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EFFECTS OF NaCl TREATMENTS ON SEED GERMINATION AND ANTIOXIDANT ACTIVITY OF CANOLA (*BRASSICA NAPUS* L.) CULTIVARS

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Abstract

Effects of salt stress on germination, seedling growth and activity of antioxidant enzymes in leaves of six cultivars of canola (*Brassica napus* L.) were investigated on two F_1 hybrids (Hyola401, Hyola330) and four open pollinated cultivars (Zarfam, Okapi, RGs003 and Sarigol). Seeds were germinated under various levels of salinity (0, 50, 100, 150 and 200 mM NaCl solutions). An increase in NaCl concentrations progressively inhibited seed germination. Hyola401 showed the highest germination percentage at all salinity levels. Seedling growth parameters were affected by salt stress particularly at 150 and 200 mM. Leaf antioxidant activities of superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) were increased by salinity increase up to 150 mM while decreased at 200 mM NaCl concentration. Although constitutive levels of activity of antioxidative enzymes were almost the same among the canola cultivars, Hyola401 induced antioxidant enzyme activities were more efficient when subjected to NaCl treatment. Among the tested cultivars, F_1 hybrid 'Hyola401' could be considered as salt tolerant as possessing higher germination percentage, better seedling growth and antioxidant activities under salinity stress. On the other hand, F_1 hybrid 'Hyola330' performed inferior to said aspects and was the most susceptible cultivar to salinity stress.

Introduction

Oilseed rape, canola (*Brassica napus* L.) is considered as the second most important source of vegetable oil providing 13% of the world´s supply (Raymer 2002). The world´s commerce is largely supplied by two species, namely *Brassica napus* L. and *Brassica rapa* L. Seeds of these species commonly contain 40% or more oil and produce meal with 35 - 40% protein.

Salinity is a major environmental stress affecting over 800 million hectares of land throughout the world which accounts for more than 6% of the world total land area (Munns and Tester 2008; Arzani 2008). Salinity, like other environmental stresses causes an increase of production of reactive oxygen species (ROS) (Rahimizadeh *et al.* 2007). Antioxidant defense systems have been developed in aerobic cells to counteract the deleterious effects of ROS (Ghassemi-Golezani *et al.* 2009). When plants are exposed to environmental stresses oxidative damage may result because the balance between the production of ROS and their detoxification by the antioxidative system is hampered (Hernández *et al.* 1993). Superoxide dismutase (SOD) is the first line of defense against ROS (Alscher *et al.* 2002), that catalyzes the dismutation of superoxide to hydrogen peroxide (H_2O_2) and oxygen (O_2).

The generation of ROS and increased activity of many antioxidant enzymes during salt stress have been reported in crop plants (Sairam *et al.* 2002, Mittova *et al.* 2002, Vaidyanathan *et al.* 2003). In sesame, activity of SOD (CAT), GR and malondialdehyde (MDA) were increased under salt stress conditions (Koca *et al.* 2007). Dai *et al.* (2009) demonstrated that the content of SOD, CAT and POD in *Brassica napus* L. have been increased under stress conditions. Crop species or genotype possessing better germination and seedling growth under salt stress will be more stress

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tolerant at later stage (Ashraf and McNeilly 2004). A positive correlation was reported between salinity tolerance at the seedling and adult plant stages in *Brassica* species (Ashraf 2001, Ashraf and Ali 2008). Evaluation of germination and seedling growth potential in saline conditions presents simple and useful parameters, provided that tolerance at the seedling stage reflects enhanced salinity tolerance at the adult plant level. The objective of present study was to determine the effects of salt stress on germination, seedling growth and activity of antioxidant enzymes in leaves of canola cultivars.

Materials and Methods

Six canola (*Brassica napus*) cultivars comprising two F_1 hybrids (Hyola401, Hyola330) and four open pollinated cultivars (Zarfam, Okapi, RGs003 and Sarigol) were used, which are predominantly grown in Iran.

