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INTERVARIETAL DIFFERENCES IN NITROGEN CONTENT AND NITRATE ASSIMILATION IN WHEAT (*TRITICUM AESTIVUM* L.) UNDER SALT STRESS

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Abstract

Effect of five NaCl salinity treatments (0, 50, 100, 200 and 300 mM) given at different growth and developmental stages on total N level and *in vitro* nitrate reductase activity (NRA) in leaves and roots of two wheat cultivars viz., Ghods and Boolani was studied in greenhouse conditions. In response to salinity treatments, total N concentrations and NO_3^- assimilation rates in Ghods, a salt-sensitive cultivar, were suppressed more than that of Boolani, a salt-resistant cultivar. However, not much differences in root N content were found between the cultivars. Our data suggest that NRA is affected under the influence of salt stress more than that of N concentration. In addition, there was no clear correlation between N levels and the activity of NR, particularly in leaves. The effect of salinity stress on N content and NRA depends on the cultivar, organ, developmental stage and degree of salt stress.

Introduction

The physiology of plant responses to salinity and their relation to salinity resistance have been much studied and frequently reviewed in recent years (Neuman, 1997). Since plants vary widely in their nutrient requirements and in their ability to absorb specific nutrients, the effect of salinity on plant nutrition differs makedly among species (Hamada *et al.*, 1992). Among the many nutrients required for the growth and development of plant cells, nitrogen is of special importance (Botella *et al*., 1993). Nitrogen comprises of up to 7% of the total dry matter of higher plants and is a constituent of many fundamental cell components such as nucleic acids, amino acids, and hence enzymes, and photosynthetic pigments (Bungard *et al.*, 1999). The evidence available suggests that the metabolism of nitrogen compounds plays a key role in the ability of plants to tolerate salinity (Botella *et al*., 1993).

Sodium chloride salinity can cause osmotic stress and specific salt toxicity by excessive accumulation of salt in plant tissues, including adverse secondary effects on nitrogen metabolism parameters, such as nitrogen uptake and NRA. Nitrogen uptake rates in plants have been found to decrease with high concentrations of NaCl salinity (Huang *et al.*, 1993).

Nitrate reductase is considered to be a limiting factor for growth, development and protein production in plants, because its activity changes directly and affect plant growth (Zornoza & Gonzales, 1998). This first enzyme of nitrate assimilation is well known to be influenced by external conditions, such as low temperature, salinity or osmotic stress (Botella *et al*., 1993). In higher plants, NR is an oligomeric and NAD (P) H-dependent complex containing FAD, heme (cytochrome b_{557}) and Mo-pterin prostetic group (Kenjebaeva & Rakova, 1995). The present paper reports the effect of NaCl stress on N level and NR activity in two wheat cultivars viz., Ghods and Boolani which were chosen for their difference in sensitivity to NaCl salinity.

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Table 1. Effect of different salinity treatments on leaves and roots N levels in two wheat cultivars.

NaCl Treatments (mM)	Leaves				Roots			
	A	B	C	D	A	B	C	D
0	GH:3125^{ij}	3145.1^{ij}	3152.5^{ij}	3137.6^{ij}	2825.7^{de}	2840.1^{de}	2839.6^{de}	2830.2^{de}
	BO:3004.5^{mn}	3025.1^{l-n}	3036.6^{l-n}	3015.2^{mn}	2831.1^{de}	2849.4^{de}	2852.1^{de}	2843.6^{de}
50	GH:3134.4^{ij}	3147.2^{ij}	3144.4^{ij}	3136.9^{ij}	2816.5^{d-f}	2839.9^{de}	2843.1^{de}	2832.5^{de}
	BO:3192.2^{hi}	3031.3^{l-n}	3034.7^{l-n}	3013.7^{mn}	2801.5^{d-f}	2885.6^{cd}	2861.9^{c-e}	2848.4^{cd}
100	GH:3437.5^{ef}	3158.2^{ij}	3089.2^{j-l}	3132.1^{ij}	2810.6^{d-f}	2887.7^{cd}	2848.2^{de}	2836.8^{de}
	BO:3456.1^{ef}	3426.1^{f}	2975.9^{n}	3008.4^{mn}	2774.4^{d-f}	3162.8^{a}	2864.5^{c-e}	2851.4^{de}
200	GH:3109.5^{jk}	3239.4^{h}	3309.9^{g}	3129^{ij}	2805.3^{d-f}	2826.6^{de}	2853.8^{c-e}	2838.5^{de}
	BO:3570.2^{d}	3492.5^{e}	3149.5^{ij}	3003.3^{mn}	2548.4^{h}	2814.3^{d-f}	2873.5^{c-e}	2854.7^{c-e}
300	GH:2991.1^{mn}	3050.7^{k-m}	3782.8^{b}	3012.2^{mn}	2656.2^{g}	2746.2^{e-g}	2811.2^{d-f}	2971.7^{bc}
	BO:4206.3^{a}	3630.1^{c}	3400.9^{f}	2990.1^{mn}	2698.1^{fg}	2794.4^{d-f}	2795.1^{d-f}	3013.9^{b}

