**Effects of Salinity and N on the Growth, Photosynthesis and N Status of Canola (Brassica napus L.)**

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**Abstract**

The effects of NaCl salinity and N on the growth, ion concentrations and photosynthesis (Pn) in three canola cultivars (‘SLM’, ‘Okapi’ and ‘Licord’) were investigated. The experiment was conducted with four NaCl levels (0, 50, 100, and 150 mM) factorially combined with three N levels (100, 200, 300 mg l\(^{-1}\)) as NH\(_4\)NO\(_3\) by adding to the half strength of Hoagland solution. The plants were grown in 121 pots filled with sand and perlite mixture (1:1) for 12 months. Salinity had a significant negative effect on all treatments, although the severity of the effect varied among the cultivars. High concentration of salinity (150 mM) decreased the leaf area by 63%, 68% and 76% in cvs ‘SLM’, and ‘Licord’ and ‘Okapi’ to that in control ([Na, N\(_\text{Na}\)])). The plants growth were improved at 200 mg l\(^{-1}\) N concentration in cvs ‘Licord’ and ‘Okapi’, but it was reduced when the N concentration increased up to 300 mg l\(^{-1}\). The growth of ‘SLM’ progressively increased with the increasing both salinity and N levels. Both Pn and transpiration rate were significantly reduced by the increase of salinity in all three cultivars. Increasing of N levels in the solution had no effect on Pn in various salinity levels in cvs ‘SLM’, and ‘Licord’, but in ‘Okapi’ cv. Pn decreased as N level increased. Salinity in the root zone led to a significant decrease in both K concentration and K/Na ratio in the leaves in all cultivars regardless of the N levels. Within each N level, leaf Na concentration increased and K concentration decreased as salinity concentration in the root zone increased from 50 to 150 mM. Nitrate reductase (NR) activity in 150 mM treated plants decreased by 27%, 58% and 52% in cvs ‘SLM’, ‘Licord’ and ‘Okapi’ respectively. The decreased activity of NR by the increased NaCl was accompanied by a decrease in total N and nitrate uptake. The deleterious effects of salinity on the plants growth appeared to be as the result of the reduction in Pn, K/Na ratio and NR activity in the salinity treated plants. It can be concluded that under salinity conditions increasing N concentration up to 200 mg l\(^{-1}\) in salt-sensitive cultivars to salinity is favorite in counteracting the adverse effects of salinity but the further increase of N concentration (300 mg l\(^{-1}\)) may be ineffective or even harmful for the canola growth. In salt-tolerant cultivars increasing N fertilization can be an effective tool to restore the decreased growth caused by high salinity.

**Keywords**: canola, salinity, nitrogen, photosynthesis, introduction

**Introduction**

Salinization of land has been received more attention because of increasing progressively throughout the world (Munns, 1993; Bybordi et al., 2010a, 1994; Kozlowski, 1997). It is estimated that approximately a third of the world’s irrigated lands and half the lands in semiarid and costal regions are affected by salinization and 10 Million hairrigated lands are abandoned annually because of excessive salinity (Epstein et al., 1980). Of the 1.5 Billion ha that is cultivated, about 5% is affected by salt (Munns, 1993). Hence, it should be found an effective way to use saline lands by the cultivation of tolerant cultivars or other agrotechniques.

The significance of salinity for the agronomical and physiological aspects of plants is enormous. All salts can affect plant growth, but not all inhibit growth. Accumulation of both Na and Cl in the roots and aerial parts is most damaging to the plants often by inhibiting photosynthesis (Pn) (Munns, 1993; Flower and Yeo, 1988; White and Broadley, 2001). In the other cases, Na is the primary cause of ion-specific damage (such as reduction in K activity). The reduction in Pn in the salinity treated plants reported by many researchers (Downton, 1977; Ball and Farquhar, 1984; Behboudian et al., 1986).

Canola is one of the major crops in Mediterranean and in some regions of Middle East. The environmental adaptability of the canola and is tolerance to salinity (has made it possible for most of these new plantations to be established in arid and marginal areas. In the last few decades, much research has been devoted to the interaction between salinity and canola cultivation, and a significant body of information has accumulated on this topic, including descriptive material and data on the characteristics and mechanisms of salt tolerance in the canola (Ayers and Westcot, 1985; Kelin et al., 1994; Bybordi et al., 2010b; Bybordi and Tabatabaei, 2009).

