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Full Length Research Paper

Influence of NO_3:NH_4 ratios and silicon on growth, nitrate reductase activity and fatty acid composition of canola under saline conditions

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Canola is a major oil seed crop worldwide and it is also an important crop in Mediterranean areas where salinity is an increasing problem. Although seed oil of standard cultivated canola is considered to be of good quality for edible purposes, the development of cultivars with oil high in oleic acid is an important breeding objective for this crop. In this paper, the effect of salinity, silicium and different nitrate and ammonium ratio were evaluated on growth, sodium and potassium content nitrate reductase activity and fatty acid composition. Canola plants were grown in a pot hydroponically system. The results revealed that, salinity significantly decreased plant growth, potassium accumulation, K/Na ratio and linolenic acid percentage while increased sodium content and oleic acid percentage. Also, silicium increased plant growth, oleic acid and linolenic acid percentage, potassium content, K/Na ratio and silicium content. Silicium prevented sodium accumulation in plants. Among different nitrate and ammonium ratios 25:75 ratio was the best treatment in respect of plant growth.

Key words: Ammonium, nitrate, salinity, silicium, canola.

INTRODUCTION

Salinity is a significant limiting factor in the agricultural productivity (Hasegawa et al., 1986). The agricultural areas affected by salt need amendment and determination of the most suitable plant species able to grow in these areas. Salinity also affects nutrient balance in plant tissues (Pessarakli et al., 1991). Increased salt treatment causes sodium and chlorine contents to rise and potassium, calcium and magnesium contents to diminish in a number of plants (John et al., 2003). Salinity has both osmotic and specific ion effects on plant growth (Dionisio-Sese and Tobita, 2000). Reduction in plant growth as a result of salt stress has also been reported in several other plant species (Ashraf and O'leary, 1997). Growth of leaf area is inhibited by salinity (Alberico and Cramer, 1993). Richardson and McCree (1985) believe that the greater ability of salinized plants to continue leaf expansion and carbon gain under water stress can be attributed primarily to a slower development of water stress, which prolonged the osmotic adjustment. Salt stress might influence also the oil fatty acid composition (Bybordi et al., 2010a). In fact, canola is a rich source of oleic and linoleic acid. These two fatty acids represent 85 – 95% of the total fatty acids in canola seeds and their relative contribution varies with genotype and environmental conditions in standard genotypes (Bybordi et al., 2010b). Canola (*Brassica napus* L.) is a very important oil seed crop, with the world harvested area of about 23 million hectares and the seed production about 31 million tons (Bybordi et al., 2010c, Bybordi and Tabatabaei, 2009.).

Silicium, which is the second most abundant in the soil, is a major constituent of many plants, but its roles in plant biology have been poorly understood (Liang et al., 1999). Although silicium has not been listed among the generally essential elements of higher plants, there have been reports of interactions between silicium supply and the responses of members of the *Poaceae* to biotic and abiotic stresses (Kaya et al., 2006).

It is evident that silicium is beneficial for the growth of many plants under various abiotic and biotic stresses (Liang et al., 2003). Some possible mechanisms through which silicium may increase salinity tolerance in plants (Liang et al., 2003) include: immobilization of toxic sodium ion (Liang et al., 2003), reduced sodium uptake in plants and enhanced potassium uptake (Liang et al., 2005) and higher potassium, sodium selectivity (Hasegawa et al., 2000). Hence, silicium has vital importance for better plant growth under salinity (Tahir et al., 2006).

Nitrogen is one of the most important mineral nutrients for plants and plants can utilise it in both anionic nitrate and cationic ammonium forms. Once absorbed, ammonium can be rapidly utilised in the synthesis of amino acids and other nitrogenous organic compounds, but nitrate must be reduced to ammonium before it can be assimilated (Barker and Mills, 1980). There is also evidence that the form of nitrogen supplied to plants not only affects their growth under non-saline conditions, but also shows an interaction with the salinity tolerance of plants (Hawkins and Lewis, 1993).

Several researchers have studied the combined effect of salinity and the nitrogen source type added to the nutrient solution on productivity, photosynthesis and nitrogen metabolism (Hawkins and Lewis, 1993; Bybordi et al., 2010). Recently, the effects of nitrate and ammonium ions and the combined effect of salinity with different nitrogen sources (Bybordi et al., 2010a) on certain biochemical adjustments to stress have been reported.

The objective of this study was to determine the changes in growth, sodium and potassium accumulation, nitrate reductase activity and fatty acid composition of canola treated by silicium and different nitrate and ammonium ratio under salt stress conditions. To achieve this purpose, the effects of silicium, different nitrate and ammonium ratio and salinity were assessed in hydroponically system. The hypothesis was to verify whether silicium may be useful to enhance the salt tolerance of canola that is mediated through improvement in plant growth.