The seeds were sterilized with 5% sodium hypochloride solution for 10 min and washed thoroughly with deionized water and then allowed to germinate on filter paper (Whatman No.1) in Petri dishes (10 cm in diam) soaked with 10 ml half-strength Hoagland's solution (Hoagland and Arnon 1950) solution for each salt treatment in the laboratory. The solutions consisted of 0.0 (control), 50, 100, 150 and 200 mM NaCl. The number of germinated seeds, length, fresh and dry weight of roots and shoots of the seedlings were recorded after ten days. One hundred seeds for each the treatment were used.

Ten seeds were sown in each plastic pot containing sand washed with sterilized water under greenhouse conditions. Seedlings were grown for 20 days in a half-strength Hoagland's solution and half-strength Hoagland's solutions containing 0, 50, 100, 150 and 200 mM NaCl were added. After ten days of salt treatment, leaves were harvested and used for antioxidant assay.

Leaf tissues (0.5 g) were homogenized with 1.5 ml of 50 mM sodium phosphate buffer (pH 7.3) containing 1 mM EDTA and 2% (w/v) polyvinylpolypyrrolidone (PVP) using chilled mortar and pestle. The homogenate was centrifuged at 14,000 g for 30 min at 4ºC and supernatant used for assays of the activities of SOD, APX and GR. For APX assay the extraction buffer was supplemented with 2 mM ascorbate.

SOD (EC 1.15.1.1) was assayed by the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) (Beauchamp and Fridovich 1971). APX (EC 1.11.1.11) activity was assayed (Nakano and Asada 1981).

GR (EC 1.6.4.2) activity was assayed from the rate of nicotinamide adenine dinucleotide phosphate (NADPH) oxidation using the procedure of Donahue *et al.* (1997) with some modifications. The assay medium contained 0.1 M tris buffer (pH 7.8), 2 mM EDTA, 50 µM NADPH, 0.5 mM oxidized glutathione (GSSG) and 20 µl of the extract. The assays were initiated by the addition of NADPH for 5 min at 25° C. The oxidation reaction for measuring GR activity was followed by monitoring the absorbance at 340 nm spectrophotometerically. The concentration of GR was calculated using an extinction coefficient of 6.2 $mM^{-1}cm^{-1}$ and was expressed as ug^{-1} FW (Fresh weight). One unit of GR is the amount of enzyme that oxidizes 1 mmol of NADPH min^{-1} under the assay conditions.

The experiment was carried out using a factorial experiment with a completely randomized design replicated four times. Data were analyzed using the general linear models (PROC GLM) of SAS Institute. Mean comparisons were conducted using Fisher's LSD test.

Results and Discussion

Effect of NaCl treatments on germination and seedling characteristics of canola cultivars was measured and its results thus obtained have been presented in Table 1. Germination percentage

was significantly affected by salinity stresses. Increased NaCl concentration caused a decrease in germination percentage. Hyola401 showed the highest germination percentage at all salinity levels. On the other hand, Hyola330 ranked as the most sensitive cultivar to salinity stress. Root length of the seedlings did not affect significantly at 50 mM NaCl, but decreased by increase in NaCl concentrations as compared to controls. Shoot lengths were also affected by salt treatment.

Table 1. Means of germination and seedling growth parameters of root and shoot lengths, root and shoot fresh and dry weights in six cultivars of *Brassica napus*.

