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Modulation of Enzymatic Antioxidants Improves the Salinity Resistance in Canola (*Brassica napus*)

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ABSTRACT

Soil salinity is one of the major obstacles hindering profitable crop production worldwide. This study was conducted to appraise the effects of salinity on germination, early seedling growth, electrolyte leakage and antioxidant activity of different canola (*Brassica napus* L.) cultivars. Three canola cultivars viz. Fornex, Alice and Modena were sown under four (0, 40, 80 & 120 mM NaCl) salinity levels. Substantial decrease in germination percentage, root length, shoots length and seedling dry weight of all canola cultivars was observed at all salinity levels in general but higher salinity level (120 mM NaCl) seemed more detrimental. Canola cultivar Modena had higher germination percentage, root length, shoot length and seedling dry weight than other canola cultivars in saline environment. Moreover, at all levels of salinity seedling electrolyte leakage, catalase (CAT) and peroxidase (POX) activities were increased in canola cultivars. However, higher activities of CAT and POX accompanied with minimum electrolyte leakage were observed in canola cultivar Modena at higher salinity levels. In conclusion, salinity stress severely affected the germination and early seedling growth of canola. Canola cultivar Modena was the most salt tolerant due to maintenance of higher germination and seedling growth under saline conditions with higher CAT and POX activities and better membrane stability.

Key Words: Salinity stress; Canola cultivars; Catalase activity; Seed germination; Root length

INTRODUCTION

Salinity, drought, nutrient imbalances and temperature extremes are among the chief abiotic stresses impairing crop productivity worldwide (Hamida & Shaddad, 2010; Farooq *et al.*, 2009, 2011). Although, salinization occurs mostly in arid- and semi-arid regions of the world but in reality no climatic zone is free from salinity (Bhutta *et al.,* 2004; Rengasamy, 2006). Salinity hampers the crop growth by creating low osmotic potential of soil solution (water stress), nutritional imbalance, specific ion effect (salt stress) and or combination of these factors (Ashraf, 1994; Marschner, 1995). Decrease in osmotic potential is linked with the accumulation of ions in soil solution, whereas nutritional imbalance and specific ion effect is linked with the higher accumulation of ions mainly Na^+ and Cl^- at toxic levels which leads to lessen the absorption availability of other essential elements like potassium and calcium etc. (El-Bassiouny & Bekheta, 2001).

Seed germination is the most critical stage in seedling establishment and determining successful crop production. High soil salinity substantially decreases seed germination and seedling growth. Salinity mainly causes hyper-osmotic stress and hyper ionic toxic effects, which leads to germination inhibition (Hasegawa *et al*., 2000). Impaired germination, early seedling establishment and seedling growth under salinity stress is earlier reported in canola (Farhoudi *et al*., 2007). Okçu *et al*. (2005) reported that reduction in germination percentage and early seedling growth of pea under salinity was attributed to osmotic stress that prevents water uptake or due to the toxic effects of Na^+ and Cl^- ions on the germinating seeds. Like other abiotic stresses, salinity also results in over production of reactive oxygen species (ROS) (Li, 2008; Gill & Tuteja, 2010). Increase in ROS production can seriously disrupt cellular homeostasis and normal metabolisms through oxidative damage to lipids, proteins and nucleic acids (Charles *et al*., 2007). Plants possess antioxidant enzymes as well as antioxidant compounds to scavenge these ROS, and antioxidant capacity of plants is directly related to their salt tolerance. Ashraf and Ali (2008) suggested that activities of antioxidant enzymes such as catalase (CAT) and peroxidase (POX) proved very effective in discriminating the canola cultivars for salt tolerance. Similarly, enhanced activities of CAT and POX enzymes under salt stress have been reported in salt-tolerant cultivars of wheat (Sairam *et al*., 2005) and millet (Sreenivasulu *et al*., 2000). Cell membrane stability has long been used an indicator of stress tolerance. Farooq

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and Azam (2006) and Munns and James (2003) suggested that the estimation of cell membrane stability is an apposite technique to screen plants for salt tolerance. Meloni *et al*. (2003) reported that salt stress damaged cell membrane and increased cellular membrane leakage in salt-sensitive rice cultivar.

Canola (*Brassica napus* L.) is moderately salt tolerant and has considerable potential to grow in salt-affected areas. Enhanced activities of some antioxidant enzymes such as SOD, CAT, and POX are key determinants of salt tolerance in different crops (Sreenivasulu *et al*., 2000). It was hypothesized that canola cultivars differs for salinity tolerance potential. This study was conducted to appraise the performance of different canola cultivars under salinity by evaluating the germination, early seedling growth, antioxidant activity and cell membrane leakage.

