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Full Length Research Paper

Growth and nitrogen metabolism changes in NaCl-stressed tobacco (*Nicotiana rustica* L. var. Souffi) seedlings

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While salinity effects have been largely documented in crop plants, little data are available on *Nicotiana rustica* species (snuff tobacco), mainly nitrogen metabolism changes. Here, tobacco (*N. rustica* L. var. Souffi) seedlings were grown for one month on control medium, and then exposed for seven days to different levels of salt stress (0, 50, 100 and 200 mM NaCl). A significant decrease in dry weight accumulation was observed only at 200 mM NaCl. Na^+ and Cl^- ions accumulation was greater in leaves relative to roots, while growth was similarly decreased in both organs. Referring to roots, leaf water content was less affected by salinity. The increase of endogenous levels of salt ions was accompanied by a drop in NO_3^- content in both leaves and roots, and a more pronounced decrease in K^+ content in leaves. Under salinity, nitrate reductase activity (NR, EC 1.6.1.6) was inhibited in both leaves and roots. The activities of nitrite reductase (NiR, EC 1.7.7.1) and glutamine synthetase (GS, EC 6.3.1.2) were inhibited in leaves but not in roots by salt stress. In stressed seedlings, NH_4^+ contents, protease activity, aminating and deaminating activities of glutamate dehydrogenase (NADH-GDH and NAD-GDH, EC 1.4.1.2) were enhanced mainly in the leaves. It could be stated that tobacco leaves and roots exhibited similar salt sensitivity in terms of growth and NO_3^- assimilation (NR activity), however they showed differential response for salt ions accumulation and NH_4^+ metabolism steps (GS and GDH).

Key words: Assimilation, growth, salt stress, nitrogen, mineral nutrition, tobacco.

INTRODUCTION

Plants are continuously exposed to abiotic and biotic stresses that endanger their survival. Among abiotic stresses, salinity is the major environmental factor limiting plant growth and productivity (Munns, 2002). Salinity has reached a level of 19.5% of all irrigated land and 2.1% of dry-land agriculture worldwide (FAO, 2005). The effect of salinity on plant growth is a complex trait that involves osmotic stress, ion toxicity, mineral deficiencies, physiological and biochemical perturbations (Hasegawa et al., 2000). High concentrations of salts in soils impose both ionic and osmotic stresses on plants and cause a reduction of shoot growth (Levigneron et al., 1995). This reduc-

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tion is, in part, due to the decrease in the water potential gradient between the plant tissues and the medium. Halophytes and some tolerant glycophytes carry out osmotic adjustment by concentrating salts in their tissues (Munns, 2002). Nevertheless the necessary quantities accumulated become quickly toxic (Munns, 1993). The water deficit always has a harmful effect, but many crop plants are chiefly sensitive to Na^+ excess (Greenway and Munns, 1980) due to its adverse effects on cytosolic enzyme activities, photosynthesis and metabolism (Ben et al., 2003). Indeed, high levels of Na^+ inhibit K^+ absorption results in a Na^+/K^+ antagonism (Tester and Davenport, 2003). Metabolic toxicity of Na^+ is largely due to its ability to compete with K^+ for binding site essential for cellular function (Aziz and Khan, 2001). Biochemical pathways leading to increased salt tolerance of plants include (i) selective accumulation or ion exclusion, (ii) control of ion uptake by roots and transport into leaves, (iii) compartmentalization of ions at the cellular and whole - plant levels, (iv) synthesis of compatible solutes, (v) changes in photosynthetic pathway, (vi) induction of anti-oxidative enzymes, and (vii) plant hormones regulation (Iyengar and Reddy, 1996).

Previous works have reported effects of salt stress on nitrogen metabolism (Carillo et al., 2005; Debouba et al., 2007). For most plants, the inorganic nitrogen uptake was in NO_3^- form. Nitrate uptake and transport appeared to be sensitive to salinity (Flores et al., 2000), and this may have severe consequences for NO_3^- assimilation in plants. Plants rapidly inactivate nitrate reductase (NR, EC 1.6.1.6) in response to several signals, including the loss of light, a decrease in CO_2 levels or when stomata close (Kaiser and Föster, 1989). Nitrite ions formed are then reduced to NH_4^+ in the chloroplast by nitrite reductase (NiR, EC 1.7.7.1). In tobacco plants, more than 80% of NO_2^- reduction occurs in leaves (Maâroufi-Dguimi et al., 2009). Ammonium originated from direct absorption, NR/NiR activities, photorespiration, dinitrogen fixation or protein catabolism, is assimilated by glutamine synthetase (GS, EC 6.3.1.2) and glutamate synthase (Fd-GOGAT, EC 1.4.7.1 and NADH-GOGAT, EC 1.4.1.14) (Schjoerring et al., 2006). Under special conditions, glutamate dehydrogenase (GDH, EC 1.4.1.2) is able to generate glutamate (Skopelitis et al., 2006). Debouba et al. (2006) have shown that increasing NaCl stress decreased tomato seedlings growth and inhibited NO_3^- reduction and NH_4^+ assimilation after ten days of treatment.