Abbreviations: A-22DAS; B-45DAS; C-58DAS; D-69DAS; GH-Ghods; BO-Boolani. The means that are not followed by the same letters are significantly different at the 1% level using Duncans Multiple Range Test (DMRT).

Table 2. Effect of different salinity treatments on leaves and roots *in vitro* NRA in two wheat cultivars.

NaCl Treatments (mM)	Leaves				Roots			
	A	B	C	D	A	B	C	D
0	GH:9.3^{e-h}	8.5^{f-j}	7^{h-q}	6.2^{j-r}	5.2^{b-e}	4.8^{c-f}	4.2^{d-h}	2.5^{j-m}
	BO:9.9^{d-g}	9.3^{e-h}	8.1^{g-k}	7^{h-q}	4.4^{d-g}	3.9^{e-j}	3.3^{g-m}	2.2^{lm}
50	GH:10.7^{d-f}	7.1^{h-p}	6.7^{i-q}	5.8^{k-s}	4.1^{e-i}	5.2^{b-e}	4.4^{d-g}	2.8^{h-m}
	BO:11.2^{c-e}	8.9^{f-l}	7.9^{g-l}	6.8^{i-q}	3.9^{e-j}	6.3^{b}	3.5^{f-m}	2.3^{lm}
100	GH:11.9^{b-d}	5.3^{n-s}	6.5^{i-r}	5.6^{l-s}	3.6^{f-l}	5.8^{bc}	3.9^{e-j}	3^{g-m}
	BO: 13.1^{a-c}	7.8^{g-m}	7.7^{g-n}	5.4^{m-s}	3.2^{g-m}	7.8^{a}	5.9^{bc}	2.6^{j-m}
200	GH: 13^{a-c}	4.2^{r-u}	6.4^{j-r}	5.1^{o-t}	3.1^{g-m}	4.1^{e-i}	3.8^{e-k}	3.3^{g-m}
	BO: 13.9^{ab}	7^{h-q}	7.5^{h-o}	4.7^{p-t}	2.7^{i-m}	3.1^{g-m}	3^{g-m}	2.8^{h-m}
300	GH:4.6^{q-t}	3.5^{s-u}	2.1^{u}	2.8^{tu}	2.7^{i-m}	3.5^{f-m}	2.5^{j-m}	5.9^{bc}
	BO:14.8^{a}	6.1^{j-r}	4.6^{q-t}	5^{p-t}	2.4^{k-m}	2.7^{i-m}	2.1^{m}	5.5^{b-d}

Abbreviations and descriptions as in Table 1.

Materials and Methods

Two wheat cultivars viz., Ghods, salt-sensitive and Boolani, salt-resistant were used. Uniformly large seeds, free of visible injury or disease, were hand selected and surface-treated with vitavax to slow down fungal growth (Dell'Aquilla & Spada, 1993).