Interaction between salinity and N affects growth and metabolism of plants in order to cope with the changes taking place in their environment (Papadopoulos and Rendig, 1983; Shenker et al., 2003). An apparent increase in salt tolerance has been noted when N levels supplied under saline conditions exceeded those that were optimum under non-saline conditions (Papadopoulos and Rendig, 1983; Bybordi et al., 2010c), implying that increased fertilization, especially N, may ameliorate the del-
eterious effect of salinity (Ravikovitch and Porath, 1967). However, factors involved in salinity-N interaction are not well documented. Reports on the effects of salinity on N metabolism have been focused on nitrate reductase (NR) activity. However, NR activity in plants has frequently been contradictory. NR was slightly inhibited by salinity in tomato roots, while leaf NR decreased sharply (Cramer and Lips, 1995). In the leaves of tomatoes and cucumbers, NR activity increased with exogenous NO$_3^-$ concentration (Martínez and Cerda, 1989).

For the canola there is not enough information concerning N assimilation. Therefore, the objective of this experiment was to determine the influence of different levels of N and NaCl on the growth, Pn and N assimilation. Furthermore, the feasibility of the increased N on the reduction in the opposing effects of salinity was studied.

**Materials and methods**

The canola (Brassica napus L.) cultivars ‘SLM$_{100}$’, ‘Licord’ and ‘Okapi’ were transplanted into the pots filled with sand and perlite (1:1). The pots were kept into the glasshouse with natural sunlight and temperature range 30±3 and 20±3°C in the day and at night, respectively. The experiment consisted of 12 treatments with four salinity concentration as NaCl (0, 50, 100, 150 mM), factorially combined with three N concentrations (100, 200, 300 mg l$^{-1}$) as NH$_4$NO$_3$, each treatment being replicated four times. The solutions were prepared by adding either NaCl or NH$_4$NO$_3$ to half strength of Hoagland solution (Hoagland and Arnon, 1950). The solution pH was adjusted to 6.5 by adding H$_2$SO$_4$. Salt and N treatment were imposed on 1 February 2007, when the plant had developed three shoots of 25-30 cm in length. The plants were irrigated once a day with 15% extra solution to prevent the building up of EC in the root zone.

The leaves removed to measure leaf area and weight. The leaf area was measured using leaf areameter (Li-Cor, model Li-1300, USA). After weighing the leaves, they were dried at 80°C in an air forced oven for 48 h.

Photosynthetic and transpiration rates of the mid-lamina of the youngest fully expanded leaves of two plants from each treatment were measured using a portable photosynthesis meter (Wålz, Model HCM-1000, Germany). The flow rate and PAR were set to 800 min and 1500 μmol m$^{-2}$ S$^{-1}$, respectively. Reference CO$_2$ concentration was set to the inside of glasshouse. The time of measurement was between 9:00 and 14:00.

Nitrate reductase (NR) was measured in the young leaves (third or forth from the top, according to Klöpper et al., 1971). The leaf tissue (0.2 g fw) was placed in reaction mixture containing 0.1 M potassium phosphate buffer (pH 7.5), 0.02 M KNO$_3$, 50% isopropanol, 0.05 chloramphenicol at 30°C for 1 h in the dark. The indicative Grease reagent containing 0.001 g/l naphthyl-ethylene diamine, 0.01 g Sulfanilic acid, and 0.9 g tartaric acid was added to each sample. The concentration of nitrite formed during the reaction was measured spectrophotometrically at 540 nm.

Nitrate in tissue samples was determined by nitrification of salicylic acid (Cataldo et al., 1975) by Perkine-Elmerse, Model 110, USA). Statistical analysis was made using analysis of variance in the SAS 8.2 software and the means were separated by LSD test at 5% level.

**Results**

The vegetative characteristics (leaf area, fresh and dry weight of leaves) as function of N concentration in the solutions at the various concentration of salinity are given in (Tab. 1) leaf area, fresh and dry weight of leaves was significantly reduced by the increasing of salinity (150 mM). However, the reduction in growth varied at 150 mM NaCl according to cultivar so that the highest and lowest reduction was observed in cvs ‘Okapi’ and ‘SLM$_{100}$’, respectively. High concentration of salinity decreased the leaf area by 63%, 68% and 76% in cvs ‘SLM$_{100}$’, ‘Licord’ and ‘Okapi’ compare to that in control (Na$_2$SO$_4$). Plant growth was improved at 200 mg N concentration in cvs ‘Licord’ and ‘Okapi’ but it was reduced when the N concentration increased (300 mg). The growth of ‘SLM$_{100}$’ progressively increased with the increasing both salinity and N levels (Tab. 1). Interactive effect of salinity and N concentration on leaf area was significant in cvs ‘Licord’ and ‘Okapi’. It indicated that increased N level up to 200 mg l$^{-1}$ promote the leaf growth at both high and low salinity concentration. However, more increasing N level (300 mg l$^{-1}$) significantly inhibited the leaf growth in both cvs ‘Licord’ and ‘Okapi’ particularly in salinity conditions. When the salinity was not limiting, the optimum N level varied with salinity. The rate of both Pn and transpiration rate were significantly reduced by the increase of salinity in all three cultivars (Tab. 2). The reduced Pn varied according to cultivar, so that the highest and lowest reduction in Pn was observed in cvs ‘Licord’ and ‘SLM$_{100}$’, respectively. Increasing of N levels in the solution had no effect on Pn in various salinity levels in cvs ‘SLM$_{100}$’ and ‘Okapi’ but in cv ‘Licord’ Pn reduced as N level increased.
Tab. 1. The effect of salinity and N levels on the vegetative characteristics of canola cultivars