MATERIALS AND METHODS

Plant material and growth conditions

Canola plants were grown in pots filled with perlite and sand (1:1 v/v) under controlled conditions in a glass house at the East Azarbyjan Agrcultural and Natural Resources Research Center Tabriz, Iran. The seeds (c.v okapi) were surface-sterilized for 5 min in sodium hypochlorite solution and then in 96% ethanol for 30 s and then rinsed with distilled water. Two sterilized seeds were sowed in each pot under conditions of 25 ± 3/18 ± 3°C day/night temperature, 60% relative humidity and supplementary photon flux density of 250 µmol m^{-2} s^{-1}.

Experimental design and treatments

After germination, the pots were arranged into three replications with 24 experimental pots per replication thus, there were three 72 experimental units. The treatments were consist of two levels of salinity (0 and 200 mM NaCl), three levels of silicon (0, 2 and 4 mM Silicic acid) and four nitrogen sources (NH_4:NO_3 ratios: 0:100, 75:25, 50:50 and 25:75). Thus, the experimental design was a randomized completed block arranged in 2 × 3 × 4 factorial.

Nitrogen, silicium and salinity treatments

Plants were fed with one of the modified Hoagland's solution following NH_4:NO_3 ratios: 0:100, 75:25, 50:50 and 25:75. The concentration of the nutrients in the solutions was as follows (in mg L^{-1}): 330 K, 170 Ca, 50 Mg, 33 P, 1.5 B, 0.1 Cu, 2 Mn, 3 Zn, 12 Fe (Fe-DTPA) and 0.1 Mo. Nitrogen was provided as NO_3^- and NH_4^+ forms at 200 mg $L^{-1}$to give NH_4:NO_3 ratios of 0:100, 75:25, 50:50 and 25:75 (Table 1). The electrical conductivity (EC) of the nutrient solution was within the range 2.7 - 2.8 dS.m^{-1}. Also the initial pH of the nutrient solutions containing NO_3 and NH_4 was adjusted to 6.5 - 6.8 by adding H_2SO_4 or KH_2CO_3. The solutions were refreshed once a day according to the nutrients and water taken up by the plants (1 liter per day). The EC and pH of drainage water from pots were checked every time and an additional solution was applied to minimize EC and pH changes in the root zone. The treatments were imposed when two true leaves of the canola plants was fully expanded. For the silicium nourishment; 0, 2 and 4 mM silicium from salicic acid was added in Hoagland solution. In order to salt stress induction, the plants were watered by drip with 200 mMNaCl solution.

Data collection

Nitrate reductase activity

Nitrate reductase activity was determined using the method described by Klepper et al. (1971). 100 mg leaf tissue was vacuum infiltrated in 2 mL of incubation solution containing 0.1 mmol.L^{-1} potassium phosphate buffer pH 7.5, 0.05 mol.L^{-1} KNO_3 and 1% v/v isopropanol.

The incubation was conducted for 1h at 30°C in the dark. To estimate the amount of nitrite formed, 1 mL each of 1% sulfanilamide in 1 mmol.L^{-1} HCl and 0.02% naphthylethylene diamine dihydrochloride were added and the test tubes vortexed. Absorbance of this resulting solution was recorded at 540 nm with a spectrophotometer (Khan et al., 1995). Nitrate reductase activity was expressed as µmol.$h^{-1}g^{-1}$FW (fresh weight).

Leaf area, fresh and dry weight

At the end of the experiment (240 days after sowing), all plants from each treatment were sampled to measure their leaf area and weight. The leaf area was measured using a leaf area-meter (Li-Cor, Moldel Li-1300, USA). After being weighed, the leaves were dried at 80°C in an air-forced oven for 48 h and dry was recorded.

Si, Na and K assay

Silicon content in leaves was determined by the blue silicomolybdate procedure as described by van der Vorm (1987). Dried leaves were placed in porcelain crucibles and incinerated for 3 h at 550°C. The ash was washed into 100 ml polycarbonate test tubes, then 50 ml of 0.08 M H_2SO_4 and 2 ml of 40% HF was added. Colour development was accomplished by adding 1.5 ml of this solution to 1.5 ml of reagent mixture of the 0.08 M H_2SO_4 and ammonium molybdate (20 g l^{-1}), and then 1.5 ml of 0.25 M tartaric acid ($C_4H_6O_6$) and finally 1.5 ml of 0.2 M ascorbic acid was added. After mixing the tubes, absorbance at 811 nm was measured and Si concentration was expressed as mg g^{-1} DW (dry weight). Sodium and potassium content were assayed by flame photometry method by flame-photometer JenWay PFP7.