Plants were grown in plastic pots containing 3kg mixture of medium textured soil, sand and manure in a ratio of 2:1:1, respectively. At tillering and boot swollen, plants were irrigated with a nutrient solution containing 200 ppm N, 92 ppm P, and 200 ppm K used added as NH_4NO_3, H_3PO_4, and KNO_3, respectively (Rascio *et al.*, 1992). The pots were kept in a completely randomised design in a controlled environment greenhouse (25 and 20±1°C day and night temperature, respectively; light intensity 400 $\mu molm^{-2}s^{-1}$ with 16/8 light/dark period, and 40-45% RH). In each pot, 10 seeds were planted but after 16 days of sowing, plants were thinned to 4 per pot. Plants were irrigated twice a week. Five levels of salinity (0, 50, 100, 200 and 300 mM NaCl) were applied (Huang *et al.*, 1993). Treatments were given at tillering, boot swollen, emergence of inflorescence (flowering), and anthesis (22,45,58,and 69 DAS) according to Zadoks code (Zadoks *et al.*, 1974). Plants were grown in saline soil for a week and then harvested (Nicolas *et al.*, 1993). Plants without salt addition served as controls. Sampling was performed from roots and sixth (22 DAS) seventh (45 DAS) and flag leaves (58 and 69 DAS).

Roots were washed by tap water, followed by distilled water and then freeze-dried (Botella *et al., 1*993). Leaves and roots were kept at- 20°C (Huang *et al.*, 1993). NR extraction and its activity assay were carried out according to the method described by Botella *et al. (*1993). NRA is expressed as $\mu mol\ NO^-_2h^{-1}g^{-1}$ Fw. Extraction and measurement of total N concentration were done using the method of Podwyszynska & Olszewski (1995) and Weatherburn (1967), respectively.

The activity of NR and N level were analyzed with analysis of variance (ANOVA) for factorial design (factor A= salinity treatment, factor B=cultivar, factor C= growth stage) with 3 replicates using Anova program of MSTATC version 2 package. The results were subjected to a statistical analysis using Duncan's Multiple Range Test (DMRT).

Results

The influence of various NaCl treatments on total N levels in foliar and root is shown in Fig.1 and Table 1. *In vitro* NRA changes in leaves and roots, as a response to salinity are shown in Fig. 2 and Table 2.

Tillering stage: In Ghods, lower NaCl applications (50 and 100 mM) increased foliar N content. However, it was decreased by the higher NaCl levels (200 and 300mM) ($P \leq 0.01$). Thus, the greatest loss of leaf N concentration, 96% of the controls, was found at 300 mM NaCl. Root N level was declined under the influence of salinity ($P \leq 0.01$). At 300 mM NaCl, it decreased to 94% of the controls. 50, 100 and 200 mM NaCl treatments had statistically similar effects on the loss of root N. Foliar NRA was enhanced by NaCl salinity ($P \leq 0.01$), except at 300mM NaCl that showed a decline to 44%. In both cultivars, root NRA was inversely related to the NaCl concentrations. Thus, Ghods and Boolani showed about 50% reduction in the activity of root NR when exposed to 300 mM NaCl.

In Boolani, foliar N content increased with rise in NaCl concentrations ($P \leq 0.01$) and at 300 mM NaCl, it was enhanced by 140%. On the contrary, NaCl stress resulted in decreased root N level. Maximum reduction of 98%, was observed at 200 mM NaCl treatment. 50 and 100 mM NaCl had statistically similar effects on the decrease of root N content.As the NaCl level rose, foliar NRA showed an increase ($P \leq 0.01$) which was enhanced by 150% at 300mM NaCl treatment (Figs. 1, 2 and Tables 1, 2).