Nicotiana rustica L. var. Souffi is the most cultivated snuff tobacco in Tunisia, mainly in regions of “Cap-bon” and “Gabes” witch are salt-affected lands. Despite the economic importance of *N. rustica* and the fact that is a plant model, like *Arabidopsis thaliana*, little interest is to study the effects of salinity on growth and nitrogen metabolism in this plant species. In this study, we aimed to assess the effects of NaCl stress on growth and activities of enzymes involved in NO_3^- reduction and NH_4^+ assimilation in tobacco (var. Souffi). The patterns of NO_3^- reduction and assimilation changes are discussed in relation to the salt accumulation and changes in metabolite contents in plant leaves and roots.

MATERIALS AND METHODS

Growth experiments

Tobacco seeds (*N. rustica* L. var. Souffi) were germinated on moistened filter paper at 25°C in the dark. The seedlings obtained were transferred to continuously aerated nutrient solutions containing 8 mM KNO_3, 2 mM $Ca(NO_3)_2$, 1 mM KH_2PO_4, 1 mM $MgSO_4$, 32.9 µM Fe-K-EDTA, and micronutrients: 30 µM H_3BO_4, 5 µM $MnSO_4$, 1 µM $CuSO_4$, 1 µM $ZnSO_4$, 1 µM $(NH_4)_6Mo_7O_{24}$. Plants were grown in a growth chamber at 26°C/70% relative humidity during the light period and 20°C/90% relative humidity during the dark period, with a photoperiod of 16 h daily with a light irradiance of 150 µmol m^{-2} s^{-2} at the plant canopy. Plants were grown for 30 days in control medium, and then the salt treatments (0, 50, 100 and 200 mM NaCl) were applied for 7 days. Plants were harvested 6 h after the beginning of the light phase, and immediately separated into leaves and roots. For further dry weight (DW) and mineral content measurements, plant tissues were dried for three days at 60°C after a rapid determination of fresh weight (FW). For enzymatic analysis, plant tissues were immediately frozen in liquid nitrogen and then stored at -80°C.

Leaf area was measured seven days after the application of NaCl using the method described by Paul (1979). The organs water contents were defined as follows:

$WC = (FW – DW) / DW$ (ml g^{-1} DW),

Where, FW is fresh weight and DW the dry weight.

Mineral analysis

Inorganic ions were extracted from dry matter with 0.5 N H_2SO_4 at room temperature for 48 h (Gouia et al., 1994). Potassium and sodium were analyzed by flame emission using an Eppendorf spectrophotometer. Chloride was quantified by colorimetry using a Digital Chloridometer HaakeBuchler (Buchler instruments Inc., New Jersey, USA). Nitrate was colorimetrically determined as described by Miranda et al. (2001). Ammonium was extracted from fresh plant material at 4°C with 0.3 mM H_2SO_4 and 0.5% (w/v) polyclar AT. Ammonium content was quantified according to the reaction of Berthelot modified by Weatherburn (1967).

Metabolites assays

Photosynthetic pigments

Chlorophyll (Chl) contents were determined by the method of Arnon (1949). After centrifugation, the absorbance of each sample was read at 460, 645 and 663 nm, then contents of Chl a, Chl b and carotenoids (cart) were calculated using the formulas of MacKinney (1941).

Protein contents

Soluble protein content was quantified using Coomassie Brilliant blue (Bradford, 1976) with bovine serum albumin as a protein standard.

Table 1. Effects of NaCl treatments (0, 50, 100 and 200 mM) during seven days on Na^+, K^+ and Cl^- content (µmol g^{-1} DW) in the leaves and roots of tobacco plants, and their related changes in the Na^+ to K^+ ratio and the leaves to roots Na^+ and Cl^- ratios.