Fatty acid composition

The seeds were separated and fatty acid compositions of the canola seed oils were determined by gas chromatography (GC) (Metcalf et al., 1966). The GC-MS analyses were performed on an Auto-System (Perkin—Elmer, USA) connected to a silexcapillary column of DB-5 (30 m × 0.25 mm × 0.25 m) made by SULPECOS. At chromatography temperature parameters were set as follows: injector at 250°C, ion source 200°C. Oven program: initial

Table 1. Analysis of variance on growth, mineral content, nitrate reductase activity and fatty acid percentage of canola under salinity stress, silicium and different proportion of ammonium and nitrate feeding.

S.O.V	d.f	FW	DW	LA	Na	K	K/Na	Si	NR	OLEIC	LEIC	LENIC
NaCl	1	2.33**	1.25**	2351.35**	78.25**	55.35**	22.13**	10.25**	3.36**	4.38**	1.23ns	3.12**
Silicium	2	3.12**	1.33**	2235.12**	35.25**	51.25**	20.13**	9.36**	0.98ns	0.98ns	1.22ns	2.35**
Nitrogen sources	3	4.11**	1.45**	2314.33**	22.35**	50.25**	19.24**	8.64**	2.25**	0.95ns	1.11ns	0.98ns
NaCl × Silicium	2	5.24**	0.99ns	2132.12**	20.12**	10.23ns	8.21ns	8.88**	0.85ns	0.93ns	1.21ns	0.97ns
NaCl × Nitrogen sources	3	4.36**	1.11*	2012.17**	10.25*	9.23ns	14.25*	8.64**	0.78ns	0.92ns	1.02ns	0.88ns
Silicium × Nitrogen sources	6	3.35**	0.84ns	2131.15**	2.32ns	8.13ns	7.25ns	3.33ns	0.74ns	0.90ns	0.86ns	0.87ns
NaCl × Silicium × Nitrogen sources	6	6.35**	0.77ns	2065.15**	2.13ns	7.11ns	6.35ns	2.38ns	0.75ns	0.88ns	0.87ns	0.78ns

FW: fresh weight($g.plants^{-1}$), DW: dry weight($g.plants^{-1}$), LA: leaf area(Cm^2), Na: sodium($mg.g^{-1}$), K: potassium($mg.g^{-1}$), K/Na: potassium sodium ratio, Si: silicium($mg.g^{-1}$),NR: nitrate reductase(µmol h-1 g -1 Fwt), OLEIC: oleic acid(%), LEIC: linoleic acid(%), LENIC: linolenic acid(%). *, ** significant at the 0.05 and 0.01 probability levels, respectively and ns no significant.

Table 2. Comparison of main effects of salinity.

Treatments (mM)		K	NR	OLEIC	LENIC
NaCl	0	51.74a	3.20a	81.73b	6.72a
	200	44.35b	2.01b	84.29a	4.91b

Within each column followed by the same letter are not significantly differences ($p < 0.05$).

temperature: 80°C for 20 s, 80 to 240°C at 4°C min−1, 240°C for 10 min. Carrier gas was helium; column pressure 100 kPa, split rate 1:30. 1.0 mm^3 sample was injected. The energy of the EI source of the Perkin—Elmer mass spectrometer was 70 eV. The contents of oleic, linoleic and linolenic acids were determined using a computing integrator. The effects of the independent variables on oil content and oleic, linoleic and linolenic acid concentrations of the oil were analyzed on a percentage basis.

Data analysis

All data were analysed from analysis of variance using SAS software. Duncan's multiple range tests was used to measure statistical differences between treatment methods and controls.

RESULTS

Analysis of variance generally showed a significant effect of the salinity for all examined traits except linoleic acid percentage. Silicium application had no significant effect on nitrate reductase activity, oleic and linoleic acid percentage. Effect of different ratio of nitrate and ammonium were significant on fresh and dry weight, leaf area, Na, K, Na/K, Si content and nitrate reductase activity (Table 1). When interactions were not significant comparison was done on main effects (Tables 2, 3 and 4). The data showed that salt stress significantly reduced potassium content, nitrate reductase activity and linolenic acid percentage while increased oleic acid percentage (Table 2). It is observed that, silicium increased potassium content but decreased linolenic acid percentage (Table 3). The comparison of means revealed there were no differences between 2 and 4 mM silicium and 0 and 2 mM silicium on potassium content and linolenic percentage, respectively, (Table 3).