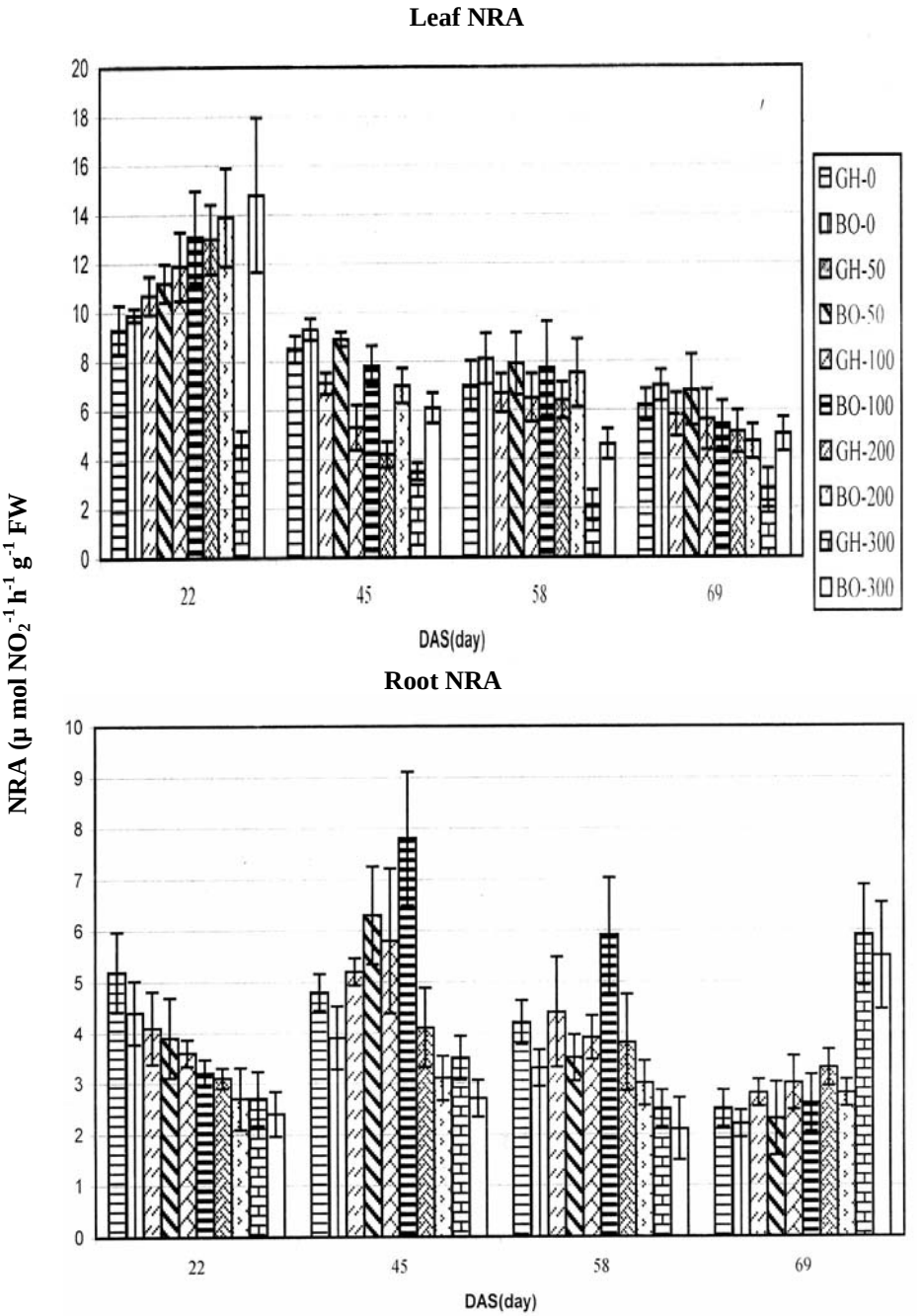

Fig. 1. Effect of 5 levels of NaCl treatments on N contents in leaves and roots of Ghods and Boolani. GH, Ghods; BO, Boolani; 0, no NaCl; 50,50 mM NaCl; 100, 100 mM NaCl; 200, 200 mM NaCl; 300, 300 mM NaCl.

