International Journal of Farming and Allied Sciences
Available online at www.ijfas.com
©2014 IJFAS Journal-2014-3-7/767-771/ 31 July, 2014
ISSN 2322-4134 ©2014 IJFAS

Evaluation of antioxidant enzymes activity in canola under salt stress

Jafar Abili[1] and Sajjad Zare[2*]

1. Islamic Azad University, Abhar Branch, Abhar, Iran
2. Agricultural Biotechnology Research Institute of Iran, PO Box 31535-1897, Karaj, Iran

Corresponding author: Sajjad Zare

ABSTRACT: Salinity is one of the major stresses in arid and semi-arid regions causing adverse effects at physiological, biochemical, and molecular levels, limiting crop productivity. In this research, three canola cultivars (Licord, Talayeh, Zarfam) were compared at 5 salinity levels (control, 50, 100, 150 and 200 mM) for their catalase, guaiacol peroxidase, superoxide dismutase activity, proline and yield in a completely randomized design with 3 replications. In our study, we found that NaCl concentrations greater than 150 and 200 mM caused the irreversible disorders. Increased salt concentrations led to significant changes in the levels of antioxidative enzymes and proline in three canola cultivars. Also, yield rates in three varieties decreased in the presence of NaCl concentrations.

***Keywords*:** Catalase, Peroxidase, Stress, superoxide dismutase

INTRODUCTION

Salinity is one of the major stresses in arid and semi-arid regions causing adverse effects at physiological, biochemical, and molecular levels, limiting crop productivity, also Salinity and drought are most important problems in Iran's agriculture. Salinity induces water deficit even in well watered soils by decreasing the osmotic potential of soil solutes, thus making it difficult for roots to extract water from their surrounding media (Sairam, 2002). Although, Salinity contributes to formation of reactive oxygen species (ROS), superoxide radical (O2-), hydrogen peroxide (H2O2) and hydroxyl radical (OH-) but plants increase the activity of antioxidant enzymes for decreasing of salinity effects (Moller, 2007). Previously, this fact reported by some researcher such as Zare and Pakniyat (2012), Ashraf and Ali (2007). The capability of scavenging ROS and reducing their damaging effects may correlate with the salinity tolerance of plants (Guo, 2006), therefore, At this article, we studied yield and antioxidant enzymes activity of three canola cultivars to salinity stress.

MATERIALS AND METHODS

Three canola cultivars (Licord, Talayeh, Zarfam) were compared at 5 salinity levels (control, 50, 100, 150 and 200 mM) for their catalase, guaiacol peroxidase, superoxide dismutase activity and proline in a completely randomized design with 3 replications. Before experimentation, all the seed samples were surface sterilized with 10% sodium hypochlorite solution for 5 min and washed three times with sterilized distilled water. Salt was applied to appropriate pots in split and in 5 stages within 5 weeks to final concentrations by irrigation based on soil field capacity, after 7 days of final salt treatment leave samples were collected and frozen in liquid nitrogen immediately and stored at –20°C before analysis. Yield per pots were determined at end of experiment.The protein content was estimated according to Bradford (1976), using bovine serum albumin as a standard. Superoxide dismutase activity, the basis of which is its ability to inhibit the phothochemical reduction of nitroblue tetrazolium (NBT) (Beauchamp and Fridovich, 1971), was determined according to the method of Dhindsa (1980). For SOD assy, the reaction

mixture contained 50mM K-phosphate buffer (pH 7.8), 13mM methionine, 75µM NBT, 0.1µM EDTA, 4µM riboflavin and required amount of enzyme extract. The reaction was started by adding riboflavin and placing the tubes under two 15 W fluorescent lamps for 15 min. A complete reaction mixture without enzyme, which gave the maximal colour, served as control. A non-irradiated complete reaction mixture served as a blank. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm, which was measured according to the method of Giannopolitis and Ries (1977). Proxidase activity was assayed adopting the method of Polle et al. (1994). According to this method POD activity was determined at 436 nm by its ability to convert guaiacol to tetraguaiacol (ε = 26.6mM−1 cm−1) The reaction mixture contained 100mM K-phosphate buffer (pH 7.0), 20.1mM guaiacol, 10mM H2O2 and enzyme extract. The increase in absorbance was recorded by the addition of H2O2 at 436 nm for 5 min. CAT activity was determined by monitoring the disappearance of H2O2 at 240 nm (ε = 40mM−1 cm−1) according to the method of Aebi (1984). The reaction mixture contained 50mM K-phosphate buffer (pH 7.0), 33mM H_2O_2 and enzyme extract.

