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Effects of Salinity Stress on the Morphology and Yield of Two Cultivars of Canola (*Brassica napus* L.)

[1]Homa Mahmood Zadeh and [2]Ma'ssoumeh Bemani Naeini
[1]Department of Biology,
[2]Department of English, Mashad Islamic Azad University, Mashad, Iran

Abstract: The present research has studied the effects of NaCl of different concentrations on some characteristics of canola. Two cultivars of canola, Okapi (tolerant cv.) and Symbol (sensitive cv.) have been studied under five salinity treatments, including 0, 3, 6, 9 and 12 ds m^{-1}, in both the laboratory and green house conditions. In the laboratory, seed germination rate and percentage and seedling growth were assayed. In the green house condition, the growth parameters were measured in vegetative, generative and harvesting phases. Results showed that the percentage and rate of seed germination, seedling growth and distance between the root hairs and root tips were all decreased by salt solutions, especially by 12 ds m^{-1} treatment. However, these parameters were increased by 3 ds m^{-1} treatment. Also, being treated with salt solutions, within the range of 12-20 ds m^{-1}, the threshold concentration for germination was determined as 14 ds m^{-1} for Okapi and 13 ds m^{-1} for Symbol. Results showed that the effects of salinity stress on the phase of germination is less than the other phases. Under greenhouse condition, the vegetative growth and yield of both cultivars were unaffected by soil salinity of a rate of up to 3 ds m^{-1}. By increasing the salinity levels above the threshold, the ratio of fresh to dry biomass, thickness of leaves, plastochronic period, thickness of pollen exine and 1000-siliqua weight increase. On the other hand, number of leaves, branches, flowers, flower primordia, siliquas and their length, number of seeds per siliqua and 1000-seed weight all decreased. The most inhibitory effect was observed in 12 ds m^{-1} treatment with more effect on Symbol than on Okapi. The length of siliquas, plastochronic period and number of flowers were more affected by salinity stress than the other parameters.

Key words: Salinity stress, canola, seed germination, yield, morphology

INTRODUCTION

Throughout civilized history, environmental stress, due to highly concentrate ions of salt in soils has endured as one of the most serious factors limiting productivity of agricultural crops, especially which are sensitive to soil salinity. Currently, elevated soil salinity affects the agricultural production in a large proportion of the world's terrestrial areas (Zhang and Hodson, 2001). Among the common methods of cultivation in such regions are: using the tolerant cultivars, reforming the soil and water to meet the needs of crops and using transgenic plants (Puppala *et al*., 1999).

One of the most sensitive phases of a plant's life to salinity is that of seed germination. Absence of germination in salinity soil is often due to the high concentration of salt in the soil where the seeds are sown. The reason is that the salt solution moves upward, following the evaporation at soil level (Bernstein, 1974). The salt disturbs both germination and the plant growth (Fowler, 1991). The research has shown that in response to soil salinity, seedlings growth, leaves area, root biomass and shoot biomass have all been reduced (Redmann *et al*., 1994).

Rapeseed (*Brassica napus* L.), from the Cruiceferae family, grows in about 42.2 milion hectars in 53 countries all over the 6 continents, yielding an average of 1451 kg ha^{-1}. Asia alone owns 59.1% of the cultivated areas, but produces only 48.6% of the whole production (Yadava and Singh, 2004). Following results from a germination experiment, researchers positioned the salt tolerance of *Cruciferae* species (including canola) between those of Poaceae (*Poa* sp.), the more tolerant and those of Fabaceae (*Vicia faba*) the less tolerant (Arshad and Rashid, 2001). Rapeseed is a transgenic plant with a low level of erucic acid (< 20 g kg^{-1}) which is widely used in producing polymers and oil. Its low rate of saturated fatty acids (< 70 g kg^{-1}) has caused canola to be increasingly used, among other important oil seeds (Puppala *et al*., 1999). Presently, it is galling at the third place after soy bean and oil palm as the most important sources of oil (Starner *et al*., 1996). This plant is also used

Corresponding Author: Homa Mahmood Zadeh, Department of Biology, Mashad Islamic Azad University, Mashad, Iran

for its synthetic material to be used as one of the elements of plastic. To do so, they transfer three genes, producing plastic from a bacterium, into the plant. Canola is also used for producing bio-gasoline (Tickell, 2000).

