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The Influence of Salt Stress on Seed Germination, Growth and Yield of Canola Cultivars

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Abstract

In order to study the salinity stress effects resulted from sodium chloride on germination, vegetative growth, elements concentration and proline accumulation in five canola cultivars, two experiments were conducted. They were carried out in germinator and greenhouse at the Research Station of the Faculty of Agriculture in Baku State University. The first factor of the experiment was canola cultivar - 'Licord', 'Fornax', 'Okapi', 'Elite', 'SLM$_{046}$', and the second factor was salinity stress level: 0, 50, 100, 150 and 200 mM NaCl in a germination experiment and 0, 75, 150, 200, 250 and 300 mM NaCl in a pot experiment. The results showed that different salinity stress levels had significant effect on germination percentage, germination speed, shoot and root length. In the pot experiment there was a significant effect on plant height, leaf area, dry matter, elements concentration, proline accumulation and seed yield due to salinity stress. A significant effect of the cultivar was observed on investigated traits except leaf area. Increase of salinity level decreased all variables except sodium, chlorine and proline concentration. Sodium, chlorine and proline concentration were increased by salinity stress. The response of canola cultivars was different at germination and vegetative growth stages. In the germination experiment, the most sensitive and most tolerant cultivars were 'Elite' and 'Licord', respectively. However, in the pot experiment, 'Licord' cv. had least growth rate and yield than 'SLM$_{046}$' and 'Okapi'. Significant differences between these cultivars were observed. It seems that the evaluation of germination properties is not useful for the assessment of salinity tolerance of canola cultivars.

Keywords: canola, germination, elements concentration, salinity stress, vegetative growth

Introduction

Salinity is one of the major abiotic stresses in arid and semi-arid regions that substantially reduces the yield of major crops by more than 50% (Bray, 2000). Salinity affects 7% of the world's land area for around 930 million ha (Munns, 2002). Salinity is one of the most serious factors limiting crops production, especially the sensitive ones (Zadeh and Naeini, 2007). Currently, high soil salinity affects the agricultural production in a large proportion worldwide (Zhang and Hodson, 2001; Bybordi *et al.*, 2010b).

Although salt stress affects all growth stages of a plant, seed germination and seedling growth stages are known to be more sensitive for most plant species (Cuartero *et al.*, 2006). Furthermore, germination and seedling stage are predictive of plant growth responses to salinity (Blum, 1985; Cuartero *et al.*, 2006). Therefore, seeds with more rapid germination under salt stress and/or normal conditions may be expected to achieve a rapid seedling establishment and more salt tolerance and hence higher yields (Munns, 2002; Bybordi and Tabatabaei, 2009).

Salt tolerance in plants is a complex phenomenon, which depends on a number of inter-related factors based on morphological, biochemical and physiological processes (Ashraf, 1993). Salinity reduces the ability of plants to take up water, leading to growth reduction as well as metabolic change similar to those caused by the water stress (Munns, 2002; Bybordi *et al.*, 2010c). A high salt concentration in the root affects the growth and yield of many important crops (Taffouo *et al.*, 2006). Salinity may reduce crop yield by upsetting water and nutritional balance of the plant (Khan *et al.*, 2007). Water availability and nutrient uptake by plant roots are limited because of high osmotic potential and toxicity of Na and Cl ions (Al-Karaki, 1997; (Bybordi *et al.*, 2010a).

Saline soils and saline water irrigation present potential hazards to canola production. Germination failures on saline soils are often the results of high salt concentrations in the seed planting zone because of upward movement of soil solution and subsequent evaporation at the soil surface.

The most common adverse effect of salinity on the crop of *Brassica* is the reduction in plant height, size and yield as well as deterioration of the quality of the product (Kumar, 1995). Seed germination has been reported to decline with increasing salinity levels (Houle *et al.*, 2001). Within canola cultivars, there are cultivar differences in sensitivity to salinity. The genetic role in seed germination resistance to salinity is probably one of the most important advantages that can be used in breeding programs. Significant variation in seed germination between canola cultivars grown under salinity condition was reported by

Zheng *et al*. (1998), Puppala *et al*. (1999) and Bybordi *et al*. (2010).