RESULTS AND DISCUSSION

Catalase Activity

according to the results, catalase activity decreased with increasing in salinity stress, these were observed in Licord, Talayeh and Zarfam under 200 in comparison with the control (43, 40 and 22%, respectively)(Fig 1). The decline in CAT activity is regarded as a general response to many stresses (Herbinger, 2002; Bakalova et al. 2004; Jung 2004; Guo et al. 2006; Pan et al. 2006; Gunes et al. 2008; Liu et al. 2008; Abedi and Pakniyat 2010; Zare and Pkniyat 2012). The reduction of CAT activity is supposedly due to the inhibition of enzyme synthesis or change in the assembly of enzyme subunits under stress conditions. It may also be associated with degradation caused by induced peroxisomal proteases or may be due to the photo-inactivation of the enzyme (Abedi and Pakniyat 2010; Zare and Pkniyat 2012). In normal condition, Licord had the highest catalas activity but this decrease was slowly in Zarfam cultivar.

Peroxidase Activity: A significant increase ($P < 0.01$) was observed in proxidase activity under stress especially 200 mM for all three cultivars Licord, Talayeh and Zarfam (10, 8.1 and 9.23%, respectively) when compared to the control (Fig 2). The increase in expression of guaiacol peroxidase could be part of the oilseed rape's response to the oxidative damage caused by the increasing levels of salt stress (Zare and Pkniyat 2012). Some previous studies, as parallel with our results, mentioned the increased POD activity under salinity stress conditions in other plants such as pea (Hernandez, 2000) rice (Lee, 2001) wheat (Sairam, 2002).

Superoxide Dismutase Activity: Highest activity was observed at 100mM treatment but activity of this enzyme reduced by 150 and 200 mM treatments (Fig 3). This may be related to the low potential of canola plants to remove O_2^- under high concentration of salt. Cultivars were similar regarding their superoxidase activity at all salt levels. According to this fact that SOD processing is known to be substrateinducible (Tsang, 1991), an increase in the SOD activity may be attributed to the increased production of active oxygen species as substrate that lead to increased expression of genes encoding SOD (Abedi and Pakniyat 2010).

Proline Content: At all NaCl concentrations, Proline content recorded in three varieties increased significantly compared to control. For plants grown in the presence of 200 mM NaCl, the proline content in Licord, Talayeh, Zarfam increased 1.52, 1.55 and 1.61 times compared to control plants, respectively (Fig 4). Proline is known to accumulate under drought and saline conditions (Misra and Gupta, 2005; Jaleel et al., 2007; Yang and Lan, 2009).

Yield:

According to results, yield rates in three varieties decreased in the presence of NaCl concentrations. In the presence of 200 mM NaCl, the yield in Licord, Talayeh, Zarfam decreased 85, 89 and 91% compared to control plants, respectively, (Fig 5). As a result, the induction of antioxidant enzyme activities is a general adaptation strategy which plants use to overcome oxidative stresses (Foyer & Noctor 2003). In our study, we found that NaCl concentrations greater than 150 and 200 mM caused the irreversible disorders. In this study, increased salt concentrations led to significant changes in the levels of antioxidative enzymes in three canola cultivars. Peroxidase and superoxidase increased by salinity stress but catalas activity decreased. Similar responses have been observed in B. maritima and B. vulgaris (Bor, 2003), cotton (Meloni , 2003), maize (Neto, 2006), and cabbage (Posmyk, 2009). We found that the 200mM treatment had a higher proline concentration, a positive correlation between abiotic stress tolerance and free proline accumulation has been reported (Martinez, 2003) Also, our result demonstrated that Licord, Talayeh, Zarfam had similar response patterns to salinity.

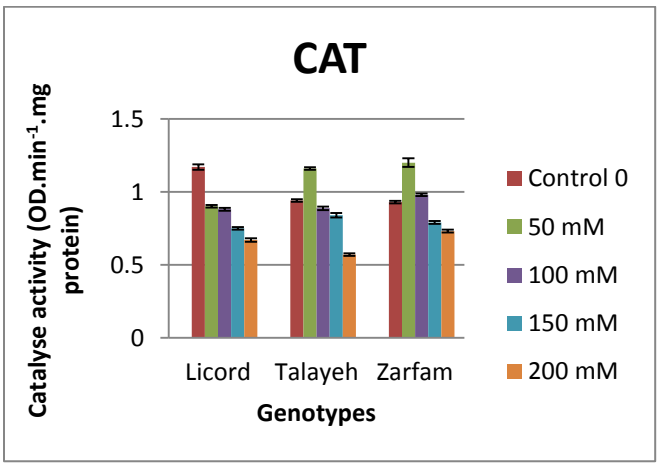

Figure 1. Effect of NaCl treatments on CAT activity in leaves of three cultivars of Brassica napus (Means ±SE)