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\begin{array}{|c|c|c|c|c|c|c|c|c|}
\hline
\text{Treatment} & \text{Leaf fw (g plant}^{-1}\text{)} & \text{Leaf dw (g plant}^{-1}\text{)} & \text{Leaf area (cm}^2\text{)} \\
\hline
\text{N*NaCl} & \text{SLM} & \text{Licord} & \text{Okapi} & \text{SLM} & \text{Licord} & \text{Okapi} & \text{SLM} & \text{Licord} & \text{Okapi} \\
\hline
\text{Na} & 137.50 & 31.73 & 19.83 & 13.65 & 9.08 & 7.19 & 927.39 & 660.09 & 167.88 \\
\text{Na*NaCl} & 124.98 & 24.36 & 7.01 & 10.30 & 6.84 & 2.34 & 1018.51 & 451.74 & 81.10 \\
\text{Na} & 121.58 & 19.55 & 9.81 & 7.64 & 5.30 & 3.62 & 497.24 & 342.17 & 53.18 \\
\text{Na*NaCl} & 92.80 & 52.07 & 5.42 & 4.39 & 16.83 & 2.09 & 289.19 & 229.83 & 49.16 \\
\hline
\end{array}
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Analysis of variance (F values)

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\begin{array}{cccccc}
\text{NaCl} & 8.5 & 6.3 & 15.4 & 10.7 & 15.6 & 6.4 & 6.0 & 10.2 & 17.1 \\
\text{N} & 3.5^* & 3.7^* & 6.5^** & 4.4^* & 4.4^* & 4.4^* & 1.1ns & 5.6^* & 7.2^* \\
\text{N*NaCl} & 0.2ns & 3.0ns & 9.8^* & 0.6ns & 9.3^* & 2.6ns & 0.1ns & 6.0^* & 11.7^* \\
\end{array}
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* Significance at 0.05 probability level, ** Significance at 0.01 probability level, ns: non significance

The data of leaf ion concentration of the plants in relation to salinity and N levels are presented in (Tab. 3 and 4). Salinity in the root zone led to a significant decrease in both K concentration and K/Na ratio in the plant tissue in all cultivars regardless of the N levels. The lower concentrations of K occurred in the treatments with the highest NaCl and N levels. Difference in K selectivity among cultivars was also observed in the K/Na ratios (Tab. 3). Reduction in leaf K/Na ratio was became more pronounced in cvs 'Licord' and 'Okapi' within each N level, leaf Na concentration increased and K concentration decreased as salinity concentration in the root zone increased from 50 to 150 mM. The interactive effect of salinity and N levels was significant in both cvs 'Licord' and 'Okapi' so that the increased N levels at high salinity concentration reduced K/Na ratio. The highest concentration of Na in the roots was observed in cv. 'Licord' at 150 mM Na concentration.

Tab. 2. The effect of salinity and N levels on the both Pn and transpiration rate of canola cultivars