The present study was undertaken to assess the effect of salt stress on some characteristics of five canola cultivars, such as seed germination, seedling growth as well as some morphological traits and yield. The tolerance of canola to salinity during germination has not been reported. Therefore, the objective of this study was to evaluate the germination response of canola cultivars to different levels of salinity.

Materials and methods

Germination experiment

The five canola cultivars (*Brassica napus* L. c.v's 'Licord', 'Fornax', 'Okapi', 'Elite', 'SLM_{046}') were collected from Seed and Plant Improvement Institute (SPII) Karaj, Iran. The same size seeds were surface-sterilized for 5 min in sodium hypochlorite solution (10%) and then they were 3-5 times rinsed with distilled water. After sterilization, 25 seeds were transferred into 9 cm sterile Petri dishes on filter paper and then were wetted with 7 ml distilled water (control) or saline water solution at 0, 50, 100, 150 or 200 mM NaCl. To prevent infection and evaporation of solution, all of the plates were closed with parafilm. All operations were performed under laminar flow. The Petri dishes were labeled and incubated in a germinator at 25°C and 18/6 h day/night illumination. Computation of germinated seeds was done daily until the end of the seventh day. After that, five germinated seeds were removed and their morphological traits were assayed. The germination percentage and germination speed were calculated.

$$\text{Germination percentage (\%)} = \frac{n}{N} \times 100$$

where n: number of germinated seed on the seventh day and N: number of seeds.

$$\text{Germination speed} = \frac{\sum Dn}{\sum n}$$

where n: number of germinated seed on the sightly day and D: days after beginning.

Pot experiment

The seeds were surface-sterilized for 5 min in sodium hypochlorite solution (10%) and then were rinsed with distilled water for 3 to 5 times. Then five seeds were sowed in a plastic pot (35 cm height and 30 cm diameter) contained perlite and vermiculite (1:1) at depth of 1 cm. Pots were transferred to glasshouse under conditions of 25/18°C day/night temperature and natural light. The pots were irrigated at field capacity level by nutrient solution (Hoagland's solution). After full germination, the number of plants was reduced to two seedlings per pot.

Salinity stress induction was done when fourth leaf was completely expanded. Different concentration of NaCl solution was added to each pot gradually.

In order to vernalize the plants, the pots were transferred at the beginning of January to outdoor for 60 days at 5°C.

Morphological traits, yield and yield components

At the physiological maturity stage, plant height, leaf area, number of silique in plant, number of seed in silique, 1000 seed weight and final yield were measured. In order to evaluate dry matter, harvested plants were oven-dried at 70°C for 48 h to constant weight. Dry matter was calculated according to the equation:

$$\text{Dry matter (\%)} = \frac{d}{w} \times 100$$, where d: dry weight and w: fresh weight

Elements concentration assay

Plant samples were grounded in a mill and then passed through a 2 mm sieve. Sub-samples of plant material were digested by a Kjeldhal method before analysis for total nitrogen. The digestion mixture included selenium as catalyst, sulfuric acid, oxygen peroxide and salicylic acid. Total N content was determined by means of the Kjeldhal method. Total P, K and Na were determined by the calorimetric method through a spectrophotometer, (Motic, CL-45240-00, China) and flame photometry by flame-photometer (Perkin Elmer, Model 110, USA). Chlorine assay was performed by means of a chloride meter.

Proline assay

Proline content in leaves was determined according to the modified method of Bates *et al*. (1973). Samples of leaves (0.5 g) were homogenized in a mortar and pestle with 10 ml sulphosalicylic acid (3% w/v), then centrifuged at 18 000 g for 15 min. 2 ml of the supernatant was then added to a test tube, to which 2 ml glacial acetic acid and 2 ml freshly prepared acid ninhydrin solution (1.25 g ninhydrin dissolved in 30 ml of glacial acetic acid and 20 ml of 6 M orthophosphoric acid) were added. The test tubes were incubated in a water bath for 1 h at 100°C and then allowed to cool to room temperature. Then were added 4 ml of toluene to the tubes and then mixed on a vortex mixer for 20 s. The test tubes were allowed to stand for at least 10 min to allow separation of the toluene and aqueous phases. The toluene phase was carefully pipetted out into a glass test tube and its absorbance was measured at a wave length of 520 nm in a spectrophotometer. The content of proline was calculated from a standard curve.