NaCl	Leave				Root				Na^+ (leaves) / Na^+ (roots)	Cl^- (leaves) / Cl^- (roots)
(mM)	Na^+	K^+	Cl^-	Na^+ / K^+	Na^+	K^+	Cl^-	Na^+ / K^+		
0	955.55 ± 33.45[a]	2445.05 ± 82.15[a]	36.88 ± 4.07[a]	0.39 ± 0.01	491.59 ± 58.98[a]	1412.74 ± 139.21[a]	2.09 ± 1.88[a]	0.33 ± 0.02	2.00 ± 0.17	15.14 ± 1.34
50	3518.48 ± 285.25[b]	1356.27 ± 91.79[b]	1097.77 ± 128.60[b]	2.61 ± 0.31	1046.47 ± 85.40[b]	1662.09 ± 141.62[a]	614.09 ± 59.39[b]	0.63 ± 0.03	3.39 ± 0.48	1.80 ± 0.25
100	4336.26 ± 230.23[c]	1306.00 ± 114.72[b]	1542.29 ± 226.71[c]	3.33 ± 0.20	1842.26 ± 152.05[c]	1556.79 ± 201.58[a]	882.92 ± 90.32[c]	1.19 ± 0.06	2.37 ± 0.29	1.77 ± 0.36
200	3883.53 ± 121.75[b]	1490.15 ± 186.96[b]	1840.99 ± 17.83[d]	2.64 ± 0.29	1689.31 ± 266.53[c]	948.96 ± 119.06[b]	788.47 ± 109.13[c]	1.81 ± 0.39	2.34 ± 0.26	2.38 ± 0.31

Data are the average of five replicates ± confidence limits; means sharing at least one letter are not significantly different; Tukey's test at $P < 0.05$ level.

Enzymes assays

Nitrate reductase (NR) activity

Nitrate reductase (NR) enzyme extraction was performed by homogenizing frozen plant material (PMF) in a chilled mortar and pestle with 100 mM potassium phosphate buffer (pH 7.4) containing 7.5 mM cystein, 1 mM EDTA and 1.5% (w/v) casein. The homogenate was centrifuged at 30 000 x *g* for 15 min at 4°C. Nitrate reductase activity (NRA) was determined according to the method described by Robin (1979). Briefly, 0.1 ml of the extract was incubated in a reaction mixture containing 0.5 ml of 0.1 M potassium phosphate buffer (pH 7.4), 0.1 ml of 0.15 mM NADH, and 0.1 ml of 0.1 M KNO_3 at 30°C for 30 min. The extract was incubated with 10 mM $MgCl_2$ (for actual NRA determination) or with excess 15 mM EDTA (for maximum NRA determination). The reaction was stopped by the addition of 0.2 ml of 1 M zinc acetate. Nitrite ions were assayed after diazotation with 1 ml of 5.8 mM sulfanilamide, 1.5 N HCl, and 1 ml of 0.8 mM N-naphthyl-ethylene-diamine-dichloride. The activation state of NR is given as a percentage ratio between the NRA measured in the presence of $MgCl_2$ and the NRA measured with EDTA.

Nitrite reductase (NiR) activity

Nitrite reductase (NiR) extracts were prepared as described above for nitrate reductase. Nitrite reductase was assayed using the method of Losada and Paneque (1971). The extract of 0.1 ml was incubated in a solution containing 0.4 ml of 0.1 M potassium phosphate buffer (pH 7.4), 0.1 ml of 15 mM sodium nitrite, and 0.2 ml of 86.15 mM sodium dithionite in 190 mM $NaHCO_3$. The reaction was stopped by agitation on a vortex. NO_2^- ions were assayed as described for the NRA assay.

Glutamine synthetase (GS) activity

For glutamine synthetase (GS) extraction, frozen samples were homogenized in a cold mortar and pestle with a grinding medium containing 25 mM Tris-HCl buffer (pH 7.6), 1 mM $MgCl_2$, 1 mM EDTA, 14 mM β-mercaptoethanol and 1% (w/v) polyvinylpyrrolidine (PVP). The homogenate was centrifuged at 25 000 x *g* for 30 min at 4°C. GS activity was determined using hydroxylamine as the substrate, and the formation of γ-glutamylhydroxamate (γ-GHM) was quantified with acidified ferric chloride (Wallsgrove et al., 1979).

