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# Effects of Salinity and $NO_3$:$NH_4$ Ratio on Yield and Quality in Canola (*Brassica napus* L.)

Ahmad BYBORDI[1], Jalal TABATABAEI[2], Ali AHMADOV[3]

[1] *Baku State University, Faculty of Biology, 23 Z. Khalilov Street, 370148 Baku, Azerbaijan; abaybordy@yahoo.com*

[2] *University of Tabriz, Faculty of Agriculture, Tabriz,51664, Iran; tabatabaei@tabriz.ac.ir*

[3] *Baku State University, Faculty of Biology, 23 Z. Khalilov Street, 370148 Baku, Azerbaijan; aa-1925@yahoo.com*

**Abstract**

The effect of salinity and $NO_3$: $NH_4$ ratio (0:100, 75: 25, 50:50 and 25:75) in the nutrient solution on growth, yield quality and N metabolism in hydroponically grow canola (*Brassica napus* L.) was evaluation. Both fresh and dry weights of leaves were significantly lower when a high concentration of either $NO_3$ (100%) or $NH_4$ (75%) was the sole N source in the nutrient solution. In nonsaline condition, increasing of both $NH_4$ and $NO_3$ ratio in the nutrient solution reduced photosynthetic (pn) rate, however in salinity condition the reduction of pn became more pronounced at a higher ratio of $NH_4$ in the nutrient solution. The yield in terms of fresh and dry weight of seed per plant was significantly increased at the 75:25 ($NO_3$: $NH_4$) treatments. Total fat in nonsaline condition was increased with increasing $NH_4$ ratio in the nutrient solution, however in saline condition it was reduced, at high $NH_4$ ratio in the nutrient solution. The increase of tissue N concentration was nearly proportional to the $NH_4$ concentration in the nutrient solution.The activity of nitrate reductase (NR) was increased by increasing $NH_4$ form 0 to 50% and then reduced at a higher ratio of $NH_4$ in the solution. Salinity increased $NH_4$ concentration so that the saline condition had nearly twice high $NH_4$ concentration in the leaves. The increase of $NH_4$ concentration induced by salinity could be parially the reduction of $NH_4$ assimilate because of the shortage of carbohydrate.

***Keywords:*** nitrogen form, nutrient solutions, salinity, photosynthesis, canola

## Introduction

Plants take up nitrogen mainly as nitrate and ammonium. When both are present in solution ammonium uptake is preferred. Under salt stress, nitrate uptake is slowed down and salinity reduces $NO_3$ assimilation with the possible consequence of N deficient in the plant (Oliel *et al.*, 2005). The $NO_3$:$NH_4$ ratio in the root zone offers an important means for the controlling of the relative uptake on nutrients (Sonneveld, 2002). Although the major form of N supply in the nutrient solution in the soil- less culture is NO3 based fertilizer, addition of some $NH_4$ to the nutrient solution seems to be beneficial to the plant (Gashaw and Mugwira, 1981)( Sonneveld *et al.*, 1999), and helps to decrease the pH of the solution (Ikeda and Osawa, 1983). The studies show that the response of plants to either $NO_3$ or $NH_4$ concentration varies with the species, temperature, and salinity (Edwards and Horton, 1982). The assimilation of $NO_3$ usually occurs mostly in the leaves of plants, however the stage of $NO_3$ assimilation seem to be linked closely enough to preclude accumulation of $NH_4$ (Pilbeam and Kirkby, 1992). Modification of $NH_4$:$NO_3$ ratio in the nutrient solution modulates the relative uptake of anions and cations (Clay *et al.*, 1984). The increase of $NH_4$ to $NO_3$ ratio in the root zone impairs growth and reduces the yield (Guo *et al.*, 2002). The presence of high concentration of $NH_4$ has been shown to induce a decreased concentration of cations such as K, Ca and Mg, while $NO_3$ has the opposite effect (Alan, 1989; Kotsiras *el al.*, 2002). Under intensive fertigation, nitrogen not only affects plant growth, it may also alter the salinity tolerance of plants depending on its ionic form. The beneficial effects of nitrate under saline conditions have been attributed to the antagonism between $NO_{3-}$ and $Cl^-$ ions (Feigin, 1990). The balance of the cation to anion uptakeratioo by secretion of $H^+$ , $HCO_{3-}$, or organic anion from root cells also has an effect on the rhizospere. Adjusting the $NO_3$:$NH_4$ ratio from the total cation to anion uptake ratio and maintain pH within desired range (Kafkafi *et al.*, 1982).

