bulking, one third upper leaves were done. Dyndsa method was used to enzyme extracts preparation for determination of antioxidant enzymes activity (Sairam et al., 2002). For determining the amount of SOD, Sairam et al. (2001) method was used. For determination of catalase enzyme in extracts, Chns and Mhly (Sairam el at., 2002) method was used. For determine the amount of glutathione reductase, Smith and colleagues (Arora et al., 2002) method was used. To evaluate physiological traits responses to salinity stress, every 10 days interval, the value of stomatal conductivity and the youngest developed leaf transpiration rate during plant growth was measured with LCI Portable Photosynthesis System device.

For determination of leaf relative water in pod filling phase, the youngest developed leaf sampling from the upper third was used (Sairam and Srivastava, 2001).

Analysis of variance was done by MINITAB software and means compared with Duncan multi-range test at 5 percent probability level with software MSTATC and plotting figures were done by EXCEL.

RESULTS AND DISCUSSION
Super oxide dismutase

Super oxide dismutase enzyme activity showed significant difference among cultivars so SLM046 showed highest and Elite showed minimum SOD enzyme activity (Figure 1). Increasing plant age from vegetative stage to maturity stage decreased SOD activity in SLM046 and Licord. But the amount of this enzyme activity in Elite in flowering stage was higher than vegetative stage. While, the highest enzyme amount during the growth period belonged to SLM046. With increasing salt in nutrient solution from 150 mM to 200 mM NaCl, the amount of enzyme per leaf weight unit decreased (Figure 2). This could be due to destruction of SOD producer structures when plants exposed to NaCl. Due to local reduction of Na+ in the leaves, reduced enzyme activity in resistant cultivars than susceptible cultivars was seen (Vaidyanathan et al., 2003). With increasing salinity, the plant antioxidant system activated and increasing enzyme super oxide dismutase activity is a first defense barrier against attack of oxygen radicals (Mirmohammadi Meybod et al., 2002) and until plant can inhibit produced superoxide, this process continues.

The results of researchers on other plants also showed that the SOD activity in resistant cultivars is higher than susceptible cultivars to salinity.

The amount of enzyme increased with increasing salinity and plant age (Reddy and Srivastava, 2003). Results of study on catalase showed that the highest amount of enzyme belonged to Elite, where there was no significant difference between SLM046 and Licord (Figure 3).
Figure 1. Average rate of superoxide dismutase activity in canola cultivars

Figure 2. Comparison the effect of salinity on SOD activity in the three growing stage S1, S2, S3, S4 and S5, respectively salt 0, 50, 100, 150, and 200 mM

Figure 3. Comparison of the mean catalase activity in different levels of salinity

Figure 4. Changes in catalase activity of different canola cultivars
Studies of Kafi et al. (2003) showed that increasing salinity significantly decreased catalase enzyme activity. Comparison of catalase activity showed the highest catalase activity in plant was at flowering stage and minimum activity in the vegetative growth stage. Meanwhile, the maximum catalase activity in different growth stages of canola plant was different. Results of Sairam and Srivastava (2001) also showed that with increasing plant age up to stage 50% of pollination, the amount of catalase increased, the amount of catalase in the salinity of 200 mM significantly reduced (Figure 5). Catalase converts hydrogen peroxide enzyme to water and molecular oxygen and as a result of salt stress, the rate of catalase decreases in leaves and roots (Gramer 2002). Similarly, comparison of enzyme activity at different levels of salinity showed increasing levels of salinity from 0 mM to 100 mM declined the catalase activity. But, with increasing salinity to 150 mM increased the enzyme activity was observed again (Figure 4).

**Glutathione reductase**

The lowest activity of Glutathione reductase (GR) was observed in SLM046 but Okapi and Licord showed highest enzyme activity (Figure 6). The results showed that the lowest GR enzyme activity related to the salinity level was 100 mM (Figure 7). The minimum GR activity was observed in SLM046 at pod maturity stage, because it can coordinate activities of catalase and glutathione reductase and hydrogen peroxide to inhibit the activity of SOD. In all studied enzymes, the level of enzyme activity increased with plant age (Figure 8).

![Figure 5. Effect of salt stress on catalase activity balance the developmental period S1, S2, S3, S4 and S5, respectively salt 0, 50, 100, 150, 200 mM](./images/figure5.png)

![Figure 6. Comparison of reductase glutathione activity in different salinity levels](./images/figure6.png)

![Figure 7. The average activity of glutathione reductase canola cultivars](./images/figure7.png)
Relative water content and transpiration rate decreased in all cultivars as a result of increasing salt (Figs 9 and 10). The results of other researchers also confirmed this (Mahmoud et al., 2003). With salinity stress, leaf relative water content decreased with increasing sodium chloride in Elite, Okapi, SLM046 and Licord as 20.06, 20.41, 20.58 and 20.43 %, respectively. Other studies also have shown that resistant wheat to cultivars, resistant relative leaf, less than half of resistant cultivars and plant age difference between resistant cultivars and half are less resistant, high salinity are not significantly difference (Sairam et al., 2001).

Transpiration rate with transition from 0 mM to 150 mM, decreased 65.49 %, and further increase in salinity caused no significant changes in transpiration rate. Transpiration differences rate among cultivars was no significant, but in all varieties with increasing growth period, after 25 days of implantation, a significant change in transpiration rate was showed (Figure 11).
Figure 11. Changes in transpiration rate with increasing plant age in different cultivars of canola

Figure 12. Interaction between salinity and the type of canola on the rate of stomatal conductance S1, S2, S3, S4 and S5, respectively salt 0, 50, 100, 150, 200 mM

**Stomata conductivity**

Stomata conductivity affected environmental effects such as light intensity, concentration of CO$_2$, and soil environment moisture and genetic factors influence intensity of photosynthesis and growth via stomata conductivity (Mashuf et al., 2003). In this experiment, with increasing salinity, plants react quickly and to reduce the effects of secondary salinity, attempt to close the stomata and reduce withdrawal of water from plant as transpiration. Therefore, in all four varieties stomatal conductance reduction at 50 mM compared to control was observed and only Licord showed that increasing salinity level to 150 mM has no specific reaction by stomata (Figure 12).

Regression relationship between SOD activity and leaf relative water content among different cultivars was significant and negative, relationship revealed that SLM046 with highest super oxide dismutase enzyme activity and the lowest leaf relative water content was resistant and Elite with the lowest enzyme activity and the highest rate of leaf water content was salt sensitive cultivars (Figure 13). The results showed that, because the SOD enzyme as the first barrier of defense against active oxygens attack (Mirmohammadi Meybod el at., 2002), salt stress increased cell ROS production and SOD activity until 100 mM NaCl.
Therefore, produced SOD volume in this level of salinity, can inhibit oxidative agents. Simultaneous with SOD activity and followed production of H₂O₂, glutathione reductase also increased its activity until 150 mM to inhibit produced hydrogen peroxide in environment. With increasing salinity level to 150 mM two cases arise: First, parallel to the amount of SOD activity, active catalase and glutathione reductase unable to reduce H₂O₂ level in medium to reduce partially the activity of SOD in 150 mM. The highest amount of SOD enzyme activity and the lowest leaf content was observed in SLM046, so it seems that SLM046 more resistant to salinity among cultivars could be as compatible plant with desired performance in saline land and recommended for field studies.

![Graph](image.png)

Figure 13. Regression between RWC and SOD enzyme activity between different rapeseed cultivars

REFERENCES


