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Effect of salt stress on antioxidant activity and seedling growth of three canola (*Brassica napus* L.) cultivars

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Abstract: This research was carried out in order to assess the effect of salt stress (0 , 40, 80 and 120 mM NaCl solution) on seedling growth and antioxidant enzyme activity of three canola (*Brassica napus*) cultivars 'Consul', 'Zarfam' (a new Iranian cultivar) and 'Okapi'. Salt stress decreased canola cultivar root length, shoot length and seedling dry weight but increased seedling EL and CAT and POD activity. 'Okapi' appears to be salt tolerant since it showed the highest seedling dry weight (0.15 mg), highest CAT (14.2 mgH2O2/g.pro/min) and POD (63.4mgH2O2/g.pro/min) activity and lowest electrolyte leakage (32.8%) under 120 mM NaCl Solution.

Key words: *Salt stress; Canola; Antioxidant activity*

1. Introduction

High salinity in soil or irrigation water is a common environmental problem affecting seed germination and plant growth. Salinity mainly causes hyper-osmotic stress and hyper ionic toxic effects; the consequence can be plant death (Hasegawa et al. 2000). Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and reduced seedling establishment. Salt stress decreases germination percentage, shoot length and root length of canola (Farhoudi et al, 2007). Kaya and Day (2008) reported that salt stress decreased sunflower germination and seedling growth.

The increasing evidences also suggest that high salinity induces oxidative stress which is a key underlying component of most abiotic stresses. Reactive oxygen species (ROS) are generated as by-products of plant cellular metabolism and are also important as signaling molecules (Mittler 2002). The production of ROS in cells increases during abiotic and biotic stresses, as does the level of ROS-induced damage. Elevated production of ROS can seriously disrupt cellular homeostasis and normal metabolisms through oxidative damage to lipids, proteins, and nucleic acids (Charles et al. 2007). Membrane injury induced by salt stress in different plant species is also related to an enhanced production of ROS (Sairam et al., 2005). 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. For example, while determining the role of various antioxidants enzyme in the salt tolerance of tomato, Mittova et al. (2004) found that higher salt tolerance of wild tomato (*Lycopersicon pennelli*) as compared to cultivated tomato was correlated with increased activities of antioxidant enzymes. Ashraf and Ali (2008) activities of antioxidant enzymes (CAT and POX) proved to be 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 seedlings of millet (*Setaria italica*) (Sreenivasasulu et al., 2000).

Cell membrane stability has long been an indicator of stress tolerance. Farooq and Azam (2006) and Munns and James (2003) suggested that the assessment of cell membrane stability is an appropriate technique to screen plants for salt tolerance. Salt stress increased cell membrane damage in rice (*Oryza sativa*) cultivars but this increase was higher in salt-sensitive cultivars (Bhattacharjee and Mukherjee, 2002). Meloni *et al.* (2003) reported that salt stress damaged cell membrane and increased cellular membrane leakage in salt-sensitive rice cultivar.

Brassica oilseed species now hold the third position among the oilseed crops and are an important source of vegetable oil (Ashraf and McNeilly, 2004). The canola has considerable potential to grow in salt-affected areas (Ashraf and Ali, 2008). enhanced activities of some antioxidant enzymes such as SOD, CAT, and POD have been found to be key determinants of salt tolerance in different crops (Sreenivasasulu et al., 2000; Meloni et al,2003; Sairam et al., 2005)This study aims to investigate the effect of salt stress on seedling growth, antioxidant activity and cell membrane leakage of canola cultivars.

2. Material and methods

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The present study was conducted to investigate the influence of salinity on seedling growth, membrane leakage and antioxidant activity in three canola (B. napus L.) cultivars, ('Consul', 'Zarfam' (a new Iranian cultivar) and 'Okapi'). The seeds of three canola cultivars were obtained from the oil plant research Institute, Karaj, Iran. The experiment was carried out under a two-factorial (cultivar × salinity level) completely randomized design with four replications. Sodium chloride was used as a source of salt. Salinity levels were 40, 80 and 120 Mm NaCl solution and distilled water served as the control (0 ds/m).

The experiment was conducted in the plant physiology laboratory of the Islamic Azad University Shoushtar branch, during 2009. Seeds were superficially sterilized with 0.1% HgCl2 solution for 5min., and then thoroughly washed for 5 minutes. Canola seeds were sown at 15×100 mm Petri dishes on top of one sheet of moistened filter 12 h photoperiod (1000μ molm−2 s−1) and 12 h dark, day/night relative humidity 60/75%, and temperature 24/17 ◦C. A seed was considered germinated when the emerging radicle elongated to 3 mm. Seed germination was recorded every 24 h for 12 days. Radicle length, shoot length and seedling dry weights were measured on the 12th day of experiment.