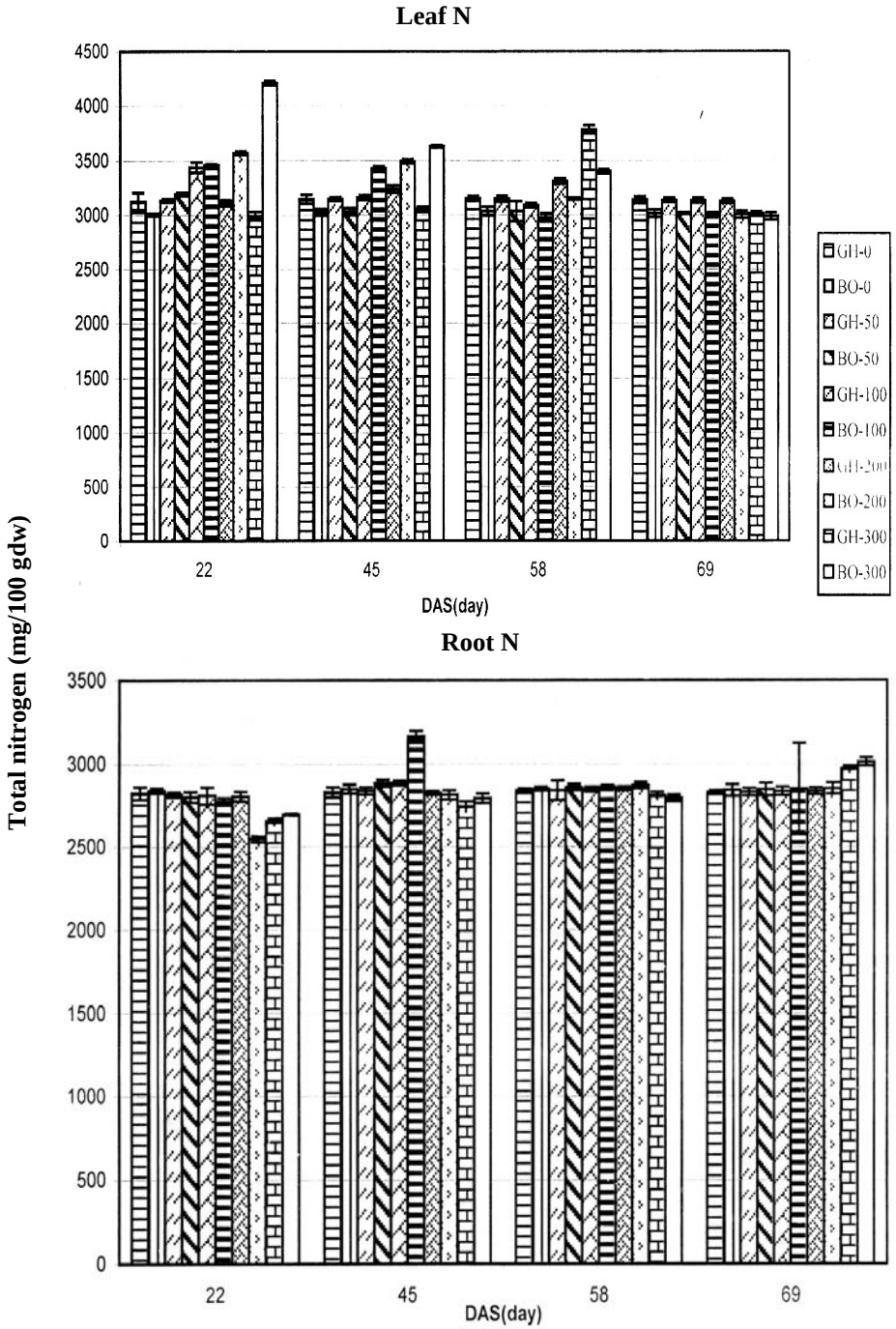

Fig. 2. Effect of 5 levels of NaCl treatments on *in vitro* NRA in leaves and roots of Ghods and Boolani (symbols and abbreviations as in Fig. 1).