The mechanism of salinity effect on plants has long been investigated by researchers, leading to remarkable results. However, a lot has remained to be learned about the function of salinity of NaCl on plants, especially on those strategic ones like canola which has been increasingly cultivated in Iran. The present research has studied the effects of NaCl of different concentrations on some characteristics of canola, such as seed germination and seedling growth as well as some morphological qualities and the yield of sensitive and tolerant cultivars like Symbol and Okapi.

MATERIALS AND METHODS

Two cultivars of canola, Symbol and Okapi were used for this study, the former known as sensitive to salt and the latter as tolerant. The seeds were supplied by the Agricultural Research Center of Khorasan. The experiment was conducted in faculty of Science, Islamic Azad University of Mashad, Iran (2004). In order to investigate the effects of NaCl on seed germination and seedling growth, the seeds were first sterilized in the laboratory, using Sodium Hypochlorite of 7% for 20 min and washed three times with sterile distilled water; then, cultured in Petri dishes containing sterile sand. The experiment was arranged in a completely randomized factorial design. In each treatment, four Petri dishes were used for sowing 25 seeds in each. Solutions of sodium chloride of different concentrations, i.e., 0, 3, 6, 9, 12 ds m^{-1} were provided through 7 mL of the solution were added to each one of the Petri dishes, placed at 25°C. we kept a record of and compared the percentage and rate of seed germination. After 5 days, seedlings were collected to be assessed and recorded length of radicle and hypocotyl and distance between the root hairs and the root tips. To determine the concentration at which seed germination stops for the cultivars, the solution of Sodium Chloride with concentrations between 14 to 20 ds m^{-1} was provided to treat the seeds of the mentioned cultivars. Since there was an increasing effect on both seed germination and seedling growth in 3 ds m^{-1} treatment, tests on seed germination were also conducted with the solutions of 2 and 4 ds m^{-1} to determine the desirable concentration of salinity for such stimulating effects.

Experiments on sowing seeds in five treated groups were conducted in green house. There were a control group which received no solution of sodium chloride plus four other groups which were treated with salt solutions of 3, 6, 9 and 12 ds m^{-1}. We provided flower pots of 50 cm high and some containers with different concentration of NaCl in each. Having sowed seeds of both cultivars, we watered the pots every four days. To study general condition of vegetative growth, some parameters such as number of leaves, thickness of the leaves, number of branches, plastochronic period and ratio of fresh to dry biomass were assessed. Then, means were calculated in three replications. Data was collected on the flowering parameters were measured and compared. These factors include: number of flower primordia, number of flowers for each bush and thickness of pollen exine. At the phase of harvesting, number and length of siliquas, 1000-seed weight and 1000-siliqua weight as well as number of seeds per siliqua were assessed and the means were recorded for three replications. In order for dry biomass to be weighed, the three-month plants were first weighed; then, having been placed in oven at 80°C for 48 h, they were weighed for a second time.

Data were statistically analyzed using analysis of variance (ANOVA) by SPSS.

RESULTS AND DISCUSSION

The experiments on the starting of seed germination, percentage and rate of seed germination all indicated that the seeds in the control start swelling right after water absorption and germinating at 25°C about 8 h after sowing. The optimum rate of germination was about 22 h after sowing. There was not much difference between the two cultivars (Okapi and Symbol). Within 16 to 22 h after sowing, the percentage of seed germination increased from 45 to 100% for Okapi and from 60 to 100% for Symbol. The percentage of seed germination was increasingly affected in 3 ds m^{-1} treatment so that within 16 h after sowing there was an increase of germination up to 20 and 45% for Symbol and Okapi, respectively (Fig. 1 and 2). For 2 ds m^{-1} treatment, the percentage of seed germination was as much as for the control, while for 4 ds m^{-1} treatment, there was a decrease of germination in proportion to the control for both cultivars (Fig. 2). Threshold concentration (i.e., the concentration at which the percentage of seed germination gets as low as less than 50%) for both cultivars, Symbol and Okapi, was determined as 13 and 14 ds m^{-1}.It was found that there was an indirect relationship between the concentration of sodium chloride and seed germination rate. For both cultivars, seed germination started 8 h after sowing in the control group; while for 12 ds m^{-1} treatment, it began after 12 and 10 h, respectively for Symbol and Okapi. In 20 ds m^{-1} treatment, the rate of germination was zero for both cultivars (Fig. 3).