Different nitrate and ammonium ratio had different effects on potassium content and nitrate reductase activity (Table 4). Potassium content was increased in plants treated with 50:50 and 25:75 ratios but there was no significant difference between them. Also, between100:0 and 75:25 was not difference about potassium content. The highest nitrate reductase activity was observed when plants were treated by 50:50 and 25:75 nitrate and ammonium ratios (Table 4).

Interaction among salinity, silicium and nitrogen was

Table 3. Comparison of main effects of silicium.

Treatments (mM)		K	LENIC
	0	45.63b	6.35a
Silicium	2	48.63a	5.94a
	4	49.86a	5.17b

Within each column followed by the same letter are not significantly differences ($p < 0.05$).

Table 4. Comparison of main effects of nitrogen sources.

Treatments		K	NR
	100:0	44.61b	2.00b
Nitrogen sources	75:25	46.00b	2.50ab
	50:50	50.80a	2.83a
	25:75	50.76a	3.10a

Within each column followed by the same letter are not significantly differences ($p < 0.05$).

significant on fresh weight (Figure 1). The result showed that, salinity stress obviously decreased fresh weight. In salt stress conditions and normal conditions silicium increased fresh weight when plants were treated by any ratio of nitrate and ammonium (Figure 1). In normal conditions the highest fresh weight was produced from plants treated with 4 mM silicium and 25:75 nitrate and ammonium ratio. Also the highest fresh weight was obtained from plants treated with 4 mM silicium and 25:75 nitrate and ammonium ratio under conditions of salt stress. The lowest fresh weight was observed in plants treated by 200 mM NaCl without silicium and 100:0 nitrate and ammonium ratio. In sum, increase of silicium concentration and ammonium ratio to nitrate increased fresh weight in both normal and salt stress conditions (Figure 1).

Dry weight was affected by salinity and different nitrate and ammonium ration. Salinity decreased dry weight while increase of ammonium increased it. Interaction between salt stress and different nitrate and ammonium ration was significant (Table 1). Figure 2 shows that, salinity significantly decreased dry weight while enhancement of ammonium increased dry weight (Figure 2). The highest dry weight was obtained from plants grown in normal conditions and 25:75 nitrate and ammonium ration. Decrease in dry weight due to salinity stress and non ammonium application is shown in Figure 2.

Leaf area was decreased due to salt stress while increase of silicium and ammonium concentration improved leaf area. These results are shown in Figure 3. The maximum leaf area was related plants treated with 4 mM silicium and 25:75 nitrate and ammonium ratio (Figure 3). However salinity decreased leaf area but silicium application or increased of ammonium concentration improved leaf area under salinity stress. This positive effect was observed under normal conditions too (Figure 3).

Salinity leads to increased of sodium content in plants. Sodium content was decreased due to silicium application under salinity stress but under normal conditions silicium had not significant effect on sodium accumulation (Figure 4). Similar results were observed when stressed plants were fed with high ammonium concentration solutions (Figure 5). Increase of ammonium decreased sodium accumulation in salt stressed plants but under normal condition there was significance difference among different ratios (Figure 5). Potassium to sodium ratio was decreased by salinity stress in other word salinity increased sodium content in plant tissues (Figure 6). Silicium increased this ratio by increased of Potassium absorption. Furthermore, ammonium had additive effect on Potassium to Sodium ratio. Interaction between salinity and different nitrate and ammonium ration is shown in Figure 6.

Salinity decreased silicium content in plants. While silicium application increased silicium content in both normal and salt stress conditions (Figure 7). Also, increment of ammonium concentration in nutrition solution improved silicium content in stressed plants (Figure 8).

DISCUSSION

In this study fresh weight, dry weight and leaf area were decreased by salt stress. Salinity caused reduction in plant dry matter, seed and oil yield (Cheng, 1984), leaf area expansion (Rawson and Munns, 1984), plant height, leaf area and number (Rehman and Hussain, 1998). Quereghi et al. (1991) reported greater reduction in shoot length and biomass as compared to not parameters, while Hussain and Ismail (1994) reported reduction in relative growth rate in to parent NaCl solution. Salinity ultimately influences the shoot and root growth in maize (Moussa, 2006) plants in the presence of NaCl added into nutrient solution. These results are supported by Gong et al. (2006), who observed an enhanced shoot dry and fresh weight under no-salt stress in barley whereas, Yeo et al. (1990) observed similar results in rice crop only under saline conditions. We observed that, silicium application increased significantly fresh weight, dry weight and leaf area. It is also reported that exogenously applied silicium increased the growth of a number of monocot and dicot species under salt free conditions (Adatia and Besford, 1986). In the present study, the growth of salt stressed canola plants is improved by the addition of silicium. It has been reported that the alleviation of salt toxicity by silicium addition results from a reduction in the sodium content in the shoots of rice(Gong et al. 2006). Also, in sorghum (*Sorghum bicolor*), application of silicium increased relative water content and dry mass of plants (Hattori et al. 2001).