Boot swollen stage: In Ghods, foliar N level was enhanced with 50,100 and 200 mM NaCl, but it was decreased at 300 mM NaCl treatment ($P \leq 0.01$). There was no statistical difference between 50 and 100 mM NaCl treatments. Root N content was increased in the presence of 50 and 100 mM NaCl, but it declined with 200 and 300 mM NaCl ($P \leq 0.01$). In both cultivars, NRA in leaves was negatively altered by salt concentrations. Thus, at 300 mM NaCl, in Ghods and Boolani, foliar NRA decreased to 41% and 66%, respectively. The presence of NaCl at the lower concentrations of 50 and 100 mM increased root NRA in both the cultivars. This rise was not observed at 200 and 300 mM NaCl treatments. At 300mM NaCl, NRA in roots of Ghods and Boolani decreased to about 70%.

In Boolani, increased levels of NaCl showed an increase in leaf N content ($P \leq 0.01$). Thus, the greatest foliar N level, 120% of the controls, was observed at 300 mM NaCl. Foliar N concentration was statistically unaffected by 50 mM NaCl. Root N level was enhanced by 50 and 100 mM NaCl, but it decreased at 200 and 300 mM NaCl (Figs. 1,2 and Tables 1, 2).

Flowering stage: As shown in Figs. 1 & 2, in both the cultivars, flag leaves N content decreased at the lower NaCl applications (50 and 100 mM) but at the higher salinity levels (200 and 300 mM), it began to increase ($P \leq 0.01$). At 300 mM NaCl, in Ghods and Boolani, foliar N levels were enhanced by 110% and 120%, respectively. In Ghods, similar to tillering stage, 50 mM NaCl treatment had statistically no effect on foliar N level. Root N content was enhanced at 50, 100 and 200 mM NaCl. However, the increase at 50 and 100mM NaCl treatments was not significant. At 300 mM NaCl, root N level was decreased to 98%. In both the cultivars, the loss of leaf NRA in response to salinity was greater than that of boot swollen. At 300 mM NaCl, foliar NO_3^- reduction levels in Ghods and Boolani, declined to 30% and 57%, respectively. In Ghods, root NRA was enhanced at 50 mM NaCl, but it declined at 100, 200 and 300 mM NaCl treatments ($P \leq 0.01$). At 300 mM NaCl, root NRA decreased to 59%. There was no marked difference between 100 and 200 mM NaCl treatments in their effect on the decrease of root NRA (Tables 1, 2).

In Boolani, 50,100 and 200 mM NaCl caused a statistically similar rise in root N level, but 300 mM NaCl resulted in loss of its content to 99%. Root NRA was enhanced at 50 and 100 mM NaCl, but 200 and 300 mM NaCl caused a decrease in its level ($P \leq 0.01$). At 300 mM NaCl, root NRA declined to 64% (Figs. 1, 2 and Tables 1, 2).

Anthesis stage: In Ghods, a negative correlation was found between flag leaves N content and NaCl concentrations. At 300 mM NaCl, foliar N level decreased to 96%. At 300 mM NaCl root N content was enhanced by 105%. Both in leaves and roots, 50,100 and 200 mM NaCl had no significant effect on N content. In both cultivars, the highest values of foliar NRA were found in control plants. In Ghods and Boolani, 300 mM NaCl treatment inhibited NRA to an extent of 45% and 71%, respectively. In contrast, root NRA progressively enhanced with an increase in concentration of NaCl ($P \leq 0.01$). Thus, at 300 mM NaCl, in Ghods and Boolani, root NRA was stimulated by 240% and 250%, respectively. Similar to flowering stage, 100 and 200 mM NaCl had statistically similar effects on root NRA in Ghods cultivar. In Boolani, flag leaves N content showed a decrease with the addition of NaCl. However, this loss was not statistically significant. Furthermore, 50 and 100 mM NaCl treatments did not markedly affect root N content,

while at 200 and 300 mM NaCl, it was enhanced ($P \leq 0.01$). At 300 mM NaCl, root N level was increased by 106% (Figs. 1, 2 and Tables 1, 2).