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\begin{array}{|c|c|c|c|c|c|c|c|c|}
\hline
\text{Treatment} & \text{Pn (μ mol m}^{-2}\text{s}^{-1}\text{)} & \text{Tr (μ mol m}^{-2}\text{s}^{-1}\text{)} & \text{Stom. Con. (μ mol m}^{-2}\text{s}^{-1}\text{)} \\
\hline
\text{N*NaCl} & \text{SLM} & \text{Licord} & \text{Okapi} & \text{SLM} & \text{Licord} & \text{Okapi} & \text{SLM} & \text{Licord} & \text{Okapi} \\
\hline
\text{NaCl} & 6.60 & 5.72 & 4.32 & 1.13 & 0.43 & 1.04 & 10.47 & 3.01 & 5.33 \\
\text{NaCl} & 2.31 & 2.24 & 1.59 & 0.25 & 0.09 & 0.06 & 5.66 & 1.34 & 1.50 \\
\text{NaCl} & 2.10 & 2.3 & 1.83 & 0.08 & 0.09 & 0.05 & 1.77 & 1.63 & 1.13 \\
\text{NaCl} & 1.54 & 1.38 & 0.41 & 0.07 & 0.74 & 0.09 & 1.63 & 1.55 & 1.27 \\
\hline
\text{NaCl} & 3.68 & 3.94 & 1.69 & 0.53 & 0.18 & 0.14 & 6.23 & 1.66 & 5.31 \\
\text{NaCl} & 2.73 & 3.06 & 1.25 & 0.10 & 0.14 & 0.06 & 2.16 & 2.30 & 1.61 \\
\text{NaCl} & 1.67 & 2.36 & 1.10 & 0.05 & 0.10 & 0.05 & 1.14 & 1.18 & 1.19 \\
\text{NaCl} & 1.24 & 1.12 & 1.08 & 0.05 & 0.49 & 0.04 & 1.19 & 2.58 & 1.08 \\
\hline
\text{NaCl} & 3.41 & 1.89 & 1.02 & 0.40 & 0.34 & 0.27 & 5.92 & 1.68 & 1.41 \\
\text{NaCl} & 2.50 & 1.28 & 1.05 & 0.08 & 0.25 & 0.24 & 1.99 & 1.67 & 1.53 \\
\text{NaCl} & 2.40 & 1.07 & 0.93 & 0.08 & 0.16 & 0.28 & 1.76 & 1.16 & 0.88 \\
\text{NaCl} & 1.30 & 0.9 & - & 0.07 & 0.13 & - & 1.50 & 1.01 & - \\
\hline
\end{array}
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Analysis of variance (F values)

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\begin{array}{cccccc}
\text{NaCl} & 15.7^* & 11.5^* & 14.5^* & 13.7^* & 16.8^* & 6.9^* & 26.4^* & 15.4^* & 5.2^* \\
\text{N} & 1.3ns & 1.8ns & 3.5^* & 2.9^* & 3.3^* & 4.4^* & 6.6^* & 2.7^* & 3.6^* \\
\text{N*NaCl} & 1.5ns & 2.7ns & 7.7^* & 1.7ns & 1.6ns & 6.5^* & 1.7ns & 1.2ns & 5.6^* \\
\end{array}
\]

* significance at 0.05 probability level, ** significance at 0.01 probability level, ns: non significance
From the data given in Table 4 on both N and NO$_3^-$ concentrations of all cultivars, it is clear that raising the NaCl concentration in the solution reduced N and NO$_3^-$ concentrations of the leaves. The concentration of N in cv Mission was not affected by N levels but in cvs ‘Licord’ and ‘Okapi’ increasing N levels from 100 to 300 mg l$^{-1}$ increased the N concentration in the leaves, but not statistically significant. Leaf NO$_3^-$ concentrations in all cultivars was increased by raising N level up to 200 mg l$^{-1}$ in the nutrient solution and higher concentration was ineffective in either N or NO$_3^-$ concentration.

NR activity in 150 mM treated plants decreased by 27%, 58% and 52% in cvs ‘SLM’, ‘Licord’ and ‘Okapi’ respectively. Decreased activity of NR by NaCl stress was accompanied by a decrease in total N and nitrate uptake (Table 4). As shown in Table 4, both nitrate and total N concentration as well as NR activity was the highest in NaO and N$_{200}$ treatments and decreased as the salinity increased.
creased. Although a clear relationship was found between Na concentration of leaves and NR activity in all cultivars, the trend of decreasing NR caused by the increased salinity varied among the cultivars. Toxicity symptoms such as dead leaf edge and drop were recorded in cvs 'Licord' and 'Okapi' at 100 mM which became more sever at 150 mM and necrosis of stem tip was observed. The plants treated with Na$_{300}$, Na$_{300}$ in cvs 'Okapi' and 'Licord' completely died at the end of the experiment. No symptoms of toxicity of salinity were observed in cv 'SLM$_{066}$'.