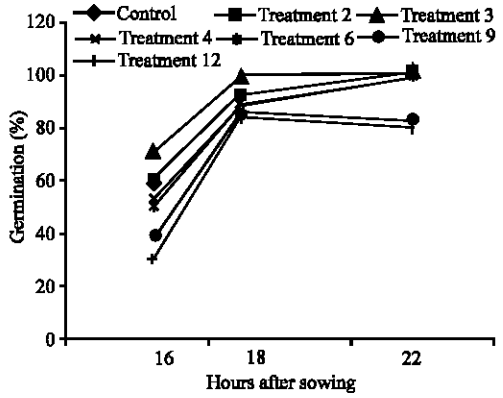


Fig. 1: The changes of seed germination of cv. okapi of canola under salinity stress

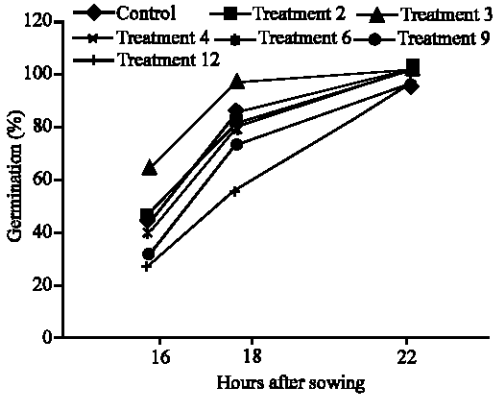


Fig. 2: The changes of seed germination of cv. symbol of canola under salinity stress

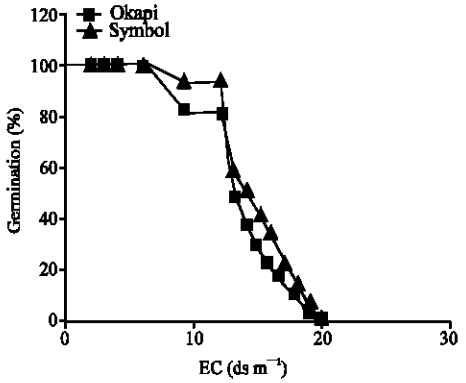


Fig. 3: The chronic changes of seed germination of canola under salinity stress

The length of both radicle and hypocotyl reduced as the rate of salinity increased (Table 1). Such a reduction in both cultivars was more for the length of radicle than for the hypocotyl.

The salt-tolerant cultivar (Okapi) for the length of radicle in 12 ds m^{-1} treatment showed a reduction of 70% as compared to the one sensitive to salinity (Symbol), which reduced by 96% in comparison with the control (Table 1). As for the length of hypocotyl both Okapi and Symbol had a decrease of 21 and 30%, respectively.

Table 1: Effect of different conc. of NaCl on seed germination and seedling growth of rapeseed (*Brassica napus*) (values are mean±SE)

Salinity cultivar (ds m^{-1})	Germination (%)	Length of hypocotyl (cm)	Length of radicle (cm)	Length of root hair from root tip
Okapi				
0	100±0	3.85±0.76	10.87±1.21	11.5±0.84
3	100±0	8.12±1.2*	15±2.5*	7.75±0.52*
6	100±0	3.72±0.62	11.15±0.87	7.25±0.87*
9	94±2.5*	3.05±0.61	6.55±1.4*	5.75±0.62*
12	94±4.2*	072±0.31*	3.72±0.86*	4±2.1*
Symbol				
0	100±0	4.57±0.33	11.87±0.92	10.75±5.3
3	100±0	8.45±1.28	13.8±0.81	8.5±3.2
6	100±0	5.65±0.72	8.75±0.87*	7.25±0.87
9	82±4.2*	2.47±0.22*	4.33±0.56*	4.25±0.82*
12	80±4.3*	1.5±0.15*	2.22±0.21*	4.25±1.1*