Cultivar	NaCl treatment (mM)	Germination (%)	Shoot			Root		
			Height (cm)	Fresh wt. (g)	Dry wt. (g)	Height (cm)	Fresh wt. (g)	Dry wt. (g)
Hayola330	0.0 (control)	87	3.903	0.294	0.020	5.273	0.052	0.006
	50	45.25	3.377	0.247	0.017	5.508	0.055	0.006
	100	36	1.293	0.067	0.007	0.475	0.009	0.001
	150	22.75	0.200	0.014	0.003	0.200	0.002	0.001
	200	20.75	0.200	0.014	0.003	0.200	0.002	0.001
Hyola401	0.0 (control)	100	5.248	0.392	0.027	9.073	0.101	0.011
	50	98.75	6.493	0.452	0.030	9.598	0.104	0.011
	100	91	4.580	0.362	0.024	8.380	0.093	0.010
	150	83.25	2.733	0.184	0.014	3.550	0.039	0.004
	200	80.75	1.478	0.087	0.008	2.040	0.022	0.003
Okapi	0.0 (control)	98.25	3.833	0.304	0.021	6.918	0.069	0.008
	50	98.5	6.193	0.417	0.028	7.405	0.074	0.008
	100	86.5	4.520	0.324	0.022	6.423	0.064	0.007
	150	74.75	2.483	0.175	0.013	4.038	0.041	0.005
	200	63.25	1.823	0.106	0.009	2.238	0.023	0.003
RGs003	0.0 (control)	98.75	3.823	0.289	0.020	7.445	0.074	0.008
	50	99.5	5.708	0.401	0.027	7.530	0.076	0.008
	100	83.75	4.360	0.328	0.022	6.865	0.069	0.008
	150	79.5	2.325	0.131	0.011	4.120	0.042	0.005
	200	63.5	1.493	0.076	0.007	2.060	0.022	0.003
Sarigol	0.0 (control)	98.5	4.310	0.313	0.022	6.928	0.068	0.007
	50	96	5.933	0.404	0.027	7.878	0.080	0.009
	100	90.75	4.085	0.285	0.020	7.098	0.071	0.008
	150	80.5	3.033	0.203	0.015	5.925	0.059	0.006
	200	44.25	1.355	0.068	0.007	2.055	0.023	0.003
Zarfam	0.0 (control)	99.75	3.438	0.230	0.016	7.593	0.076	0.008
	50	99.5	4.980	0.375	0.025	8.203	0.081	0.009
	100	92.25	4.240	0.340	0.023	7.275	0.073	0.008
	150	79	2.445	0.147	0.012	3.423	0.035	0.004
	200	74.25	1.298	0.218	0.015	1.495	0.018	0.002
	$LSD_{0.05}$	10.39	1.097	0.111	0.006	2.18	0.023	0.0024

Shoot length increased at 50 mM but decreased significantly at 150 and 200 mM NaCl concentrations. This adverse effect of salt stress on shoot and root was greater in Hyola330.

Although shoot dry weight decreased with 150 and 200 mM NaCl concentrations in the tested cultivars, the rate of decline was greater in Hyola330. Root dry weight also decreased under salt treatment except at 50 mM NaCl. Shoot dry and fresh weights increased significantly at 50 mM. Hyola401 possessed the highest shoot dry weight under 50 mM NaCl treatment while Hyola330 had the lowest shoot dry weight under 150 and 200 mM NaCl treatments. Increase in salt treatments caused a significant reduction in fresh shoot and root weights except at 50 mM. Reduction in fresh shoot and root was relatively greater in Hyola330.

The activity of SOD increased in all the cultivars (Fig. 1). Maximum activity of this enzyme was found with 150 mM for Hyola401, Sarigol, Zarfam and Okapi but treatment of 100 mM NaCl and 50 mM NaCl shows maximum activity of SOD for Hyola330 and RGs003, respectively. The activity SOD increased by 67, 101, 133 and 92% in Hyola401 treated with 50, 100, 150 and 200 mM NaCl, respectively as compared to control (Fig. 1). The decline in SOD activity indicated that the oxygen scavenging function of SOD was impaired or may be the results of plant adaptation to salt stress. Constitutive and induced levels of SOD activity reflect higher dismutationg capacity of Hyola401 as compared to other cultivars (Fig. 1). This result is agreement with that of other researchers who observed the increase in SOD activity with the increase of NaCl concentrations (Costa *et al.* 2005, Dai *et al.* 2009). The activity of SOD in Zarfam and Sarigol was also increased significantly with the increasing NaCl concentrations. SOD activity in Hyola330 significantly increased at 100 mM, the activity did not significantly alter at 50, 150 and 200 mM NaCl as compared to the control. Activity of SOD in RGs003 cultivar only increased at 50 mM as compared to the control significantly (Fig. 1). In Okapi treatment of 50, 100, 150 and 200 mM NaCl increased SOD activity by 54, 108, 140 and 83% compared to the control, respectively.