Protease activity

Protease activity was measured using the method of Weckenmann and Martin (1984), using azocasein as substrate. Absorbance of the released-azo-dye was measured at 340 nm and one unit of activity was defined as the activity producing an increase of 0.01 unit of absorbance during the 1 h incubation.

Glutamate dehydrogenase (GDH) activity

Glutamate dehydrogenase (GDH) extraction was performed according to the method described by Magalhaes and Huber (1991). Frozen samples were homogenized in a cold mortar and pestle with 100 mM Tris-HCl (pH 7.5), 14 mM β-mercaptoethanol and 1% (w/v) PVP. Extracts were centrifuged at 12 000 x *g* for 15 min at 4°C. NADH-dependent aminating and NAD-dependent deaminating activities of GDH were determined by following the absorbance changes at 340 nm (Loyala-Vergas and De Jimenez, 1984).

Statistical analysis

The data are presented in the figures and in the tables as the average of five replicates per treatment and means ± confidence limits. Significant differences between means were calculated using Tukey test at $P < 0.05$ level by SPSS Release 18 for Windows.

RESULTS

Mineral, water and growth status

Tobacco plants treated for one week with different NaCl concentrations (0, 50, 100 and 200 mM) showed a significant increase in the endogenous Na^+ and Cl^- contents in both leaves and roots (Table 1). Particularly, Na^+ was more accumulated in leaves than in roots. At 200 mM NaCl, the amounts of Na^+ and Cl^- in the leaves were two times greater than that of roots (Table 1). However, the differential accumulation of salt ions between leaves and roots does not reflect any variation in the growth response of these organs to salinity. Dry weight (DW) was similarly decreased