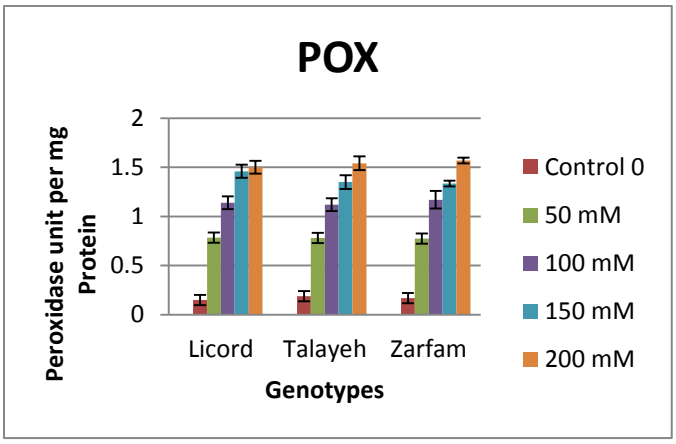

Figure 2. Effect of NaCl treatments on Peroxidase activity in leaves of three cultivars of Brassica napus (Means ±SE)

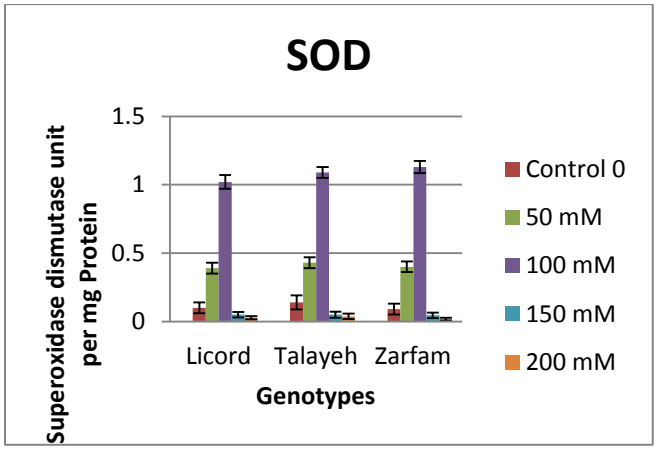

Figure 3. Effect of NaCl treatments on Superoxide dismutase activity in leaves of three cultivars of Brassica napus (Means ±SE)

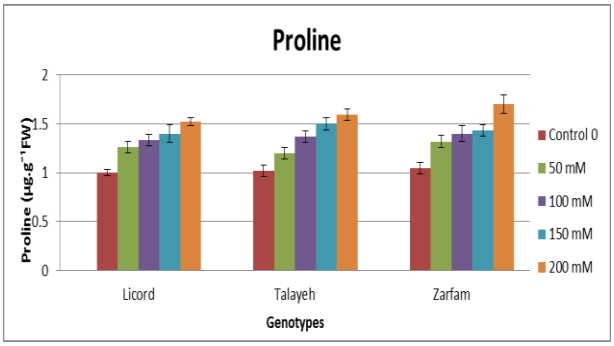

Figure 4. Effect of NaCl treatments on Proline content in leaves of three cultivars of Brassica napus (Means ±SE)

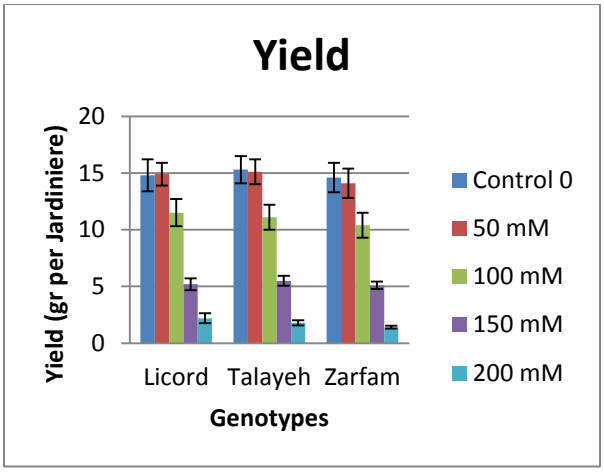

Figure 5. Effect of NaCl treatments on Yield of three cultivars of Brassica napus (Means ±SE)

REFERENCES

Abedi T and Pakniyat H. 2010. Antioxidant Enzyme Changes in Response to Drought Stress in Ten Cultivars of Oilseed Rape (Brassica napus L.). Czech J. Genet. Plant Breed., 46, 2010 (1): 27–34

Ashraf M and Ali Q. 2008. Relative membrane permeability and activities of some antioxidant enzymes as the key determinants of salt tolerance in canola (Brassica napus L.). Environ. Exp. Bot., 63: 266-273.