Experimental design and analysis of data

The experimental designs were Completely Randomized Design for germination experience and Randomized Complete Block for pot experiment. The treatments were

arranged in factorial ones with four replications. All data were analyzed by SPSS software and Duncan's Multiple Range Test was used to determine significance of differences between variables ($p<0.05$).

Results and discussion

Germination experiment

Germination percentage and speed

The results demonstrated that the effects of cultivars and salinity levels were significant for shoot length, root length, germination percentage and germination speed (Tab. 1).

Tab. 1. Analysis of variance on germination traits under salinity stress

Variance	d.f	Shoot length	Root length	Germination percentage	Germination speed
Cultivar	4	22.673**	38.265**	3769.700**	1.248**
Salinity	4	42.492**	118.468**	3285.731**	0.138**
Cultivars × Salinity	16	8.531 ns	8.023 ns	482.523**	0.009**
Error	72	8.479	9.541	40.758	0.0003
C.V		44.2	33	8.18	0

*,** Significant at the 0.05 and 0.01 probabilitylevels, respectively ns, not significant

Interaction effect between cultivar and salinity level was significant on germination percentage and germination speed. Different levels of salinity had a different effect on these traits, and there were differences between cultivars. 'Elite' cv. showed the lowest germination percentage and germination speed compared with the other cultivars (Tab. 2).

Thus, this cultivar was known as sensitive to salinity stress. An insignificant difference was found between 'SLM_{046}' and 'Licord' cvs. with respect to germination percentage and germination speed, but 'Licord' cv was introduced as a tolerant cultivar (Tab. 3).

The reduction of germination percentage was significant at 150 and 200 mM. Also, germination speed decreased when the salinity level was raised (Tab. 3).The results confirmed the report of Moss and Hoffman (1977) that high salinity level leads to ion imbalance, osmotic regulation disorders and finally decrease in water absorption by seeds.

Tab. 2. Comparison of main effect of cultivars on germination traits

Cultivar	Shoot length (cm)	Root length (cm)	Germination percentage	Germination speed
SLM_{046}	3.6250^{b}	6.1425^{b}	89.0200^{a}	0.6695^{b}
Okapi	3.7630^{b}	5.8630^{b}	72.1050^{b}	0.3855^{c}
Licord	5.4990^{a}	8.3890^{a}	89.8200^{a}	0.8895^{a}
Fornax	3.5815^{b}	6.5795^{b}	73.2500^{b}	0.3914^{c}
Elite	3.0388^{b}	5.3253^{b}	53.4230^{c}	0.3730^{d}

Means within each column followed by the same letter are not significantly different ($p < 0.05$)

Tab. 3. Comparison of main effect of salinity levels on germination traits

Salinity levels	Shoot length (cm)	Root length (cm)	Germination percentage	Germination speed
0 mM NaCl	6.2363^{a}	9.8800^{a}	92.0^{a}	0.6829^{a}
50 mM NaCl	5.3312^{a}	8.1431^{ab}	91.68^{a}	0.6275^{b}
100 mM NaCl	2.9506^{b}	6.6656^{b}	89.77^{ab}	0.5794^{c}
150 mM NaCl	2.8466^{b}	4.6147^{c}	59.25^{b}	0.5331^{d}
200 mM NaCl	2.8712^{b}	3.2037^{c}	54.0^{c}	0.4550^{e}

For a given means within each column followed by the same letter are not significantly differences ($p < 0.05$)

Root and shoot length

It was observed that the effects of salinity and cultivar were significant for root and shoot length (Tab. 1). The 'Licord' had the highest root and shoot length, while there were not differences between other cultivars in terms of root and shoot length. The enhancement of salinity decreased shoot and root length for all of cultivars (Tab. 3). The lowest shoot length and root length were found for 100, 150 and 200 mM and 150 and 200 mM, respectively (Tab. 3). It seems that root length is more affected by salinity than shoot length.

Pot experiment

Elements content

The effect of cultivar and salinity level was significant on the content of all elements and proline accumulation in plants (Tab. 4).