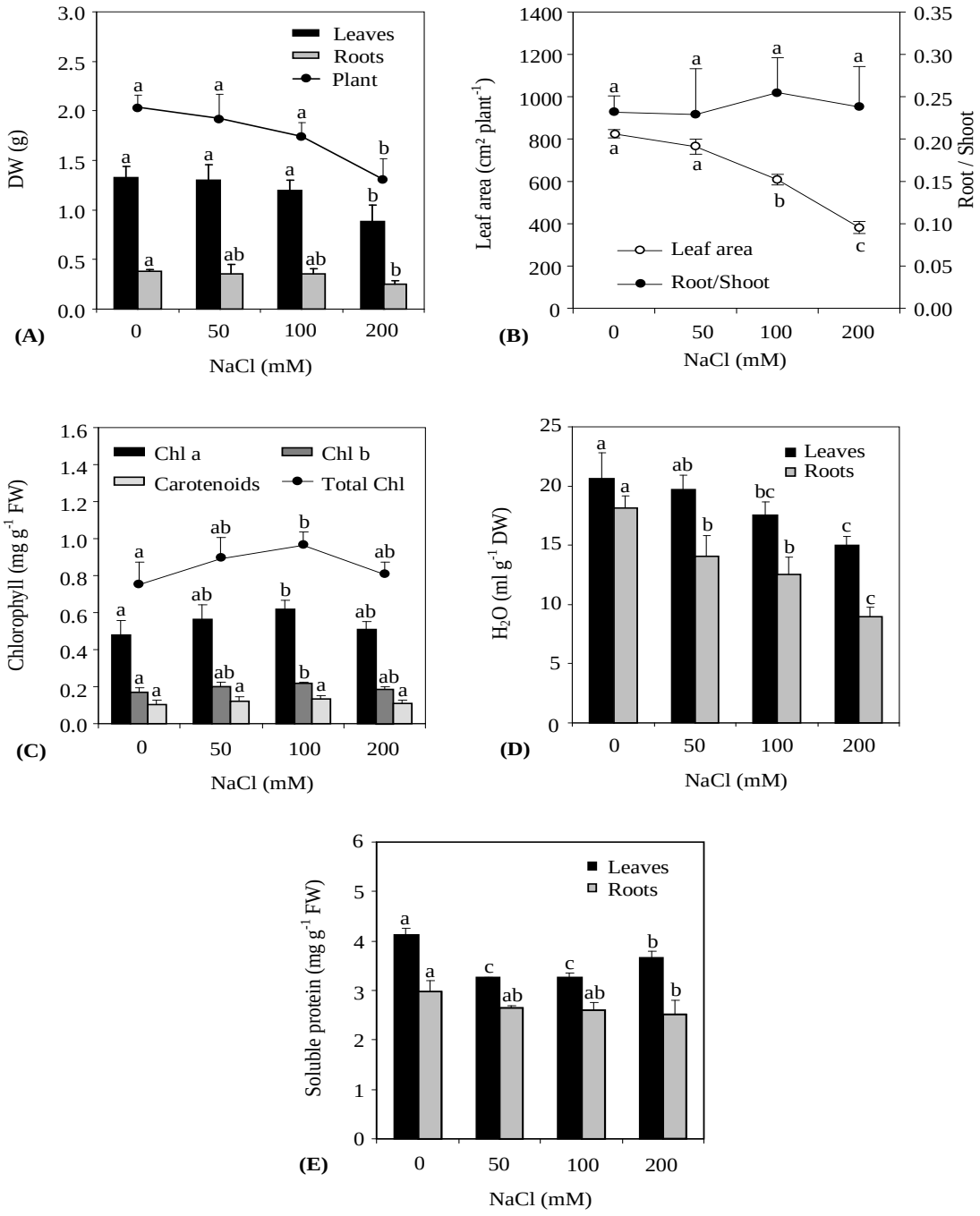

Figure 1. Effects of NaCl treatments (0, 50, 100 and 200 mM) during seven days on (A) dry weight (DW), (B) leaf area and root to shoot ratio, (C) chlorophyll (Chl) a, Chl b, total Chl and carotenoids content, (D) water content, and (E) soluble protein content in tobacco plants. Columns are the average of five replicates ± confidence limits. Column values sharing at least one letter are not significantly different; Tukey's test at $P < 0.05$ level.

In leaves and roots with increasing NaCl treatments (Figure 1A). In both organs, this decrease was significant only at high salt concentration (200 mM) and reached 35% of the controls. The similar leaf and root response to salinity leads to a constant root to shoot DW ratio (R/S ratio) (Figure 1B).

The leaf surface area gradually decreased with increasing salt concentrations (Figure 1B). Significant NaCl effects on the leaf surface area were recorded at 100 and 200 mM NaCl treatments with a decrease of 26 and 53%, respectively, compared to the controls. In contrast, salinity enhanced the total chlorophyll content

Table 2. Changes in nitrate reductase (NR) activity (µmol NO_2^- formed g^{-1} FW h^{-1}), NR activation state (%), nitrite reductase (NiR) activity (µmol NO_2^- reduced g^{-1} FW h^{-1}), NiR to NR activities ratio, glutamine synthetase (GS) activity (µmol γ-glutamylhydroxamate (GHM) g^{-1} FW h^{-1}) and NO_3^- (µmol g^{-1} DW) and NH_4^+ (µmol g^{-1} FW) contents in leaves and roots of tobacco plants subjected to seven days of increasing NaCl concentrations (0, 50, 100 and 200 mM).

Plant part	NaCl (mM)	NR activity	NR activation state	NiR activity	NiR / NR activity	GS activity	NO_3^-	NH_4^+
Leaves	0	83.00 ± 6.26^a	43.27 ± 4.78^a	2653.80 ± 69.56^a	32.06 ± 3.40	28.48 ± 1.73^a	1440.66 ± 103.24^a	0.41 ± 0.00^a
	50	24.32 ± 2.53^b	43.60 ± 10.65^a	2218.00 ± 64.75^b	83.31 ± 7.46	20.17 ± 1.78^b	1041.18 ± 146.06^b	0.75 ± 0.02^c
	100	30.66 ± 0.85^b	45.81 ± 6.80^a	2363.40 ± 47.63^b	77.43 ± 5.32	18.57 ± 0.66^b	950.62 ± 123.97^b	0.75 ± 0.01^c
	200	24.11 ± 2.09^b	70.80 ± 10.88^b	2232.60 ± 37.87^b	92.31 ± 8.08	20.56 ± 1.19^b	867.85 ± 139.38^b	0.52 ± 0.01^b
Roots	0	34.98 ± 4.46^a	47.09 ± 9.94^a	2095.60 ± 96.87^a	60.42 ± 6.80	8.64 ± 1.46^a	595.24 ± 55.29^a	0.77 ± 0.00^a
	50	15.09 ± 1.05^b	53.47 ± 2.04^a	1985.90 ± 72.77^a	132.44 ± 5.49	9.82 ± 1.05^a	347.17 ± 68.65^b	0.93 ± 0.03^b
	100	10.59 ± 1.67^c	54.73 ± 11.87^a	1944.80 ± 76.76^a	185.23 ± 25.00	8.37 ± 1.14^a	329.14 ± 64.14^b	0.95 ± 0.03^b
	200	12.16 ± 1.08^b	47.28 ± 8.03^a	1939.60 ± 88.09^a	167.41 ± 26.34	9.30 ± 1.43^a	283.54 ± 12.54^c	0.91 ± 0.01^b