MATERIALS AND METHODS

Experimental details: This study was conducted under controlled conditions in the laboratory of Department of Agronomy, Faculty of Agriculture, Islamic Azad University, Shoushtar Branch, Iran. Three canola (*Brassica napus* L.) cultivars viz. Fornex, Alice and Modena were sown under four (0, 40, 80 & 120 mM NaCl) salinity levels. The experiment was laid according to completely randomized design (CRD) with factorial arrangements and replicated four times. The seeds of all three canola cultivars were obtained from the Oil Plant Research Institute, Karaj, Iran.

Seeds were surface sterilized with 0.1% $HgCl_2$ solution for 5 minutes and then thoroughly washed with water for 5 min. Canola seeds were sown in Petri dishes (15×100 mm) on top of one sheet of moistened filter paper. Petri dishes were put in growth rooms with 12 h photoperiod (1000 µ $molm^{-2}$ s^{-1}) and 12 h dark period and day/night relative humidity and temperature were maintained as 60/75% and 24/17°C, respectively. A seed was considered germinated when the emerging radicle elongated to 3 mm. Seed germination was recorded every 24 h till constant count. The experiment was terminated 12 days after sowing and root length, shoot length and seedling dry weights were measured from ten random selected seedlings from each replicate. Final germination was computed by dividing total germinated seeds to total sown seeds and expressed in percentage.

Estimation of electrolyte leakage: Electrolyte leakage was measured as described by Valentovic *et al*. (2006). 0.3 g of fresh seedling epicotyls washed with deionized water, placed in tubes with 15 mL of deionized water and incubated for 2 h at 25°C. Electrical conductivity was then determined (EL_1). Samples were autoclaved at 120°C for 20 min and the final electrical conductivity was measured following equilibration at 25°C (EL_2). The electrolyte leakage was computed as a ratio of EL_1 and EL_2 and was expressed in percentage (Valentovic *et al*., 2006).

Estimation of catalase (CAT) and peroxidase (POX) activity: CAT (EC:1.11.6) and POX (EC:1.11.1.13) were extracted by homogenizing frozen fresh seedling material in ice-cold solution containing 100 mM Tris (pH 7.0), 10 mM d-isoascorbic acid, 20 g L^{-1} PVP-10, 1.5 g insoluble PVP, 0.1 mM EDTA and 2 mL L^{-1} Triton X-100. CAT activity was determined following Chance (1995) 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. POX activity was determined following the protocol of Chance (1995) using guaicol as a reactant. POX activity was measured by monitoring the H_2O_2-dependent oxidation of reduced 2, 3, 6-trichloroindophenol at 675 nm using a UV–VIS spectrophotometer (Model U-2001, Hitachi, Tokyo, Japan).

Statistical analysis: The collected data was analyzed by using the Fisher's analysis of variance technique under completely randomized design (CRD) and the treatments means were compared by Duncan test at 0.01 probability level (Steel *et al*., 1997).

RESULTS

Salt stress caused substantial reduction in final germination percentage (FGP) in all canola cultivars at all levels but higher salinity level (120 mM NaCl) was found more damaging; and different canola cultivars behaved differently (Table I). More FGP was observed in Modena than other cultivars at each salinity level (Table I). Likewise sizeable reduction in root and shoot length, and seedling dry weight of all canola cultivars was observed under salinity stress particularly at 80 and 120 mM NaCl level (Table I); but considerable differences in performance of different cultivars were also noted (Table I). Significantly more root and shoot length, and seedling dry weight was observed in canola cultivar Modena especially at higher salinity levels than both other canola cultivars under study (Table I).

Increasing salinity levels enhanced seedling electrolyte leakage (EL) in all canola cultivars in general but notable differences among cultivars were noted; and significantly lower seedling EL was observed in canola cultivar Modena at all salinity levels than other cultivars used in the study (Table II). With increasing salinity levels, a gradual rise in catalase (CAT) and peroxidase (POX) activities of seedlings of all canola cultivars were observed except at 120 mM NaCl salt stress level, CAT activity was decreased; and canola cultivars behaved differently in this regard (Table II). At elevated salinity levels i.e., 80 and 120 mM NaCl, higher activities of CAT and POX were observed in Modena while lower activities CAT and POX were noted in Fornex cultivar (Table II).

DISCUSSION

Results of the study indicated that salinity induced decrease in seed germination and early seedling growth of

Table I: Effect of salinity on germination and early seedling growth of different canola cultivars

Salinity levels	Final germination percentage (%)			Shoot length (mm)			Root length (mm)			Seedling dry weight (g)		
(mM NaCl)	Modena	Alice	Fornex	Modena	Alice	Fornex	Modena	Alice	Fornex	Modena	Alice	Fornex
0.00	91.00 a	90.00 a	94.00 a	44.50 a	41.00 b	44.10 a	85.10 a	75.60 b	82.00 a	0.27 a	0.27 a	0.26 a
40.00	89.00 a	80.00 b	81.00 b	40.00 b	30.10 d	35.00 c	40.50 d	45.20 c	47.90 c	0.24 ab	0.23 ab	0.24 ab
80.00	72.00 c	50.00 d	61.00 d	31.40 d	28.10 e	27.10 e	35.70 e	31.40 f	27.10 fg	0.22 ab	0.15 b	0.11 c
120.00	61.00 d	39.00 e	25.00 f	22.10 f	22.40 f	20.20 f	28.80 g	10.10 h	15.50 h	0.12 c	0.06 d	0.05 d