The results demonstrated that, salinity incensed sodium content but silicium and ammonium decreased

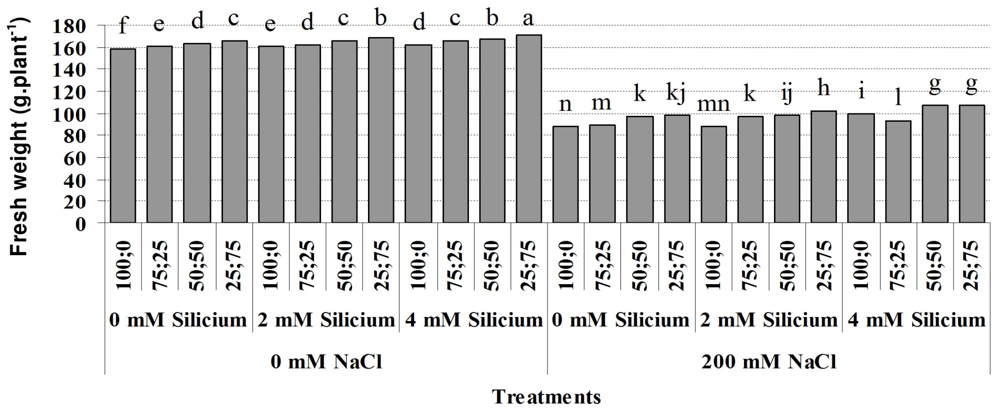

Figure 1. Interaction among salinity stress, silicium application and different nitrate and ammonium ratio on fresh weight.

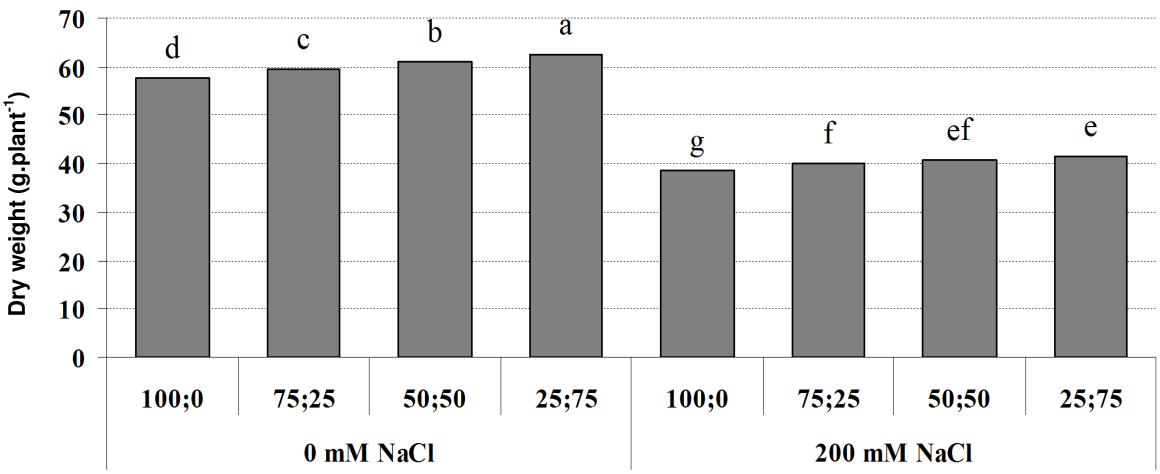

Figure 2. Interaction between salinity stress and different nitrate and ammonium ratio on dry weight.