Data are the average of five replicates ± confidence limits; means sharing at least one letter are not significantly different; Tukey's test at $P < 0.05$ level.

in the whole foliar plant system (Figure 1C). This increase was most significant at 100 mM NaCl treatment being about 27% compared to the controls. However, carotenoid content appeared to be unchanged by salinity.

Salinity significantly affect the leaf hydration only at the highest NaCl concentrations (100 and 200 mM) when a decrease by 15 and 27%, respectively, was observed relative to control plants (Figure 1D). Whereas, the root water content was significantly decreased from the lowest salt treatment (50 mM) by 23%, compared to controls, and reached 51% at 200 mM (Figure 1D).

Ion analysis showed that all the salt treatments significantly decreased, approximately with similar extent (40% relative to controls), to the K^+ content of tobacco leaves (Table 1). However in roots, a significant decrease of the K^+ content was recorded only at 200 mM NaCl by about 33% with respect to controls. Consequently, the leaf Na^+/K^+ ratio was more increased than that of roots by salinity (Table 1). NO_3^- contents were gradually and significantly decreased by increasing salinity in both organs, mainly in roots (Table 2). At 200 mM NaCl, this decrease reached 40 and 50% in the leaves and roots, respectively, compared to the controls.

NO_3^- and NO_2^- reduction

In the control tobacco seedlings, we found that Maxim- um NR activity was at least 2.3 times greater in leaves (83.00 ± 6.26) than in roots (34.98 ± 4.46) (Table 2). In both organs, NR activity was significantly and similarly inhibited by all salt treatments (about 70% compared to controls) (Table 2). However, NR activation state seems to be unaffected by salinity, except in leaves at 200 mM NaCl when it was significantly enhanced by 63% (Table 2).

The reduction of NO_2^- ions formed by NR activity is carried out primarily in the leaves (Table 2). In control plants, the NiR activity was higher than NR activity in the leaves (32-fold) and in the roots (60-fold) (Table 2). In addition, leaf GS activity was at least 3 times higher than that of roots (Table 2). Interestingly, the inhibitory effects of NaCl treatments on NiR and GS activities appeared only in the leaves, whereas no effect was recorded in the roots. At 200 mM NaCl concentration, leaf NiR and GS activities were decreased by about 16% and 28%, respectively, referring to controls (Table 2).

NH_4^+ assimilation

In tobacco seedlings, salinity led to a significant increase in the endogenous levels of NH_4^+ mainly in the leaves (Table 2). In the leaves, this increase was highest at 50 and 100 mM NaCl and reached 83% as compared to controls, while in the roots; it was similar under all salt concentrations by about 23%. In contrast, salinity significantly decreased the total soluble protein content in both leaves and roots (Figure 1E). At both 50 and 100 mM NaCl, the levels of soluble protein content were decreased by about 21 and 12% in the leaves and roots, respectively, compared to controls.