Tab. 4. Analysis of variance on elements and proline under salinity stress

Variance	d.f	Cl	Na	K	P	N	Proline
Cultivar	4	0.391**	0.337**	5.480**	0.126**	8.668**	1.692**
Salinity	5	0.225**	1.662**	1.791**	0.024**	1.603**	18.712**
Replication	3	0.119**	0.049ns	2.987**	0.002ns	2.696**	0.056ns
Cultivars × Salinity	20	0.044ns	0.042ns	0.138ns	0.002ns	0.053ns	0.213*
Error	87	0.022	0.025	0.234	0.002	0.136	0.099
C.V		35.5	28.2	33.1	14.01	20.1	18.4

*, ** Significant at the 0.05 and 0.01 probability levels, respectively ns, not significant

Also, the comparison of the main effects showed that, the highest elements content and proline accumulation were related to 'SLM_{046}' cultivar, while the lowest were observed in 'Licord' and 'Elite' cultivars (Tab. 5).

Increase of salinity level to 250 and 300 mM increased chlorine; sodium and proline content, while the content of other elements decreased with by increasing salinity level

Tab. 5. Comparison of main effect of cultivars on elements and proline

Cultivar	Cl	Na	K	P	N	Proline
'SLM$_{046}$'	0.5568 ^{a}	0.6918 ^{a}	1.9075 ^{a}	0.3368 ^{a}	2.5927 ^{a}	2.2575 ^{a}
'Okapi'	0.4431^{b}	0.5198 ^{b}	1.2442 ^{b}	0.2623 ^{b}	1.8093 ^{b}	1.8592 ^{b}
'Licord'	0.3143^{c}	0.5048 ^{b}	1.0513 ^{bc}	0.2181^{c}	1.3964 ^{c}	1.7729 ^{bc}
'Fornax'	0.3022^{c}	0.5132^{b}	1.0412^{bc}	0.2111^{c}	1.3896^{c}	1.7524^{bc}
'Elite'	0.2822^{c}	0.4118 ^{c}	0.7896 ^{c}	0.1743 ^{d}	1.2658 ^{c}	1.6679 ^{c}

For a given means within each column followed by the same letter are not significantly differencet ($p < 0.05$)

(Tab. 6). The highest and the lowest sodium content were observed in 'Elite' and 'SLM$_{046}$' cvs. In addition, sodium content increased with salinity level increment. The highest and the lowest sodium content were achieved from 300 mM and control treatments, respectively (Tab. 6).

Tab. 6. Comparison of main effect of salinity levels on elements and proline

Salinity levels	Cl	Na	K	P	N	Proline
0 mM NaCl	0.2956 ^{b}	0.1650 ^{c}	1.8050 ^{a}	0.2880^{a}	2.2475^{a}	0.4350^{e}
75 mM NaCl	0.3238 ^{b}	0.3087 ^{d}	1.3969 ^{b}	0.2769^{a}	1.9856^{b}	0.9688^{d}
150 mM NaCl	0.3313 ^{b}	0.3906^{d}	1.3356 ^{bc}	0.2519^{b}	1.7413^{bc}	1.3438^{c}
200 mM NaCl	0.3812 ^{b}	0.5169 ^{c}	0.9900 ^{cd}	0.2550^{b}	1.6662^{cd}	2.0700^{b}
250 mM NaCl	0.4969 ^{a}	0.8356 ^{b}	0.9213 ^{d}	0.2244^{c}	1.4038^{d}	2.8813^{a}
300 mM NaCl	0.5900 ^{a}	0.9694 ^{a}	1.0400 ^{cd}	0.2269^{c}	1.5475^{cd}	3.1375^{a}

For a given means within each column followed by the same letter are not significantly differences ($p < 0.05$)

With the increase of the salinity level, potassium content gradually decreased until 250 mM salinity level, but at 300 mM it increased (Tab. 6). Ashraf (1993) reported that sodium and chlorine accumulation in tolerate cultivars is lower than in sensitive cultivars and potassium content was higher in tolerate cultivars. Also Teak and Zamin (2008) showed that potassium content decreased due to salinity in sensitive cultivars. It seems that the decrease in potassium content is due to an antagonistic effect between sodium and potassium. Greenay and Munns (1980) confirmed the antagonistic effect between these elements. Potassium was gradually reduced until 250 mM, while, in a case of a high level (300 mM) potassium reduction was stopped and potassium content increased. Increase of a chlorine ion is a reason for this phenomenon. Marschner (1995) showed that chlorine is absorbed by a plant and it had a higher mobility than sodium, as it is transported on both short and long distances.