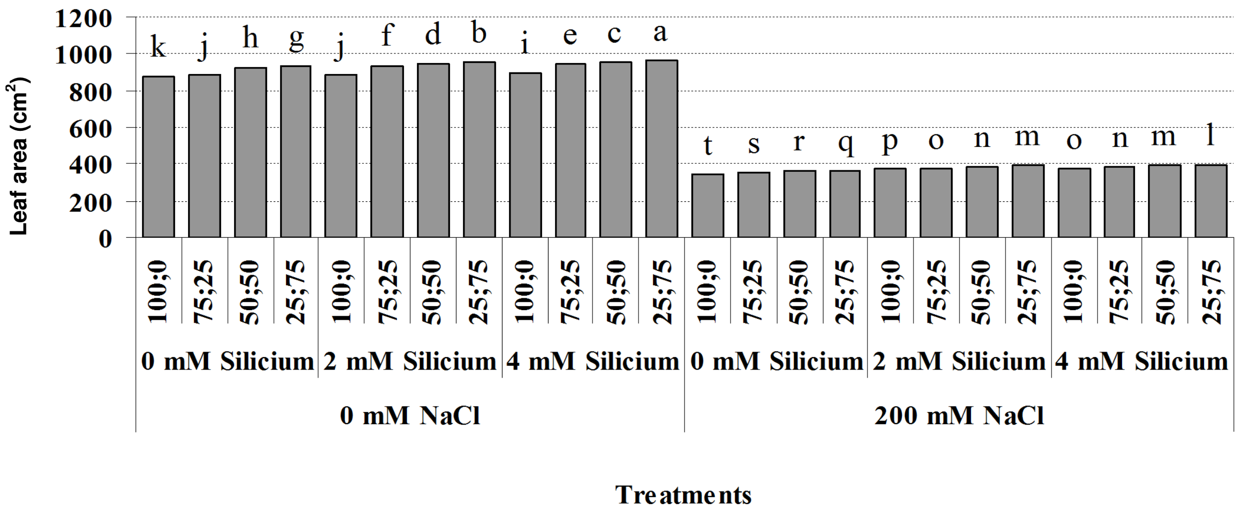

Figure 3. Interaction among salinity stress, silicium application and different nitrate and ammonium ratio on leaf area.

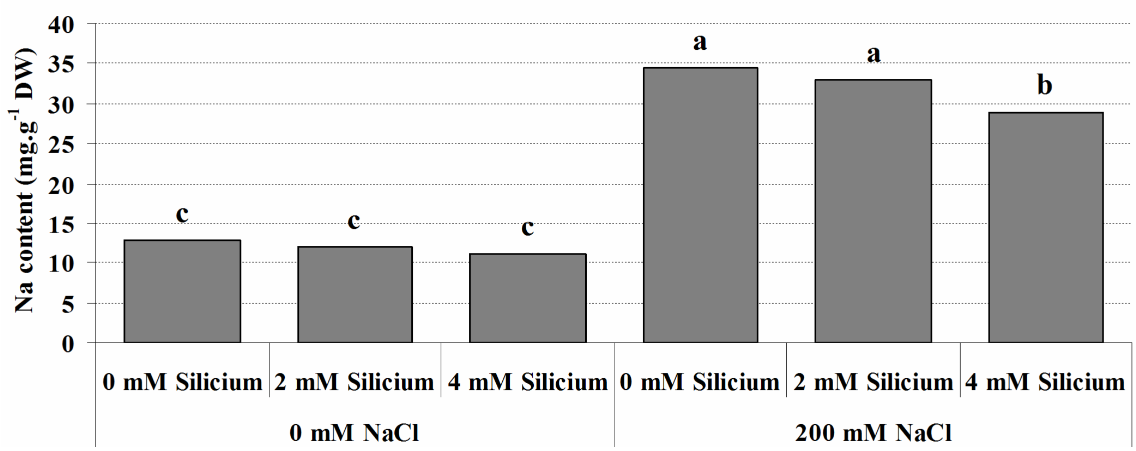

Figure 4. Interaction between salinity stress and silicium application on sodium content.

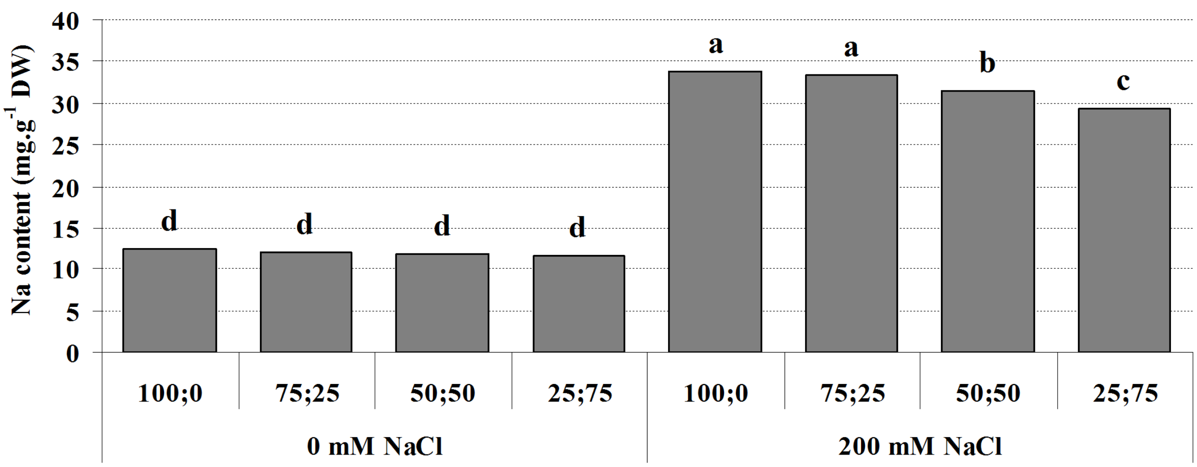

Figure 5. Interaction between salinity stress and different nitrate and ammonium ratio on sodium content.