**Discussion**

Canola plants grown in the various levels of salinity were affected differently by the N rising in the nutrient solutions. The decline in leaf growth is an earliest response of the plants to salinity (Munns and Termaat, 1986). Leaf abscission at high salinity (150 mM) occurred in cvs 'Licord' and 'Okapi' to further reduction in leaf area. This may caused by ions accumulation in the leaves, particularly old leaves (Greenway and Munns, 1980). The reduction in plant growth was due to the reduced leaf growth, which agrees with finding of Cramer (2002). At the highest concentration of salinity, however, N concentration not only had no effects on growth promotion, but reduced the plants growth. It implies that other factors (such as osmotic pressure) involved in plant growth. The direct factor might be salinity (such as osmotic effect, Cl or Na toxicity) as reported by Bongi and Loreto (1989); Xu et al. (2000). Reduction in both K and K/Na at high salinity is another opposing effect of salinity, which impairs the function of K in the salinised plants. Devitt et al. (1981) and Jackson and Volk (1997). The increased salinity concentration in roots with increasing salinity in the root zone implies exclusion mechanism. This mechanism is most likely to act in low or moderate salinity as reported by Chartzoulakis et al. (2002). Remarkable genotypic differences were found on Na transport to leaves so that Na concentration of leaves in cv. 'SLM$_{066}$' remained at low level at higher salinity concentration. It could be salt tolerance in canola is associated with the ability to reduce uptake and/or transport of saline ions.

The results clearly showed that a concentration dependent decrease in NR activity, nitrate and N concentration in the leaves. As NR is a substrate inducible enzyme (Marschner, 1995) and is decreased activity under salinization has been attributed by some researchers to decreased NO$_3$ uptake by plants under salt stress (Lacuesta et al., 1990; Abdelbaki et al., 2000). The decreased of NO$_3$ is accompanied by a high Cl uptake (Parida et al., 2004) and low rate of xylem exudation in high osmotic conditions either by NaCl or other nutrients (Tabatabaei et al., 2004). Either the reduced NO$_3$ uptake or translocation leads to lower NO$_3$ concentration in the leaves, consequently reducing NR activity of leaves under salinity conditions. This finding agrees with Cramer and Lips (1995), who indicated that salinity may control NR activity through NO$_3$ uptake since NR activity is largely determined by NO$_3$ flux into the metabolic pool, rather than by tissue NO$_3$ content itself. Most researchers have focused on the inhibitory role of Cl on NR activity, however the effect of Na on NR activity has not been known.

A clear relationship between Na concentration of leaves and NR reduction was observed; suggesting that Na may has an indirect effect on NR activity. It is most likely that in low level of NaCl treatments the increased concentration of N and K in the leaves may be responsible for favorable growth of the plants. In general, the results suggest that high salinity causes a depression in NR activity, nitrate and N concentration and K/Na ratio in salt-sensitive cultivars and the adverse effects of salinity is become more pronounced in both high and low levels of N concentration in the solution. Increasing N from 100 to 200 mg l$^{-1}$ in the solution increases NO$_3$ concentration in the leaves, however it was ineffective at 300 mg l$^{-1}$ in restoring the decreased in the leaf NO$_3$ caused by the increased salinity. This indicates that the effect of salinity could be an independent factor according to cultivar from that of N if that N is not the limited factor. The results clearly demonstrated that under salinity conditions increasing N fertilization in salt-sensitive cultivars is ineffective in counteracting the adverse effects of salinity, which may build up during the growing period or high salinity conditions. However, in salt-tolerant cultivars increasing N fertilization can be an effective tool to restore the decreased growth caused by high salinity.

The lowest inhibition was observed in 'SLM$_{066}$', which had lower Na concentration in the leaves. The positive relationship between K/Na and Pn suggests that the Pn rate is associated with salt content, which disagree with Tatini et al. (1995) who reported that Pn rate is independent from salt content. The dependence of salinity and Pn rate is varied according to cultivar as reported by Chartzoulakis et al. (2002). The reduction of Pn would be partly the rustle of the lower water availability in salinity conditions. Loreto et al. (2002) demonstrated that the limitation of Pn in salinity conditions in canola cultivars as a rustle of the low chloroplast CO$_2$ concentration, caused by both stomatal and mesophyll conductance reduction. As shown in Tab. 2, the reduction of stomatal conductance and transpiration rate in cv. 'SLM$_{066}$' occurred less than those in 'Licord' and 'Okapi', therefore this explain why 'SLM$_{066}$' is apparently salt-tolerant. At high salinity in most cultivars, Na is transported and accumulated to the aerial parts, resulting toxicity symptoms.

The decline of K concentration under salinity conditions has been reported by Greenway and Munns (1980) and Devitt et al. (1981). This was also the case for canola particularly in salt-sensitive cultivars. Hence, maintain of relatively high K concentration or K/Na ratio in the leaves acts an important role in regulation mono-valent cationic osmotic and physiological function of K in either Pn or N assimilation.
References