Based on Duncan's test, values headed by *differ significantly ($p<0.05$)

Seedling growth of both cultivars in 3 ds m^{-1} treatment, as compared to the control, showed an increase. Okapi showed an increase of 50% for the radicle and that of 100% for the hypocotyl, while the percentage of growth for Symbol was 17 and 86% respectively for radicle and hypocotyl.

The distance between the root hairs and root tips reduced as the concentration of NaCl increased. This characteristic for Okapi reduced from 11.5 mm in the control to 4 mm in 12 ds m^{-1} treatment. For the other cultivar, Symbol, such a length was measured as first being 10.75 mm and then reducing to 4.25 mm (Table 1).

Seed germination of canola is epigaeous and lateral roots begin to form four days after sowing. There was no difference in treatments of Okapi samples, as compared to the control. However, in 12 ds m^{-1} treatment, there was 1 day delay of lateral roots emergence. Figure 4 illustrates the seedlings growth on the third day of sowing. As a whole 3 ds m^{-1} treatment caused a relative improvement of seedling growth at this stage, while 6, 9, 12 ds m^{-1} treatments caused a delay (Fig. 5).

Both cultivars showed more tolerance for salinity at the phase of seed germination, as compared to the later growth phases. As found by Redmann *et al.* (1994) some cultivars of canola like HcN92 and Legend were more tolerant to salinity at the phase of germination among other phases of growth. The reason is that probably stored nutrients existing in the seed support the growth at the early stages.

A decrease in both percentage and rate of seed germination at high levels of salinity (12 ds m^{-1}) could be attributed to the process of osmosis, ionic toxicity, lack of nutrients in the soil or a combination of them all (Redmann *et al.*, 1994).

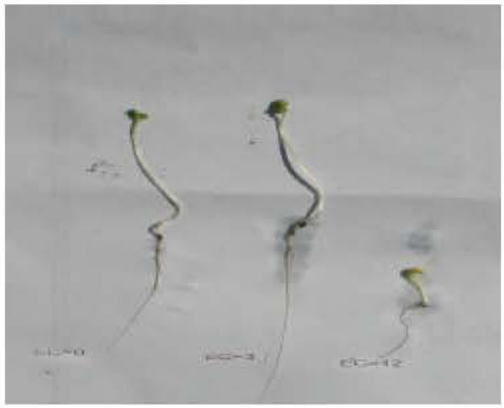
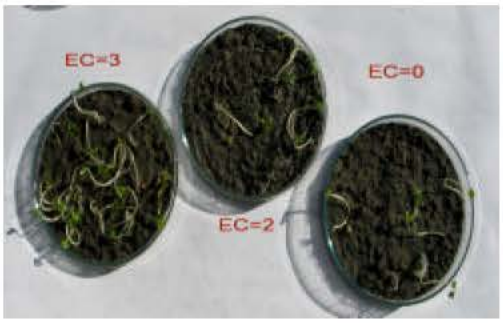


Fig. 4: Seedlings growth of canola, 3 days after sowing under salt stress



(a)

(b)

Fig. 5: Seedlings growth of cv. Okapi of canola at different salinity levels (a, b)

Generally speaking, some growth phases are more sensitive to salinity. For instance, seedlings growth of rice, survival and fertility are affected by salinity more than 1.9, 3.5 and 4.5 ds m^{-1}, respectively (Zeng, 2000). Further, some other research studies show different cultivars of canola to be different in their degree of sensitivity to salinity and they remarkably vary in their seed germination under salt stress conditions (Puppala *et al*., 1999; Zheng and Gao, 1998). Increase in the percentage of seed germination and seedling growth in 3 ds m^{-1} treatment observed may be because of in this salt concentration, the ratio of Na+ to K+ is ideal for metabolic functions; thus, more effective for germination and growth.