Discussion

The increase in foliar N level during tillering under the influence of salinity, according to Huang *et al*., (1993) is due to a leaf growth limitation by osmotic stress and salt toxicity, and therefore a reduced sink demand. However, in wheat variety Ghods, at tillering, the loss of foliar N concentration was observed at 200 and 300mM NaCl. This reduction according to Roggate *et al*., (1999) can lead to a larger decrease in leaf-elongation rate and a decrease in final leaf size. Similar to our results, Huang *et al*., (1993) have reported the loss of root N (or nitrate) uptake in NaCl-treated plants during vegetative growth stage (tillering). Aslam *et al.*, (1984) reported that this decrease was caused by antagonistic interaction between Cl^- and NO_3^- in their uptake processes in root. They have also shown that NO_3^- uptake is inhibited by salinity through a restriction of the NO_3^- transporter activity,which is more sensitive to Cl^- than Na^+.

During anthesis, the increase in root N concentration and loss of flag leaves N level are in good agreement with the experimental results of Huang *et al*., (1993). They found that these changes are due to a decrease in N transport from root to shoot. According to Helal *et al*., (1975) and Cramer *et al*., (1995), lower accumulation of foliar N correlates with lower N uptake rates in salinized than in control plants. Erskine *et al*., (1996) have reported that NO_3^- accumulated in roots under salt stress may act as an osmoticum and thus facilitate the adjustment to low soil water potentials. The same nitrate pool may then function as a quantitatively significant internal source of N for growth.

Reports in the literature concering the effect of salinity on NRA are conflicting (Cramer *et al*., 1995). Both inhibition and stimulation have been reported (Gouia *et al*., 1994). As shown by Kondrat'ev *et al*., (1995), in control plants, the loss of foliar NRA at later growth stages (particularly anthesis) was related to inner (senescence), and not external (nitrate presence) factors.

The physiological comparison carried out in the present work showed that the loss of NRA in leaves was accompained by its increase in roots (Fig.2). This finding is consistent with those reported by Kondrat'ev and Lebedinskaya (1995). They found that a mechanism evidently operates in plants to maintain the required capacity for nitrate assimilation. In the present study we observed that in control plants, NRA in leaves was more than that of roots. These results are in consistence with the findings of Botella *et al*., (1993) who have reported that in cereals more of NO_3^- reduction occurs in leaves.

NO_3^- reduction in leaves was lowered by exogenous NaCl in a higher proportion than that of roots (Fig. 2). Gouia *et al*., (1994) have reported that the main factor that represses NRA in leaves may be the effect of decreased xylem transport rate of NO_3^- to shoot on decreasing availability of NO_3^- at the site of reduction. Botella *et al*., (1993) believed that foliar NRA decrease may be due to an excessive Cl^- shoot tissue content which affect directly nitrate transport from vacuole to cytoplasm, since NO_3^- is considered to be stored in vacuole, but NR is a cytoplasmic enzyme. The differences in NRA between saline treatments were more pronounced than those between the cultivars (Table 2). This infers NRA to be trait that is not under straight genetic control, environmental factors being more important (Kondrat'ev *et al*., 1995).

Most of the authors did not find a clear relation between N levels in organs and NRA (Botella *et al*., 1993). In the present study, some changes of NRA occurred independently of total N concentrations. In roots, NRA was highly correlated with N levels more than that of leaves. This was more evident in Boolani than in Ghods. In leaves, a direct correlation between N content and NRA was found only at tillering and then an inverse relation was observed between them. The results of the present study would suggest that under the influence of salinity treatments in general, (1) there were not much differences in root N level between the cultivars, (2) NRA was affected more than that of N content, (3) NaCl altered the activity of NR and N content in leaves more than that of roots, (4) the effect of salt stress on N level and NRA varied with cultivar, organ, developmental stage, and degree of salt stress and (5) Compared to Ghods, Boolani has generally more ability in maintaining higher NRA and N level.

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References

Aslam, M., R.C. Huffaker and D.W. Rains. 1984. Early effects of salinity on nitrate assimilation in barley seedlings. *Plant Physiology*, 76: 321-325.

Botella, M.A., C. Cruz, M. Martins-Loucao and A. Cerda. 1993. Nitrate reductase activity in wheat seedlings as affected by NO_3^-/NH_4^+ ratio and salinity. *Journal of Plant Physiology*, 142: 531-536.