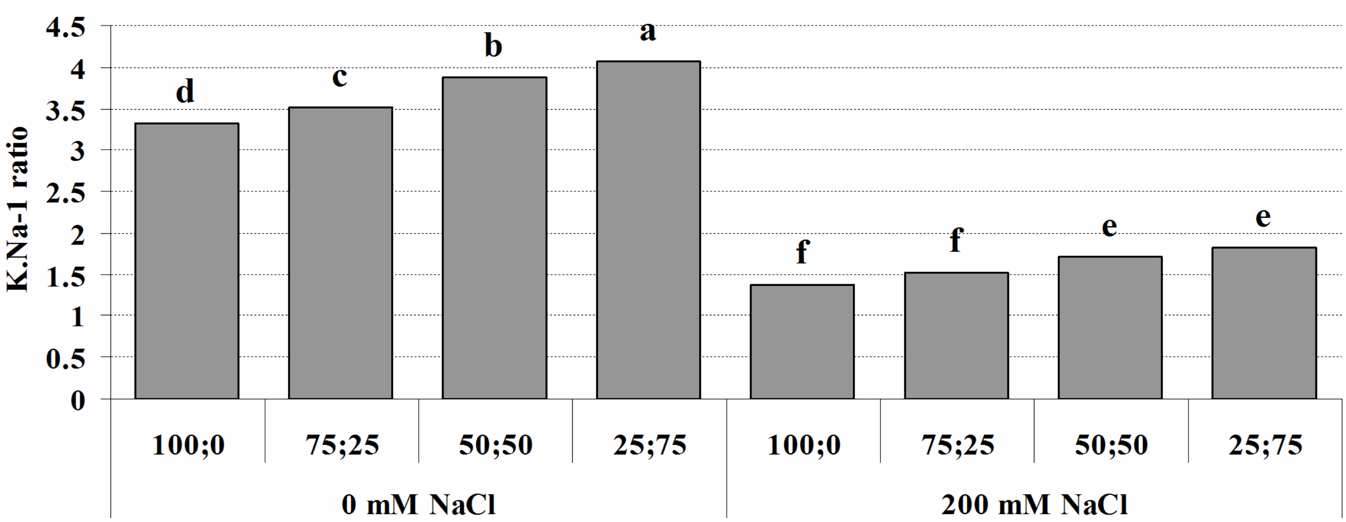

Figure 6. Interaction between salinity stress and different nitrate and ammonium ratio on K/Na ratio.

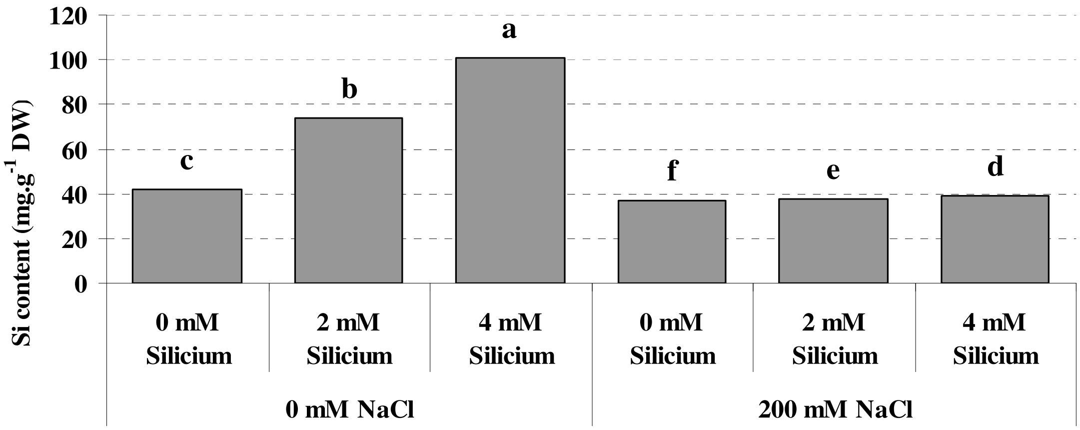

Figure 7. Interaction between salinity stress and silicium application on silicium content.