As for the results of morphological changes, we found out that general structure and morphology of canola show some changes due to the effects of salinity treatments. The first leaves began to emerge 8 days after sowing the seeds where there was no significant difference between the treatment and the control.

Salinity treatments cause a reduction for number of leaves for both cultivars (Table 2). The highest reduction occurred in 12 ds m^{-1} treatment. The one more sensitive to salt (Symbol) showed more reduction.

Being affected by the salt solution, leaves thickness of the two cultivars, Symbol and Okapi, was more by 22 and 28%, respectively. Also the plastochronic period of both cultivars positively increased, in proportion to the concentration of salt solution. Here, it was Okapi which was more affected by the salinity treatments so that its plastochrone in 12 ds m^{-1} treatment increased as much as twice as the control.

As a whole, having been affected by salinity treatments, the ratio of fresh to dry biomass increased, the maximum rate of which belonged to 12 ds m^{-1} treatment. The cultivar sensitive to salinity showed an increase of 32%, while for the tolerant one it was only 11%. Branching decreased for the plants treated with salt solution so much so that it was calculated as 55.4 and 53.4% for Symbol and Okapi, respectively.

In respect to the generative phase, the comparative study between the treatments and control, indicate that flowering in 12 ds m^{-1} treatment delayed for 4 days. There was no difference between the two cultivars.

The number of flower primordia as well as the number of flowers in each bush were different in the treatments from those in the control (Table 3). The most

Table 2: Effect of salt stress on morphological changes of two cultivars of *Brassica napus* (values are mean±SE)

Salinity cultivar (ds m^{-1})	No. of leaves	Thickness of leaves (μ)	Plastochrone (h)	Fresh biomass/dry biomass	Branching
Okapi					
0	0.97.75±0.9	65.35±3.4	47.75±4.3	5.405±0.6	7.6±1.2
3	8.1±1.1	64.1±2.5	47.75±2.1	5.228±0.58	6.5±1.5
6	7.25±0.85	70.875±3.2	48±0.93	5.938±0.52*	4.8±1.7
9	7±0.8	89.125±3.8	93.25±4.5*	5.998±0.57*	3.4±0.9*
12	6.25±0.93*	91.2±2.2*	94±3.7*	6.133±0.41*	3.2±0.9*
Symbol					
0	7±0.82	69.125±4.1	120.25±3.7	4.465±0.51	8.35±0.85
3	7.5±0.6	68.25±4.5	119.5±2.4	4.108±0.43	7.5±1.4
6	7±0.39	81.6±3.7*	119.25±1.7	5.17±0.67	6.7±1.7
9	7±0.41	82.1±2.4*	147±4.8*	5.525±0.61	5.8±0.96*
12	5.75±0.5*	88.65±3.5*	148.25±3.9*	6.582*±0.68	3.2±0.83*

Based±on Duncan's test, values headed by *differ significantly ($p<0.05$)

Table 3: Response of canola (*Brassica napus*) at flowering stage to salt stress (values are mean±SE)

Salinity levels (ds m^{-1})	No. of flowers primordia	No. of flowers in per bush	Thickness of exine (μ)
Okapi			
0	10.4±2.1	20±1.8	3.35±0.54
3	10.2±1.7	20.25±0.8	--------
6	9.5±0.93	17.75±1.1	--------
9	7.3±0.89*	13.25±0.76*	--------
12	7.6±0.87*	14.25±1.8*	3.43±0.48
Symbol			
0	11.2±1.8	22.75±1.4	3.2±0.42
3	11±1.4	20±1.7	--------
6	8.2±0.93	15.5±0.77	--------
9	6.3±0.76*	10.5±1.2*	--------
12	6.8±0.71*	8.5±0.65*	3.8±0.41

Based on Duncan's test, values headed by *differ significantly ($p<0.05$)