Bungard, R.A., A. Wingler, J.D. Morton and M. Andrews. 1999. Ammonium can stimulate nitrate and nitrite reductase in the absence of nitrate in *Clematis vitalba*. *Plant, Cell and Environment*, 22: 859-866.

Cramer, M.D., A. Schierholt, Y.Z. Wang and S.H. Lips. 1995. The influence of salinity on the utilization of root anaplerotic carbon and nitrogen metabolism in tomato seedlings. *Journal of Experimental Botany*, 46: 1569-1577.

Dellaquill'a, A. and P. Spada. 1993. The effect of salinity stress upon protein synthesis of germinating wheat embryos. *Annals of Botany*, 72: 97-101.

Erskine, P.D., G.R. Stewart, S. Schmidt, M.H. Turnbull, M. Unkovich and J.S. Pate 1996. Water availability-a physiological constraint on nitrate utilization in plants of Australian semi-arid mulga wood lands. *Plant, Cell and Environment*, 19: 1149-1159.

Gouia, H., M.H. Ghorbal and B. Touraine. 1994. Effects of NaCl on flows of N and on NO_3^- reduction rate within whole plants of salt-sensitive bean and salt-tolerant cotton. *Plant Physiology*, 105: 1409-1418.

Hamada, E.A.M., M.A. Hamond, M.A. El-sayed, R.C. Kirkwood and H. El-Sayed. 1992. Studies on adaptation of selected species of the family *Gramineae*. *Fedds Repertorium*, 103: 87-98.

Helal, M., K. Koch and K. Mengel. 1975. Effect of salinity and potassium on the uptake of nitrogen and on nitrogen metabolism in young barley plants. *Physiologia Plantarum*, 35: 310-313.

Huang, L., F. Murray and X. Yang. 1993. Responses of nitrogen metabolism parameters to sublethal SO_2 pollution in wheat [*Triticum aestivum* cv-Wilgoyne (Ciana/Gallo)] under mild NaCl stress. *Environmental and Experimental Botany*, 33: 479-493.

Kenjebaeva, S. and N. Rakova. 1995. Multiple forms of nitrate reductase and their role in nitrate assimilation in roots of wheat at low temperature or high salinity. *Physiologia Plantarum*, 93: 249-252.

Kondrat'ev, M.N. and S.O. Lebedinskaya. 1995. Nitrate reductase and protease activities in wheat during reproductive growth. *Russian Journal of Plant Physiology*, 42: 350-357.

Neumann, P. 1997. Salinity resistance and plant growth revisited. *Plant, Cell and Environment*, 20: 1193-1198.

Nicolas, M.E., R. Munns, A.B. Samarakoon and R.M. Gifford. 1993. Elevated CO_2 improves the growth of wheat under salinity. *Australian Journal of Plant Physiology*, 20: 349-360.

Podwyszynska, M. and T. Olszewski. 1995. Influence of gelling agents on shoot multiplication and the uptake of macroelements by *in vitro* culture of Rose, Cordyline and Homalomena. *Scientia Horticultrae*, 64: 77-84.

Rascio, A., C. Platani, N. Fonzo and G. Wittmer. 1992. Bound water in durum wheat under drought stress. *Plant Physiology*, 98: 908-912.

Roggate, U., A.J.S. Mcdonald, I. Stadenberg and U. Schurr. 1999. Effects of nitrogen deprivation on cell division and expansion in leaves of *Ricinus communis* L. *Plant, Cell and Environment*, 22: 81-89.

Weatherburn, M.W. 1967. Phenol-hypochlorite reaction for determination of ammonia. *Analytical Chemistry*, 39: 971-973.

Zadoks, J.C., T.T. Chang and C.F. Konzak. 1974. A deciaml code for the growth stages of cereals. *Weed Res*., 14: 415-421.

Zornoza, P. and M. Gonzales. 1998. Intraspecific differences in nitrogen assimilating enzymes in spinach under contrasting forms of nitrogen supply. *Journal of Plant Nutrition*, 21: 1129-1138.

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