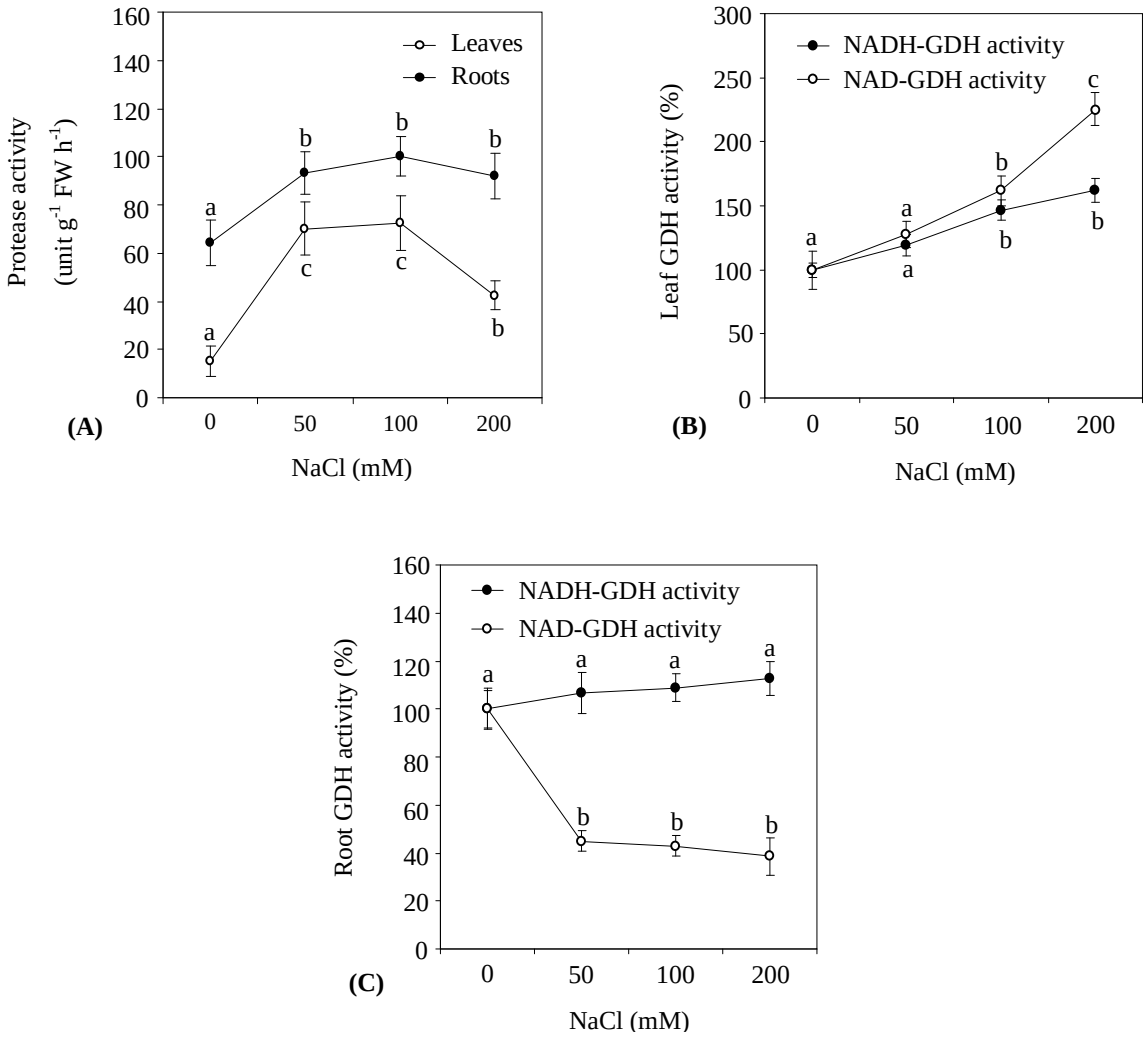

Figure 2. Effects of NaCl treatments (0, 50, 100 and 200 mM) during seven days on (A) protease activity (unit. g^{-1} FW. h^{-1}) in leaves and roots of tobacco plants, and GDH activity (µmol NAD. g^{-1} FW. h^{-1}) in (B) leaves (100% NADH-GDH = 0.231 ± 0.012 and 100% NAD-GDH = 0.069 ± 0.010), and (C) roots (100% NADH-GDH = 1.627 ± 0.050 and 100% NAD-GDH = 0.013 ± 0.003). Each point is the average of five replicates ± confidence limits; means sharing at least one letter are not significantly different, Tukey's test at $P < 0.05$ level.

All salt treatments significantly enhanced the protease activity in both organs (Figure 2A). This enhancement was more important in roots relative to leaves. At 100 mM NaCl, the increase of protease activity reached 5-fold and 1.5-fold in leaves and roots, respectively, referring to controls.

In control plants, root aminating GDH activity (NADH-GDH) was about seven times greater than in leaves (Figures 2B and C). In contrast, deaminating GDH activity (NAD-GDH) was about five times higher in the leaves than in roots (Figures 2B and C). In both organs, GDH had dominant anabolic function (NADH-GDH). In the leaves, aminating and deaminating GDH activities were gradually and significantly increased by increasing NaCl concentrations (Figure 2B). At 200 mM NaCl, NADH-GDH and NAD-GDH activities were enhanced by about two-fold, respectively, with respect to controls. However, in the roots NADH-GDH activity showed to be unaffected by salinity whereas NAD-GDH activity was significantly decreased (Figure 2C).