Table II: Effect of salinity on peroxidase and catalase activities, and electrolyte leakage of seedlings of different canola cultivars

Salinity levels	Peroxidase activity (mg H_2O_2 g^{-1} pro min^{-1})			Catalase activity (mg H_2O_2 g^{-1} pro min^{-1})			Electrolyte leakage (%)		
(mM NaCl)	Modena	Alice	Fornex	Modena	Alice	Fornex	Modena	Alice	Fornex
0.00	28.10 d	20.10 d	21.00 d	11.00 c	12.00 c	8.20 de	10.00 f	11.00 f	12.80 f
40.00	29.00 d	30.30 c	30.00 c	15.10 b	16.20 b	14.00 bc	20.10 e	36.80 c	27.90 d
80.00	42.10 b	37.20 c	30.70 c	17.70 a	17.20 a	9.40 d	27.10 d	42.40 b	43.10 b
120.00	63.70 a	49.20 ab	30.60 c	8.20 de	10.10 d	6.10 e	34.60 cd	50.20 a	53.00 a

Means not sharing the same letter within a column or row differ significantly from each other at P = 0.01 according to Duncan test

canola (Tables I & II). However, the response of different cultivars was varying and cultivar Modena performed better with maximum germination and seedling growth accompanied with least seedling electrolyte leakage even at higher salinity levels (Tables I & II). Several researchers had quoted impaired germination and earlier seedling growth in several crops (Farooq & Azam, 2006; Farhoudi *et al*., 2007). Minimal water uptake by canola seeds owing to osmotic effect or the toxic effects of Na^+ and Cl^- ions on the germinating seeds might be attributed to decreased germination of canola cultivars under salinity (Khajeh-Hosseini *et al*., 2003). Salinity has a pronounced effect on plasma membrane and lipid peroxidation, thereby affecting permeability of membranes which in turn modulates the pattern of ion leakage (Sairam *et al*., 2002). Therefore, inhibitory effects of salinity on early seedling growth of canola cultivars might be attributed to low osmotic potential of solution, specific ion effect or combination of these factors (Ashraf, 1994; Marschner, 1995) on one hand and elevated membrane damage (Table II) due to salinity induced oxidative stress on other hand. It has been suggested that decrease in membrane stability reflects the extent of lipid peroxidation caused by over production of reactive oxygen species (ROS) (Sairam *et al*., 2002). Farooq and Azam (2006) also reported rise in cell membrane injury under salt stress in different wheat varieties. Plants enhance the productions of antioxidants as a defensive mechanism in order to accommodate the hyper-production of ROS under variety of a-biotic stresses including salinity (Li, 2008; Simova-Stoilova, 2008). Superoxide dismutase (SOD), CAT and POX are the most important antioxidant enzymes in the scavenging system of ROS (Li, 2008). Therefore, notable elevation in the activities of CAT and POX in canola seedlings under salinity stress might be attributed to the defensive mechanism adopted against the salinity induced oxidative stress.

Different canola cultivars differ in their level of salt tolerance due to their different genetic makeup (Ashraf & Ali, 2008). Results of the study clearly elaborated that canola cultivar Modena observed higher seed germination and early seedling growth under higher salinity levels due to its better genetic makeup. Moreover, higher activities of antioxidant enzymes such as CAT and POX (Table II) along with lower membrane electrolyte leakage at higher salinity levels (Table II) enabled canola cultivar Modena to outperform than other cultivars under saline environment. Ashraf and Ali (2008) also concluded that salt tolerant canola cultivars had higher antioxidant enzyme activities than sensitive ones. Therefore, it is suggested that high antioxidant enzyme activity has a significant role in imparting salt tolerance in plants. In this background, the higher CAT and POX activities in the seedling of cultivar Modena under salt stress signify its high tolerance to salinity stress due to better membrane stability (Table II). These results are substantially in agreement with those of Sairam *et al.* (2005) who reported a lower decrease in membrane stability index in tolerant genotypes of wheat than in salt-sensitive ones under salt stress.

In summary, salinity minimized the germination and early seedling growth and perked up membrane electrolyte leakage of all canola cultivars with varying degree especially at higher salinity (80 & 120 mM NaCl) levels. Moreover, better germination and early seedling growth was observed in canola cultivar Modena owing to better membrane stability due to substantial increase in CAT and POX activities under salinity stress.

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