Table 4: Response of Canola (*Brassica napus*) at fruiting stage to salt stress (values are mean±SE)

Salinity cultivar (ds m^{-1})	No. of siliquas bush per	Length of siliquas (cm)	No. of seeds per siliquas	Weight of 1000 seed (g)	Weight of 1000 siliqua (g)
Okapi					
0	10.25±1.4	4.67±0.62	13.2±1.4	2.34±0.25	24.5±2.3
3	12±1.7	4.45±0.81	13.2±1.2	2.32±0.14	28.83±1.9
6	8.25±1.3	1.42±0.22*	8.3±0.94	2.30±0.3	29.6±1.02
9	4.25±0.8*	1.525±0.3*	8.1±0.66*	2.24±0.22*	32.1±2.7*
12	3.5±0.91*	1.125±0.43*	6.3±0.71*	2.2±0.17*	37.68±1.7*
Symbol					
0	16.5±1.7	4.97±1.1	15.6±1.5	2.9±0.13	25.15±1.4
3	15±0.78	4.42±0.98	14.3±1.2	2.52±0.11	28.3±1.8
6	10±1.2	2.7±0.27	9.4±1.8*	2.48±0.4*	30.6±0.95
9	5±0.14*	1.03±0.13*	7.7±0.93*	2.21±0.16*	42.4±2.1*
12	4.25±0.78*	0.9±0.1*	6.1±1.02*	2.19±0.12*	49±2.1*

-Based on Duncan's test, values headed by *differ significantly ($p<0.05$)

changes (reductions) occurred in 12 ds m^{-1} treatment, showing more decrease for Symbol than for Okapi. The thickness of pollen exine in this treatment increased by 2% for Okapi and 15% for Symbol.

Following the reduction of flower number, there was also a reduction of the number of siliquas at this stage (Table 4). The most reduction occurred for 12 ds m^{-1} treatment with 65 and 74% of reduction, respectively for Okapi and Symbol. The length of siliquas also, decreased by 82 and 75% for symbol and Okapi, respectively in the same treatment. Besides, the number of seeds per siliqua decreased for both cultivars in the salt treatments. With increasing in salinity levels, the more weight loss of 1000 seed so that a reduction of 6% for Okapi and that of 24% for Symbol was noticed in the treatment with the concentration of 12 ds m^{-1}. In the same treatment, the weight differences of 1000 siliqua and 1000-seed were calculated as 54 and 37% for Symbol and Okapi, respectively.

The effects of salinity treatments of different concentrations on morphology of canola are due to the widespread effects of salt stress on plant cell functions, including photosynthesis, the functions of different enzymes, metabolism of the cell, etc. (Redmann *et al*., 1994; Arshad and Rashid, 2001). The ionic toxicity, due to salinity, is because of the replacement of potassium by sodium through biochemical interactions as well as structural changes, lack of protein functions as a result of uptake of the ions of Cl^{-} and Na+and interference of the interactions between amino acids (Zhu, 2002). The reduction of number of flower primordia could be probably related to effects of salinity stress on generative meristem at the beginning of flowering phase, reducing the number of flower primordia, first and then, leading to a reduction of the number of flowers. Following the flower decrease, there was a fall in the number of siliquas at the phase of fruiting. Other researchers have also reported a reduction of the number of canola seeds as well as weight loss for its 1000 seed under saline conditions (Francois, 1994) enhancing of 1000 siliqua weight at the high level of concentration (12 ds m^{-1}) is a sign of lignification of the siliqua under salt stress which is not considered an ideal characterization, no matter if it may increase the siliquas resistance against advert environmental conditions.

In sum, present findings show that both cultivars of canola, whether salt sensitive (Symbol) or salt tolerant (Okapi), are less sensitive to saline stress at the phases of seed germination and seedling growth, while at the later phases of development, especially those of flowering and yield are violently affected by high level of saline concentration (i.e., 12 ds m^{-1}). Also a concentration of 3 ds m^{-1} at the phase of germination has positive stimulating effects which might provide an optimum concentration of Na+for cell metabolic functions.

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