The highest and the lowest P content were observed in 'SLM$_{046}$' and 'Elite' cvs., respectively (Tab. 5). Increase of salinity levels decreased P content for all of salinity levels (Tab. 6). According to our results, there is a complex relation between P content and salinity and it relates to a genotype. Grattan and Grieve (1999) reported that phosphorus content in plants depended on the species, growth condition and a cultivar. The results showed that salinity stress decreased phosphorus content (Tab. 6). We observed that phosphorous content increased at a 300 mM salinity level. Grattan and Grieve (1992) showed that salinity decreased phosphorus content in plant tissues but in some studies reported salinity increased phosphorus content.

The nitrogen concentration was dependent on the cultivar (Tab. 5). The highest and the lowest nitrogen content was found to be achieved from 'SLM$_{046}$', 'Elite' and 'Licord' cvs., respectively (Tab. 5). The reason for this event can be due to the interaction effect between chlorine and nitrate. Chlorine accumulation decreased nitrate content in tomato and eggplant (Sawas and Salem 1996). Nitrogen decline according to salinity stress has been reported by many researchers (Perez-Alfocea *et al*., 1993; Sawas and Salem, 1996). It seems that the decrease in nitrogen mineralization due to salinity stress is the reason for a decrease of the nitrogen content.

There was a decrease in yield components due to an increment of salinity stress (Tab. 7).

There was significant difference among cultivars in aspect of the number of silique in plant, number of seed in silique, 1000 seed weight and seed yield (Tab. 8). The highest and the lowest seed yield were achieved from 'SLM$_{046}$', 'Okapi', 'Licord', 'Fornax' and 'Elite' cvs., respectively (Tab. 8).

Tab. 7. Analysis of variance on yield and yield component under salinity stress

S.OV	d.f	Number of silique in plant	Number of seed in silique	1000 seed weight	Seed yield	Plant height	Leaf area	Dry matter
Cultivar	4	299.674**	70.930**	7.922**	19.649**	204.860**	1124.792^{ns}	68.225**
Salinity	5	11724.829**	688.831**	29.653**	223.473**	1606.919**	61412.017**	77.154**
Replication	3	6.857^{ns}	3.722*	0.024^{ns}	.346^{ns}	115.110*	1039.347^{ns}	36.854**
Cultivars × Salinity	20	16.853**	2.778**	0.365**	2.112**	13.735^{ns}	546.100^{ns}	1.732^{ns}
Error	87	2.982	.945	0.009	0.185	40.234	1347.912	2.574
C.V		6.9	8.6	4.7	9.7	16.8	26.9	14.6

*, ** Significant at the 0.05 and 0.01 probability levels, respectively ns, not significant

132

Tab. 8. Comparison of main effect of cultivars on yield and yield component

Cultivar	Number of silique in plant	Number of seed in silique	1000 seed weight	Seed yield	Plant height	Leaf area	Dry matter
'SLM$_{046}$'	29.75^{a}	13.1667^{a}	2.5858^{a}	5.5088^{a}	40.2500^{a}	150.8750^{a}	17.7917^{a}
'Okapi'	23.77^{b}	12.1458^{b}	2.1538^{b}	4.3729^{b}	39.0417^{a}	134.7083^{a}	16.0833^{b}
'Licord'	24.22^{b}	9.9792^{c}	1.6058^{c}	3.8212^{c}	37.7917^{a}	141.0000^{a}	15.2500^{b}
'Fornax'	23.85^{b}	9.6852^{c}	1.5968^{c}	3.7925^{c}	37.8512^{a}	142.2135^{a}	15.3652^{b}
'Elite'	21.41^{c}	9.5833^{c}	1.2933^{d}	3.4346^{d}	33.5417^{b}	139.0000^{a}	13.7500^{c}

For a given means within each column followed by the same letter there are no significant differences ($p < 0.05$)