Electrolyte leakage was measured as described by Valentovic et al. (2006). 0.3 g of fresh seedling epicotyls washed with deionized water and placed in tubes with 15 ml of deionized water and incubated for 2 h at 25°C. Electrical conductivity was then determined (EL1). Samples were autoclaved at 120°C for 20 min and the final electrical conductivity was measured following equilibration at 25°C (EL2). The electrolyte leakage was defined as fallowing formula (Valentovic et al. 2006):

EL (%)= 100 x EL1/EL2

Catalase and peroxidase are impartment antioxidant enzyme for scavenge plant cell. CAT (EC:1.11.6) and POD (EC:1.11.1.13) were extracted by homogenizing frozen fresh leaf material in ice-cold solution containing 100mMTris (pH 7.0), 10mMd-isoascorbic acid, 20 g L-1 PVP- 10, 1.5 g insoluble PVP, 0.1mM EDTA and 2mL L-1 Triton X-100.CAT activity was determined following Chanes (1995) by monitoring the disappearance of H2O2 by measuring the decrease in absorbance at 240 nm of a reaction mixture containing 1.9mL H2O, 1.0mL of 5.9mM H2O2 in potassium phosphate buffer (pH 7.0), and 1.0mL extract. POD activity was determined following the protocol of Chanes (1995) using guaicol as a reactant. POD activity was measured by monitoring the H2O2-dependent oxidation of reduced 2,3,6-trichloroindophenol at 675 nm using a UV–vis spectrophotometer (Model U-2001, Hitachi, Tokyo, Japan).

Analysis of variance was employed for statistical analysis of the data using a MSTATC computer package (Cohort Software, USA). The mean values were compared with the Duncan test (Armitage and Berry, 1987). P value was P < 0.01. Graphs were drawn using Excel 2000 software.

3. Results

3.1. Seedling dry weight

Seedling dry weight was significantly influence by salt, cultivar and salt and cultivar interaction (Table 1). Salt stress decreased dry weight of canola cultivars (Table 2). Results indicated under highest salinity level, highest seedling dry weight obtained from Okapi cultivar but seedling dry weight in Zarfam and Consul Cultivar was same and lower compared Okapi cultivar in120 mM NaCl. In 80 mM NaCl Zarfam cultivar seedling dry weight was higher compared Consul Cultivar.

Table 1: Analysis of variance of the traits under study

	Shoot length	Root length	Seedling dry weight	Electrolyte leakage	CAT activity	POD activity
Cultivar	907.6**	4003.6**	0.23**	688.1**	23197**	542.3**
Salt	884.8**	5221.0**	0.12**	812.9**	281.4**	601.0**
Salt*cultivar	544.0**	668.3**	0.114**	446.0**	101.02**	441.4**
Error	41.7	70.12	0.005	32.9	9.08	26.01
Cv(%)	9.1	14.1	13.6	15.0	14.1	10.1

Ns: non-significant, **: Significant at the 0.01 level of probability according to Duncan test

Table 2: Effect of salt stress on seed germination and seedling characteristics of canola cultivars*

Salt level	Shoot length (mm)	Root length(mm)	Seedling dry weight (gr)	Electrolyte leakage (%)
Control	31.0 a	77.5 a	0.40 a	25.0 d
40 mM NaCl	23.4 b	50.2 b	0.35 ab	30.5 c
80 mM NaCl	16.6 c	37.9 c	0.32 b	37.4 b
120mM NaCl	11.2 d	22.4 d	0.17c	43.4 a

Means followed by the same letter(s) are not significantly different at P = 0.01 according to Duncan test

3.2. Shoot and Root length

Canola shoot and root length were significantly influence by salt, cultivar and salt and cultivar interaction (Table1). Results showed salt stress

decreased root and shoot length of canola cultivars. The lowest root and shoot length values obtained at salinity level 120 mM NaCl (Table2). Table 3 shows no significant difference between shoot lengths of canola cultivars at highest salinity level but at 8 mM NaCl Okapi cultivar significantly had highest shoot length compared other cultivars. In all salinity levels Okapi cultivar had higher root length compared to the others (Table3). The highest root length was found with Okapi cultivar at 12 mM NaCl salinity level (Table 3).

3.3. Electrolyte leakage (EL)

Seedling electrolyte leakage (EL) was significantly influence by salt, cultivar and salt and cultivar interaction (Table1). Increasing salinity level increased seedling EL of the canola cultivars (Table2). In all salinity level Okapi cultivar EL was lower compared other cultivars. Highest seedling EL was obtained in Zarfam cultivar followed Consul Cultivar at 120 mM NaCl and Okapi cultivar had lowest EL in this salinity level (32.8%) (Table3).

3.4. CAT and POD activity

CAT and POD activity were significantly influence by salt, cultivar and salt and cultivar interaction (Table1). Increasing salinity level increasied seedling CAT and POD activity of the canola cultivars but under 120 mM NaCl salt stress decresed CAT activity (Figur 1 and Figur2). Results showed fewer than 80 and 120 mM NaCl salinity level highest POD activity found in Okapi. In Consul Cultivar under all salinity level results did not show any significant difference between POD activity. Under highest salinity level CAT activity decreased in all canolas cultivar but this decreased remarkably in Consul cultivar (Table3).