Whereas growth suppression by ammonium probably results from a shortage of sugar in the roots (Cramer *et al.*, 1995) or from inhibition of nitrate reductase activity in roots and shoots (Botell *et al.*, 1993). Some reports, however, indicate that combined $NH_4$ and $NO_3$ fertilization at an 50:50 ratio results in greater biomass accumulation compared with plants fed with either nitrogen source alone (Lips *et al.*, 1990). In canola, some of the damaging effects of salinity have been averted by partial substitution if nitrate with ammonium in the nutrient solution. In tomatoes, improved plant growth and higher yield were

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obtained with addition of low levels of $NH_4$ to the mainly nitrate containing fertigation solution (Flores *et al*., 2001) Therefore, the objective of this experiment was to assess the effect of combination of salinity and $NO_3$: $NH_4$ ratio on the growth yield and N metabolism in canola.

**Materials and methods**

The experiment was conducted at agricultural research center of Tabriz, Iran. Seedling of canola (*Brassica napus* L. var SLM046,) were planted in the propagation pots. When two leaves were fully expanded, the seedling were transferred to the glasshouse and placed into pots filled with a mixture of perlite and vermiculite (1:1, v/v). For each saline and nonsaline treatment, the experimental design was a completely randomized design with four replicates per treatment. The greenhouse was under natural sunlight during spring and summer and the temperature was set to 22°C±3 and 15°C±3 day and night, respectively. The plants were subjected to two salinity levels (0, 200 mM NaCl). The plants in different saline conditions were fed with one of the following $NO_3$: $NH_4$ ratio : 0:100, 75:25, 50:50 and 25:75. Modifying Hogland's solution (Hoagland and Arnon, 1950) was used to prepare the nutrient solution. The concentration of the nutrients in the solutions was as follow (in $mg.l^{-1}$ ):300K, 170 Ca, 50 Mg. 60 S, 33 P,1.5 B, 0.1 Cu, 2 Mn, 3Zn, 12 Fe (Fe-DTPA), and 0.1 Mo. Nitrogen at 200 mg.l-1 was provided NO3 and NH4 forms to give NO3:NH4 ratio of 100:0, 75:25, 50:50 and 25:75 (Tab. 1). To obtain identical K, Ca, Mg, total N and P concentrations in all treatments, the changes in $NO_3$: $NH_4$ ratios were balanced by varying the Cl concentration. The electrical conductivity (EC) of the nutrient solution was within the range 2.7-2.8 $dS.m^{-1}$. The balance of $NO_3$:$NH_4$ ratio changes by varying the concentration of at least one more nutrient was inevitable due to the charge balance constrain (Schrevens and Cornell, 1993; Savvas *et al*., 2003). However difference in EC within these ranges are unlikely to influence significantly plant growth (Sonneveld *et al*., 1999). The initial pH of the nutrients solutions containing $NO_3$ and $NH_4$ was adjusted to 6.5- 6.8 by adding $H_2SO_4$ (Adams, 2002).