Tab. 9. Comparison of main effect of salinity levels on yield and yield componen

Salinity levels	Number of silique in plant	Number of seed in silique	1000 seed weight	Seed yield	Plant height	Leaf area	Dry matter
0 mM NaCl	66.3438^{a}	19.1563^{a}	3.7394^{a}	10.3831^{a}	56.2500^{a}	256.2500^{a}	19.25^{a}
75 mM NaCl	50.3750^{b}	18.5000^{a}	2.9031^{b}	7.3156^{b}	38.8750^{b}	156.7500^{b}	16.93^{b}
150 mM NaCl	18.1563^{c}	10.9063^{b}	2.4762^{c}	3.3381^{c}	37.9375^{b}	125.2500^{c}	16.00^{b}
200 mM NaCl	7.3750^{d}	10.2813^{b}	1.4425^{d}	1.8650^{d}	34.4375^{bc}	125.3125^{c}	14.50^{c}
250 mM NaCl	4.0313^{e}	4.8125^{c}	0.6925^{e}	1.5725^{d}	30.2500^{cd}	77.5000^{d}	14.62^{c}
300 mM NaCl	2.4688^{f}	3.6563^{d}	0.2044	1.2319^{e}	28.1875^{d}	107.3125c	13.00^{d}

For a given means within each column followed by the same letter there are not significantly differences ($p < 0.05$)

Comparison of means demonstrated that, the highest silique per plant was related to the control treatment and the lowest silique was observed in 300 mM salinity level (Tab. 9). The highest decrease of silique number was obtained from 150 mM salinity to up levels (Tab. 9). It has already been reported by Lin (2004) that the decrease in slique number is associated to the increase of ABA and pollen death. In canola plants, time of flowering is a critical stage, on the other hand, salinity stress decreases growth period and consequently, plants decrease the silique number to attain survival. According to the results of Sinaki *et al.* (2007) salinity stress at flowering stress, decreases silique number. It seems that the most important reason for silique number reduction is low tolerance of canola plants to low salinity level. Moss and Hoffman (1977) reported that salinity stress at 10 ds.m^{-1} is the threshold of salinity tolerance for canola plants.

The decrease in seed number was started from 200 mM salinity level. According to the results, it seems that one of the reasons of seed number decrease is silique size reduction. Sakr *et al*. (2007) reported that major of growth parameters, such as seed number decreased by salinity stress

It has been observed that 1000 seeds weight decreased due to salinity stress (Tab. 9). The highest 1000 seeds weight was found for 75mM salinity level (Tab. 9). There was a gradual reduction in seed weight at a low salinity level but an increase at high levels (Tab. 9). Decrease in seed weigh can be due to prevention of assimilate transport to the seeds and decrease in growth during seed filling stage. Munns *et al*. (2006) showed that when barley plants were exposed to salinity stress, there were many disorders in reproductive stages. Gradual decrease in seed weight at low salinity levels than high salinity levels was due to the low sensitivity of canola to salinity during the vegetative growth stage. Canola is sensitive to salinity at seedling and early vegetative stage and this sensitivity decreases at the end of the growth stage, such as seed filling stage (Francois, 1994). The lowest and the highest seed yield were achieved from 300 mM salinity stress levels and control treatment, respectively (Tab. 9).

The decrease in yield components due to salinity stress lead to loss of final yield. It seems that ions accumulation in plant tissues at different growth stages is the main reason of yield decrease. According to theresults of 1000 seeds weight and number of silique in plants, these parts of yield components are more sensitive to salinity and decreased in final yield is related to their decrease.

Conclusions

In general, the results of this study demonstrate that salinity stress affects some of physiological processes in canola. These actions caused a change in the seed yield of the crop. The increase of the salinity level decreased all variables except sodium, chlorine and proline concentration. Sodium, chlorine and proline concentration increased by salinity stress. The response of canola cultivars was different at germination and vegetative growth stages. During the germination experiment, the most sensitive and most tolerate cultivars were 'Elite' and 'Licord', respectively. However, in the pot experiment, 'Licord' had the lowest growth rate and yield compared to 'SLM$_{046}$' and 'Okapi'. Significant differences between these cultivars were observed. It seems that the evaluation of germination properties is not useful for the assessment of salinity tolerance of canola cultivars.

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