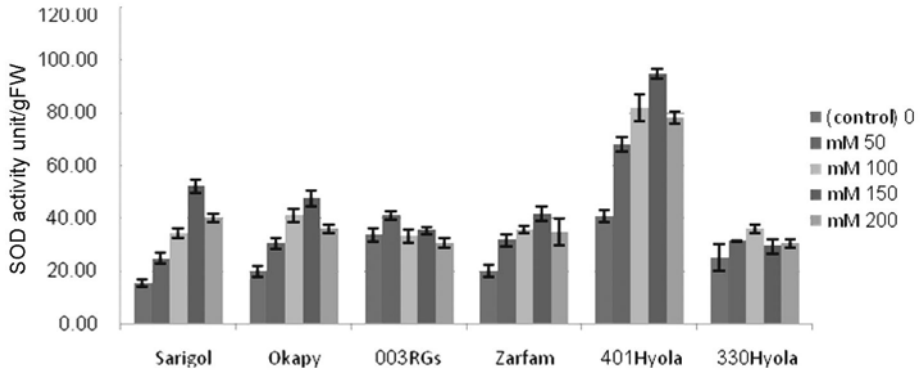

Fig. 1. Effect of NaCl treatment on SOD activities in leaves of six cultivars of *Brassica napus* (Means ± SE).

Hyola401 has the greatest APX activity with 89 unit/gFW while Hyola330 has the lowest content with 56 unit/g FW at control treatment (Fig. 2). Sarigol, RGs003, Zarfam and Okapi did not differ significantly for APX activity at control treatment. One hundred mM NaCl treatment caused a 27% APX activity in Hyola401. However, 200 mM NaCl treatment caused a 21% decrease of APX in Hyola401 as compared to control. Maximum activity of APX was found with 100 mM NaCl treatment for Hyola401. APX activity increases with salt stress, this increase is more conspicuous in the Hoyla401 which is salt-tolerant than Hyola303 (Fig. 2). Hafsi *et al.* (2010) observed increase in APX activity especially under $^{-}K/^{+}NaCl$ conditions in *Hordeum maritimum*. However, there was no significant difference in activity of this enzyme between 50

and 100 mM NaCl concentrations (Fig. 2). The activity of APX in Hyola330 increased by 19, 30, 28 and 24% with 50, 100, 150 and 200 mM NaCl treatments, respectively as compared to the control. In Sarigol and RGs003 the lower (50 mM) and higher (200 mM) concentration of NaCl did not enhanced APX activity in comparison to the control, but in Sarigol the activity increased by 19 and 40% with 100 and 150 mM NaCl respectively as compared to the control and in RGs003 increase was the 41 and 36%, respectively. APX activity in Zarfam and Okapi was distinctly higher than controls under 50 and 100 mM NaCl treatment. In contrast, the activity of this enzyme was not significantly different at 150 and 200 mM NaCl treatments over the control (Fig. 2).

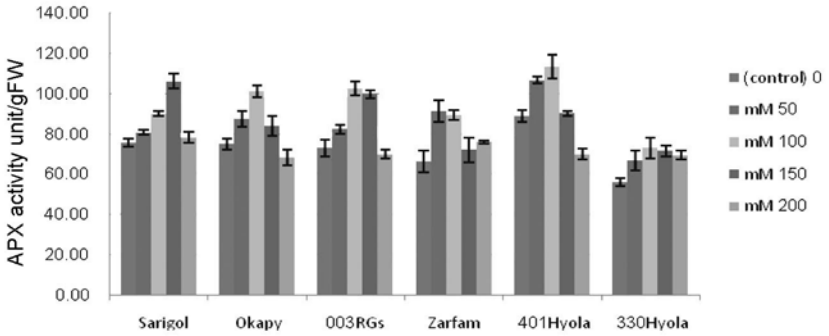

Fig. 2. Effect of NaCl treatment on APX activities in leaves of six cultivars of *Brassica napus* (Means ± SE).