Tab. 1. The concentrations of salts (mM) used to prepare nutrient solutions at $NO_3$ :$NH_4$ ratio of 100:0, 75:25, 50:50 and 25:75.

| Salts | $NO_3$: $NH_4$ ratios in the solutions | | | |
|---|---|---|---|---|
| | 100 : 0 | 75: 25 | 50 : 50 | 25 : 75 |
| $KNO_3$ | 5.7 | 5.4 | 0.0 | 0.0 |
| $Ca(NO_3)_2$ | 4.3 | 2.7 | 3.6 | 1.8 |
| $MgSO_4$ | 2.0 | 2.0 | 2.0 | 2.0 |
| $KH_2PO_4$ | 1.0 | 0.0 | 0.0 | 0.0 |
| $NH_4H_2PO_4$ | 0.0 | 1.0 | 1.0 | 1.0 |
| $NH_4Cl$ | 0.0 | 2.6 | 6.2 | 9.8 |
| KCl | 1.0 | 2.3 | 7.7 | 7.7 |
| $CaCl_2$ | 0.0 | 1.5 | 0.7 | 2.4 |

The Ec and pH of drainage water from pots were checked every week in the drainage water from the pots and an additional solution was applied to minimize Ec and pH changes in the root zone. The concentration of Ca and K in the plant tissues was measured by atomic absorption spectrophotometry (Perkin Elmer, Model 110, USA). The concentration of N and P in the youngest fully expanded leaves were determined by kjeldahl and colorimetric method, respectively, The activing of nitrate reductase (NR) in the young leaves samples was measured according to Gebauer *et al*., (1998) with some modification. At maturity all plants were harvested and seed yield per plant was recorded for each treatment. For the extraction of oil from seed, the dried seeds were grand to a uniform powder and oil was extracted in n-hexane. A weighed quantity (15g) of the seed powder was placed in an extraction thimble and extraction was carried out for 6 hr in a Soxhlet extrator, after which time the oil containing solvent was filtered through whatman No.42 filter paper. The oil was heated in an oven at 60 °C to remove last traces of the solvent. The leaf area was measured using a leaf area- meter (Licor, Model Li-1300, USA). After being weighed, the leaves were dried at 80 °C in an air-forced oven for 48.h. The photosynthetic (Pn) rate of the mid- lamina portion of the youngest fully expanded leaves (two leaves) from each experimental unit was measured with a portable photosynthesis-meter (Walz, Model Da-1010, Germany) from 9:00 to 12 :00 a.m. Reference $Co_2$ concentration was set to the inside glasshouse. The rates of Pn were measured at 450 $\mu l.l^{-1}$ $Co_2$ concentration and 800 µmol.m-2.S-1 PAR. The leaf chambers temperature was set up to 28°C and relative humidity was ranged from 70 to 80%. The data were subjected to analysis of variance (ANOVA) using generalized linear models by using the SAS 8.2 software package. The significance of the treatment effects presented as , not significant; * $P<0.05$, ** $P<0.01$. In case of significant treatment effects, comparison of means was performed using LSD at a significance level of 0.05.

**Results and discussion**

The vegetative characteristics as a function of various N forms in the nutrient solution at the salinity levels of canola plants are given in Tab. 2. Both fresh and dry weight of leaves were significantly lower when a high concentration of either $NO_3$ (100%) or $NH_4$ (75%) was the sole N Source in the solution. The highest both leaf number and leaf area were observed in 25:75 and 50:50 ($NO_3$: $NH_4$) treatment. In both saline and nonsaline condition, the index of chlorophyll was increased with increasing NH4 ratio in the solution.

Within each column, same letters indicate no significant difference between treatment ($P<0.05$).