4. Discussion

Our results showed salt stress decrease seedling growth and seedling dry weight but seedling electrolyte leakage and antioxidant enzyme activity increased (Table2). Similar results were reported in several crops (Farhoudi et al, 2007; Faroq and Azam, 2006) indicated that seed germination and seedling growth were reduced in saline condition. Salinity has a pronounced effect on plasma membrane and lipid peroxidation, there by affecting its permeability which in turn modulates the pattern of ion leakage (Sairam et al., 2002). However, stability of biological membranes has been taken as an effective screening tool to assess the salinity stress effects (Munns and James, 2003). For example, Farooq and Azam (2006) reported an increase in cell membrane injury under salt stress in different wheat varieties. It has been suggested that decrease in membrane stability reflects the extent of lipid peroxidation caused by ROS (Sairam et al., 2002). In the present study relatively lower membrane permeability was found in salt-tolerant cultivar Okapi which could be easily related to considerably higher activities of its antioxidant enzymes examined compared because this cultivar had highest shoot and root length and dry weight and lowest electrolyte leakage specially under highest salinity level. Salt-tolerant cultivars generally show higher activity of these antioxidant enzymes as compared to salt-sensitive ones (Sreenivasasulu et al., 2000).

Table 3: Effect of salt stress and cultivar on seed germination and seedling characteristics of canola cultivars

Cultivar	Salt level	Shoot length (mm)	Root length(mm)	Seedling dry weight (gr)	Electrolyte leakage (%)	CAT activity (mgH2O2/g. pro/min)	POD activity (mgH2O2/g. pro/min)
	Control	42.4 a	90.5 a	0.30 a	11.7 f	11.5 c	27.1 d
Okapi	40 mM NaCl	38.1 b	45.2 d	0.27 ab	18.3 e	15.3 bc	29.4 d
	80 mM NaCl	29.0 d	40.1 e	0.25 ab	25.5 d	19.2 a	44.1 b
	120mM NaCl	20.0 f	33.4 g	0.15 c	32.8 cd	14.2 bc	63.4 a
	Control	39.1 b	82.0 b	0.30 a	11.5 f	12.0 c	21.9 d
Zarfam	40 mM NaCl	28.9 d	50.3 c	0.25 ab	34.1 c	17.2 b	33.1 c
	80 mM NaCl	26.0 e	36.5 f	0.18 b	40.1 b	18.1 a	39.5 c
	120mM NaCl	20.8 f	14.2 h	0.09 d	48.1 a	10.1 d	50.0 ab
	Control	42.6 a	87.1 a	0.30 a	10.8 f	9.2 de	22.3 d
Consul	40 mM NaCl	33.1 c	52.6 c	0.25 ab	25.3 d	14.3 bc	33.9 c
	80 mM NaCl	25.7 e	33.4 fg	0.12 c	41.0 b	11.1 d	33.9 c
	120mM NaCl	19.0 f	16.6 h	0.07 d	51.4 a	7.7 e	32.8 c

* Means followed by the same letter(s) are not significantly different at P = 0.01 according to Duncan test

Ashraf and Ali (2008) found canola salt tolerance cultivar had highest antioxidant enzyme activity compared salt sensitive cultivar. This suggests 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 Okapi under salt stress signify its high tolerance to salinity stress. 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 our study, cell membrane leakage and antioxidant activity was found to be an effective determinant of salt tolerance in the set of canola cultivars examined in the present study, because it showed a positive correlation between antioxidants activity and seedling dry weight or shoot and root lenght but negative

correlation obtained between seedling electrolyte leakage and seedling dry weight (Table4).

Table 4: person correlation between canola seedling characteristics under salinity stress

	CAT activity	POD activity	Shoot length	Root length	Seedling dry weight	Electrolyte leakage
CAT activity	1					
POD activity	-0.06 ns	1				
Shoot length	0.55*	0.59*	1			
Root length	0.51*	0.61*	0.26ns	1		
Seedling dry weight	0.81**	0.94**	0.66**	0.56*	1	
Electrolyte leakage	-0.76**	-0.68**	-0.70*	-0.80**	-0.86**	1

Ns: non-significant
*: Significant at the 0.05 level of probability according to Duncan test
**: Significant at the 0.01 level of probability according to Duncan test

In conclusion, once excessive reactive oxygen species in plant were not removed in time, plants would be subjected to seriously oxidative damage. Therefore, enzymatic antioxidant defense system can protect plant cells from injury. POD and CAT are the most important protective enzymes to remove reactive oxygen species. Our findings revealed that the increased electrolyte leakage probably have adverse effects on dry weight and seedling length in canola under salt stress. Cultivar Okapi seems to be a salt tolerant canola cultivar since it showed the highest seedling dry weight, highest antioxidant activity and the lowest electrolyte leakage at 120 mM NaCl, other cultivars are found to be salt susceptible as they had higher electrolyte leakage at the same salinity level. Suggestively, seedling electrolyte leakage and antioxidant activity is a proper technique for seedling screening at high salinity levels.

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