GR activity increased significantly in Hyola401, Zarfam and Okapi at 50 and 100 mM NaCl as compared to the controls. Similarly salinity treatment in Hyola330 and RGs003 had no significant effect on GR activity as compared to the controls. Treatment of 200 mM NaCl caused a decrease in GR activity in Hyola330, Hyola401 and Zarfam cultivars (Fig. 3). The activity of GR decreased by 36 and 45% in RGs003 and Hyola330, respectively with 200 mm NaCl treatment. GR activity of Sarigol cultivar increased with NaCl concentrations up to 150 mM and decreased thereafter (Fig. 3). GR is a member of the flavoenzyme family that catalyzes NADPH dependent reduction of oxidized glutathione, which is important in protecting plants from oxidative stress (Foyer *et al.* 1991). Ruiz and Blumwald (2002) demonestrated that in *Brassica* wild-type plants GR activity increased by twofold at150 mM NaCl. (Seckin e*t al.* 2010) also reported that in *Hordium marinum* SOD and GR activities increased under salt stress but in *H. vulgare* SOD activity decreased at 300 mM NaCl.

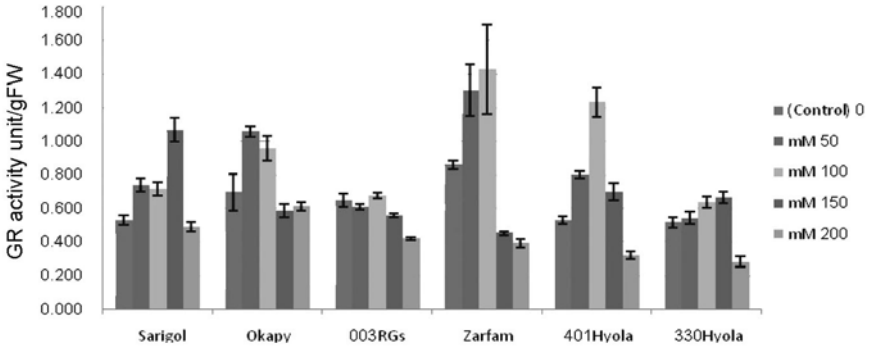

Fig. 3. Effect of NaCl treatment on GR activities in leaves of six cultivars of *Brassica napus* (Means ±SE).

Effect of salt stress on germination and seedling growth have shown in Table 1. Germination of seeds of all the cultivars decreased with increased level of salinity. Salt induced inhibition of seed germination could be attributed to osmotic stress or to specific ion toxicity (Jamil *et al.* 2005). Hyola401 has higher dry weight than other cultivar in all salt treatment. Previous researchers suggested that measuring of seedling stage was introduced as a scale for show salt tolerant and sensitive variety of rapes and a positive association between shoot biomass and seed yield was found (Ashraf 2001, Ashraf and Ali 2008). Reduction in the growth of seedling under salt stress may be attributed to the osmotic effect that cause disturbances in the water balance, water deficit, reduction of photosynthesis and consequently an inhibition of growth (Poljakoff-Mayber 1982). Furthermore, it may result from the accumulation of toxic ions, impaired uptake of essential nutrients and/or damage in cellular organelles (Torres-Schumann *et al.* 1989).

Plant subjected to abiotic stresses underwent impairment of electron transport systems of membranes that caused increase in ROS production (Navari-Izzo and Rascio 1999, Smirnoff 1993). ROS can rapidly attack all types of biomolecules such as nucleic acids, proteins, lipids, and amino acids (Luna *et al.* 1994, Mehta *et al.* 1992), leading to irreparable metabolic dysfunction and cell death. Therefore, antioxidant enzymes such as SOD, APX and GR can protect plant cells from injury. Responses of SOD, APX, GPX, CAT and GR enzymes activate the essential component of the plant antioxidative defense system as they dismutases two O^{2-} to water and oxygen (Cakmak and Horst 1991).

It appears that among the tested cultivars, F_1 hybrid 'Hyola401' could be considered as salt tolerance as possessing higher germination percentage, seedling growth and antioxidant activities under salinity stress. F_1 hybrid 'Hyola330' performed inferior to those aspects and was the most susceptible cultivars to salinity stress.

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