Tab. 2. The effects of salinity and $NO_3$: $NH_4$ ratio on the vegetative characteristics of canola plants

| Salinity | $NO_3$:$NH_4$ ratio | Leaf fresh weight (g.plant$^{-1}$) | Leaf dry weight (g.plant$^{1}$) | Leaf area (cm$^2$) |
|---|---|---|---|---|
| Non-saline | 100:0 | 273.8 b | 39.3 b | 1876 b |
| | 75:25 | 286.5 a | 43.6 a | 1988 a |
| | 50:50 | 288.7 a | 44.8 a | 1996 a |
| | 25:75 | 274.4 b | 38.7 b | 1867 b |
| Saline condition (200mM NaCl) | 100:0 | 169.3 c | 36.9 c | 8372 c |
| | 75:25 | 183.2 b | 39.3 b | 9928 b |
| | 50:50 | 185.1 b | 43.7 | 1160 ab |
| | 25:75 | 170.3 c | 35.5 d | 7687 d |

There was a significant reduction of vegetative characteristic of canola plant in saline condition (Tab. 2). Leaf area in saline condition was only 84% of that in nonsaline condition. In nonsaline condition, the increased both $NH_4$ and $NO_3$ ratio in the nutrient solution reduced pn rate. In saline conditions, the reduction in pn rate was severely impaired at a higher ratio of $NH_4$ in the nutrient solution. The yields in terms of fresh weight per plant were significantly increased at the 75:25 and 50:50 ($NO_3$: $NH_4$) treatment (Tab. 2). Both high ratios of $NH_4$ and $NO_3$ reduced fresh weight per plant. Fresh and dry weight of seeds in the saline condition was reduced by 25% and 29.5%, respectively, as compared to nonsaline condition. Fat percent was increased in nonsaline condition in (50:50), (75:25) ($NO_3$:$NH_4$) treatments Higher availability of carbohydrate was the result of the increased pn rate and fat. The concentration of Ca in the seeds was significantly reduced in 25:75 ($NO_3$:$NH_4$) treatment (Tab. 3). The data of leaf ions concentration of the plants relative to salinity and ($NO_3$: $NH_4$) ratio are presented in (Tab. 3).

Within each column, same letters indicate no significant difference between treatment ($P<0.05$).

The increase of tissue N concentration in the nutrient solution. Both high concentrations of $NO_3$ was observed in 75:25 and 100:0 ($NO_3$:$NH_4$) treatments. Salinity significantly increased $NH_4$ concentration. High ratio of $NH_4$ in the nutrient solution and salinity increased Ca concentration in the leaf tissue (Tab. 4). The activity of NR was increased by increasing $NH_4$ from 0 to 50% and then reduced at a higher ratio of NH4 in the solution (Tab. 4). The highest NR activity was observed in 50:50 ($NO_3NH_4$) treatment. The vegetative characteristics of canola were affected by the ratio of $NO_3NH_4$ in the nutrient solution and salinity. Increasing concentration of bath $NH_4$ and $NO_3$ significantly reduced the canola plant nonsaline condition. This reduction became more sever in saline condition. There was a reduction in the leaf area (40.5%) in nonsaline and saline plants when grown in 75% $NH_4$ compared with plants grown in 50% $NH_4$ our results are disagreement with the earlier report by Claussen (2002) and Tabatabaei *et al.* (2006) who showed that the $NH_4$ form of N caused a decrease in dry weight in tomato, but led to an increase in dry weight when supplied besides $NO_3$.

Within each column, same letters indicate no significant difference between treatment ($P<0.05$).

This experiment clearly demonstrated that both high concentration of $NO_3$ and $NH_4$ has an inhibitory effect on growth of canola and it becomes more pronounced in the salinity conditions. Many researchers (Gashaw and Mugwira, 1981; Salsac *et al.*, 1987) have reported the beneficial effects of two forms of N in an appropriate ratio on the growth of different plants. Lesss growth of plants fed with high concentration of $NH_4$ may be due to the unavailability of $NO_3$ as a N source and higher demand of carbohydrates channeled for $NH_4$ assimilation and detoxification. On the other hand, complete exclusion of $NH_4$ from the solution reduced the canola plant growth. Both fresh weight and reduced under 100:0 ($NO_3$:$NH_4$) and 25:75 ($NO_3$: $NH_4$) ratio and became more pronounced in salinity. The possible explanation is that high concentration of $NH_4$ reduces the uptake of cations such as Ca and K and increase the concentration of $NH_4$, which is toxic for the plant (Sasseville and Mills, 1979; Alan, 1989). The higher reduction of fresh weight in saline conditions may be due to increased $NH_4$ concentration and reduced carbohydrate level.