Bakalova S, Nikolova A and Wedera D. 2004. Isoenzyme profiles of peroxidase catalase and superoxide dismutase as affected by dehydration stress and ABA during germination of wheat seeds. Journal of Plant Physiology, 30: 64–77.

Bor M, Özdemir F and Türkan I. 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet Beta vulgaris L. and wild beet Beta maritima L. Plant Soil 164: 77- 84,

Foyer C and Noctor G. 2003. Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiologia Plantarum, 119: 355–364.

Gunes A, Pilbeam D, Inal A and Coban S. 2008. Influence of silicon on sunflower cultivars under drought stress, I: Growth, antioxidant mechanisms and lipid peroxidation. Commun. Soil Science & Plant Nutrition, 39: 1885–1903.

Guo Z, Ou W, Lu S and Zhong Q. 2006. Differential responses of antioxidative system to chilling and drought in four rice cultivars differing in sensitivity. Plant Physiology and Biochemistry, 44: 828–836.

Herbinger K, Tausz M, Wonisch A, Soja G, Sorger A and Grill D. 2002. Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. Plant Physiology and Biochemistry, 40: 691–696.

Hernandez JA, Jimenez A, Mullineaux P and Sevilla F. 2000. Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defences. Plant Cell Environ., 23: 853-862.

Jaleel CA, Gopi R and Sankar B. 2007. Studies on germination, seedling vigour, lipid peroxidation, and proline metabolism in Catharanthus roseus seedlings under salt stress. S Afr J Bot 73: 190-195,

Jung S. 2004. Variation in antioxidant metabolism of young and mature leaves of Arabidopsis thaliana subjected to drought. Plant Science, 166: 459–466.

Lee DH, Kim YS and Lee CB. 2001. The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). J. Plant Physiol., 158: 737-745

Liu J, Xie X, Du J, Sun J and Bai X. 2008. Effects of simultaneous drought and heat stress on Kentucky bluegrass. Journal of Horticultural Science, 115: 190–195.

Martinez CA, Maestri M and Lani EG. 2003. In vitro salt tolerance and proline accumulation in Andean potato (*Solanum* spp.) diff ering in frost resistance. Plant Sci 116: 117-184,

Meloni DA, Oliva MA and Martinez CA. 2003. Photosynthesis and activity of superoxide dismutase peroxidase and glutathione reductase in cotton under salt stress. Environ Exp Bot 49: 69- 76,.

Misra N and Gupta AK. 2005. Effect of salt stress on proline metabolism in two high yielding genotypes of green gram. Plant Sci 169: 331-339.
Moller IM, Jensen PE and Hansson A. 2007. Oxidative modifications to cellular components in plants. Ann. Rev. Plant Biol., 58: 459-481.
Neto ADA, Prisco JT and Enéas-Filho J. 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environ Exp Bot 56: 87-94,
Pan Y, Wu LJ and Yu ZL. 2006. Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of liquorice (Glycorhiza uralensis Fisch). Journal of Plant Growth Regulation, 49: 157–165.
Posmyk MM, Kontek R and Janas KM. 2009. Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. Ecotox Environ Safe 72: 596-602,
Sairam RK, Rao KV and Srivastava GC. 2002. Differential response of wheat genotypes to long-term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci., 163: 1037-1046.
Tsang EWT, Bowler C, Herouart D, Van CW, Villarroel R, Genetello C, Van MM and Inze D. 1991. Differential regulation of superoxide dismutase in plants exposed to environmental stress. Plant Cell, 3: 783–792.
Yang SL and Lan SS. 2009. Gong M. Hydrogen peroxide-induced proline and metabolic pathway of its accumulation in maize seedlings. J Plant Physiol 166: 1694-1699.
Zare S and Pakniyat H. 2012. Changes in activities of antioxidant enzymes in oilseed rape in response to salinity stress. International Journal of Agriculture and Crop Sciences. 4-7/398-403