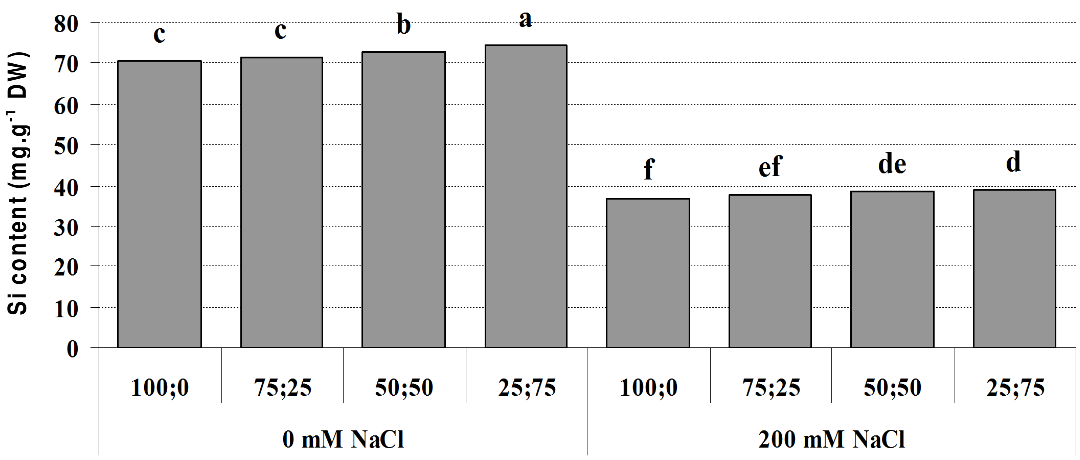

Figure 8. Interaction between salinity stress and different nitrate and ammonium ratio on silicium content.

sodium accumulation due to enhancement of potassium absorption. According to Weimberg (1987), high levels of sodium inhibit the potassium uptake and as a result of this it causes a decrease in the K/Na ratio. The decrease in the K/Na ratio in our study may be attributed to the fact that sodium causes a disturbance in the ion balance in plant by an increase in the sodium uptake. Many of the deleterious effects of sodium seem to be related to the structural and functional integrity of membranes (Kurt et al., 1986). It is suggested that the capacity of ion accumulation of plants is related to their tolerance to salt stress. It was found that tolerant species accumulated lower sodium and decreasing of potassium was lower than sensitive species (Tipirdamaz and Çakirlar, 1989). Increased potassium concentration also shows the ability of plants to combat the salinity stress that will strongly depend upon sodium and silicium content. The added silicium increased the potassium concentration than the plants grown without silicium in saline conditions. Liang et al. (1999) found that the salt tolerance due to silicium application is attributed to selective uptake and transport of potassium and sodium by plants.

Nitrate reductase activity (NRA) was significantly decreased by salinity stress treatment. Under conditions of salt stress, nitrate reductase activity could be lowered initially due to enzyme degradation/inactivation and the reduction in gene expression and nitrate reductase protein synthesis (Ferrario et al., 1998). These observations are in agreement with the findings of other authors who indicate a similar inhibition of NRA due to salinity in other crops (Khan et al., 1995). Increase of nitrate reductase activity due to enhancement of ammonium was observed.

Salt stress had significant effect on oleic and linolenic acid. In this study, the main effect of salt stress on fatty acid composition was an increase in oleic acid and a consistent decrease in linoleic acid. Silicium had significant effect on linolenic acid parentage only. The highest concentration of silicium increased linolenic acid percenttage. Different ratio of nitrate and ammonium had not any significant effect on fatty acids. It has been reported that, salinity caused reduction in oil content (Muhammad and Makhdum, 1973). Also, it reported that, water stress caused an increase in oleic acid in high oleic canola hybrids and also caused a reduction of it in standard hybrids. In fact, an increase in oleic/linoleic acid ratio was already reported under water stress conditions occurring during grain filling in different sunflower genotypes (Baldini et al., 2002). Under saline conditions, both osmotic and toxic stresses do occur and it is possible that water deficit occurring under salt stress might have caused a shortening of the lipid accumulation phase and some damage to all enzymatic activities including that of oleate desaturase. Moreover, toxic stress caused by sodium and chlorine accumulation should not be excluded, as already reported in Flagella et al. (2004).

Conclusion

In conclusion, it can be suggested that, silicium application had positive effect on canola growth under conditions of salt stress also increase ammonium and is better than nitrate in this conditions because ammonium absorption is not related to high energy. In addition, ammonium had positive effect on plant growth.

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