It is well known that salinity reduces photosynthesis, carbohydrate levels, and plant weight in various of species of crop.

Tab. 3. The effects of salinity and $NO_3$: $NH_4$ ratio on the concentration of mineral nutrients

| Salinity | $NO_3$: $NH_4$ ratio | $NO_3$(mg.g$^{-2}$) | N(mg.g$^{-1}$) | P(mg.g$^{-1}$) | K(mg.g$^{-1}$) | $NH_4$(mg.g$^{-1}$) |
|---|---|---|---|---|---|---|
| Non-saline | 100:0 | 3.7 a | 32.8 c | 3.0 bc | 44.0 b | 1.7 c |
| | 75:25 | 3.6 a | 34.6 b | 6.0 a | 47.0 a | 1.9 b |
| | 50:50 | 2.5 b | 36.4 a | 9.0 a | 48.5 a | 2.5 b |
| | 25:75 | 2.3 b | 39.4 a | 8.0 a | 41.3 b | 3.6 a |
| Saline condition (200mM NaCl) | 100:0 | 4.5 a | 32.4 c | 2.5 c | 64.5 a | 2.3 b |
| | 75:25 | 3.8 a | 34.6 b | 4.5 b | 57.5 a | 2.9 b |
| | 50:50 | 3.8 a | 40.4 a | 4.0 b | 50.2 a | 4.2 a |
| | 25:75 | 2.8 b | 42.4 a | 9.8 a | 44.6 b | 4.5 a |

Tab. 4. The effects of salinity and $NO_3$: $NH_4$ ratio on pn, Ca concentration and Nitrate reductas activity

| Salinity | $NO_3$: $NH_4$ ratio | Photosynthetic Rate (μMol.$m^{-2}$ $s^{-1}$) | Ca concentration (mg.$g^{-1}$) | Nitrate reductase activity | Activity fat (%) |
|---|---|---|---|---|---|
| Non-saline | 100:0 | 10.4 b | 4.3 d | 1.8 c | 38 bc |
| | 75:25 | 12 ab | 9.2 b | 2.4 bc | 40 ab |
| | 50:50 | 13.3 a | 8.5 bc | 3.2 ab | 41 a |
| | 25:75 | 10 b | 3.8 d | 1.6 c | 39 b |
| Saline condition (200mM NaCl) | 100:0 | 6.4 d | 7.2 c | 2.7 b | 35 |
| | 75:25 | 8.2 c | 11.7 a | 2.8 b | 38 bc |
| | 50:50 | 9.5 bc | 10.7 ab | 3.8 a | 39 b |
| | 25:75 | 6.2 d | 6.4 c | 1.7 c | 34 c |

Novoa and loomis (1981) proposed that the demand for N is determined by growth rate and the N composition of new tissue. Because of the relation between N form and photosynthesis, it has been suggested that the N concentration of leaf is adjusted to the irradiance experienced during growth to make full use of the intercepted radiation. In this experiment, the decrease of dry weight in both saline and nonsaline conditions was nearly proportional to the leaf area, as total photosynthesis sources, in higher $NH_4$ concentration in the nutrient solution. Leaves are the main photoassimilates sources for the plant (Taiz and Zeiger, 2002) and the biomass production is associated with both leaf area and the pn rate. A reduction in pn rate at the high ratio of both $NH_4$ and $NO_3$ concentration occurred.

This result agrees with the finings of Takacs and Tecsi (1992), who reported a reduction of pn in $NH_4$ fed plants. The increased activity of pn enzymes with $NH_4$ as opposed to $NO_3$ nutrition is generally the results of increased protein content of $NH_4$ fed plants (Golvano *et al.*, 1982). Some researchers have argued that the accumulation of NH4 in the leaves may cause uncoupling of the electron transport from photosphorylation in chloroplast, resulting in decreasing photosynthetic rate ( Puritch and Baker, 1967, Claussen 1986; Rothstein and Cregg, 2005).

The inhibition of pn by toxicity of $NH_4$ has been reported by many researchers (Givan, 1979; Frantz *et al.*,1982).The higher demand for carbon skeletons to detoxify excess $NH_3$ in plants supplied with $NH_4$ as sole N source imposes an opposing effect on plant growth (Beevers and Hag eman. 1969).Moreover, at high external $NH_4$ concentration, the uptake of N predominantly in cationic form suppressers the total anion uptake; this process is balanced by excessive H+ secretion from the root cells (Kirkby and Knight, 1977; Pilbeam and Kirkby, 1992). Consequently, the pH in the root environment may decline to level that are detrimental for most plant species. Although the reduction in pn at high $NH_4$ concentration has been reported in many plants, the reason for the reduced pn at high concentration of $NO_3$ has not been found. It seems that higher concentration of $NO_3$ has an inhibitory effect on pn rate. It is well known that highest growth rates are archived when an adequate supply balanced the sucrose supply of amino acids. This means that under good pn conditions a high N supply along with proper N form is required for best growth. Fresh and dry weight were in nonsaline conditions in 50:50($NO_3$:$NH_4$) treatment. Higher availability of carbohydrate was the result of the increased pn rate and higher leaf area led to better growth of nonsaline condition in 50:50 ($NO_3$:$NH_4$) treatment. Lower yield by plants under saline rendered low photosynthetic activity. This became more severest the increased concentration of $NH_4$ in the root zone (Claussen and lenz, 1999, Grindly,1997).

Both salinity and the ratio of $NO_3$:$NH_4$ in the nutrient solution altered the nutrient concentration in the leaves. The greater concentration of total N and $NH_4$ was observed with increasing $NH_4$ concentration in the nutrient solution . This is consistent with the finding lorenzo *et al.* (2000). Who reported that the addition of ammonium in a nutrient solution produced as total nitrogen uptake increase during shoot elongation of rose, while without ammonium, nitrate uptake was lower during shoot elongation. Unfavorably high concentration of $NH_4$ in the nutrient solution may lead to $NH_4$ toxicity, which considered to be the result of effects such as $NH_4$ induced nutrient deficiency caused by impaired uptake of ions, acidification of the root zone , alteration in the osmotic balance, modification of phytohormone, and impaired of N enzyme metabolism (Gerendas *et al.,* 1997; Lorenzo *et al.*, 2000). It is well known that $NH_4$ nutrition leads to the acidification of the root zone, however in this experiment the pH of solution was controlled to eliminate this factor as an impact on plant growth. Plants take up nitrogen mainly as nitrate and ammonium. (Mengel and Viro 1978; Sawas, *et al.*, 2003). $NH_4$ also reduces the uptake of Ca and K by the roots. The activity of NR in the canola plants was affected by $NO_3$: $NH_4$ ratio. An important prerequisite of adaptation of plant species and cultivars to certain environmental conditions are enzymes that are capable for catalyzing nitrogen reduction and assimilation (Lee and Stewart; 1978, Runge, 1983). The activity of NR has often been shown to decrease dramatically if plants were fed with ammonium instead of nitrate nitrogen (Claussen, 1986). A positive correlation between NR activity and $NO_3$ concentration in the leaf tissues implies that the activity of NR is induced by increasing $NO_3$ concentration, as it has been reported by Sivaasankar and Oaks (1996).

**Conclusions**

The data present in this paper suggest that salinity has effected on the yield of canola and its effects is modulated by N form in the nutrient solution. Use of 50:50 or 75:25 ($NO_3$:$NH_4$) ratio in root to cause increasing yield of canola plant in saline and nonsaline conditions.

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