DISCUSSION

The results in the present study, show that *Nicotiana rustica* exhibited tolerant behaviour at moderate (50 and 100 mM) and short term (7 days) NaCl stress. Significant growth decrease was recorded at severe NaCl stress (200 mM) (Figure 1A). Nonetheless, this was not related to higher salt ions accumulation with increasing external NaCl stress (Table 1). According to mineral analysis, similar levels of accumulated Na^+ and Cl^- ions were recorded at 100 and 200 mM NaCl treatments (Table 1).

Shoots and roots growth was similarly decreased by stress, while higher Na^+ and Cl^- ions were confined in leaves (Table 1). Such accumulation probably reflects an inclusive behavior. These plants, called “Includers”, salt was trapped and accumulated in the aerial organs cells, mainly in their vacuoles (Levigneron et al., 1995). The similar sensitivity of leaves and roots to NaCl resulted in a constant root to shoot (R/S) ratio (Figure 1B).

The increase of chlorophyll content in the foliage of tobacco by salinity (Figure 1C) indicated that the salt stress may promote the synthesis of photosynthetic pigments (Qiu et al., 2007) or this could be related to the significant decrease in the leaf area (Figure 1B). Other works have reported similar accumulation of chlorophyll content under salinity (Zheng and Lin, 1992; Chen et al., 2000). The increased pigment concentrations recorded in tobacco may be a strategy to maintain photosynthesis as an acclimation mechanism to salt stress reduced photosynthetic surface area. Strategies of salinity tolerance include the preservation of photosynthetic rate and stomatal conductance (Lakshmi et al., 1996) and the increase of chlorophyll concentration (Salama et al., 1994). Visible senescence and chlorosis symptoms detected on the oldest leaves at high NaCl treatment (200 mM) (data not shown) could be the result of the excess Na^+ and Cl^- ions accumulated (Nabil and Coudert 1995). The excessive amounts of salt will eventually rise to toxic levels in the older transpiring leaves, causing premature senescence (Munns, 2002).

Effects of salinity on growth were associated with a decrease in water content which was more pronounced in roots than in leaves (Figure 1D). The lesser water content perturbation in leaves was related to the higher salt ions accumulation in these organs relative to roots. In many plants in which salt inclusion is the principal mechanism, endogenous inorganic ions and particularly Na^+ and Cl^- were used for osmotic adjustment (Greenway and Munns, 1980; Wyn, 1981; Ashraf, 1994). At higher salt treatments (100 and 200 mM NaCl), leaf tobacco cells became enable to adjust water content (Figure 1D), suggesting that salt ions were probably accumulated in the symplasm. Symplasmic accumulation of salt ions could interfere with nutrient uptake and may inactivate enzymes in the cytosol.

Ion analysis showed that salinity stress significantly decreased K^+ contents in the leaves since the lowest NaCl treatment and in the roots only at the highest one (Table 1). This finding agrees with results shown by Cusido et al. (1987) and seems to be linked to the differential accumulation of Na^+ in leaves and roots. This result suggests that the absorption but not the transport process was not affected by competition between K^+ and Na^+. High Na^+ concentration can induce K^+ deficiency, inhibiting the activity of enzymes that require K^+. Thus, the interaction between relative K^+ and Na^+ concentration has been considered a key factor in determining salt tolerance in plants (Willadino and Câmara, 2005). In this work, Na^+/K^+ ratios above 1.0 were found in the leaves from the lowest NaCl concentration (50 mM) and in the roots from 100 mM NaCl (Table 1). Na^+/K^+ ratios equal to or smaller than 0.6 are necessary for an optimal metabolic efficiency in non-halophyte plants (Greenway and Munns, 1980).

Changes in K^+ contents generally affected NO_3^- ions absorption and transport. Our results show that NO_3^- contents in both leaves and roots progressively decreased with increasing salinity (Table 2). The salt-osmotic effects induced by NaCl can initially disrupt the root membrane integrity (Carvajal et al., 1999), inducing a restriction in the NO_3^- uptake (Klobus et al., 1988; Bourgeais-Chaillou et al., 1992), and therefore causing a reduction in the loading of NO_3^- into the root xylem (Abd-El Baki et al., 2000), leading to a decrease in plant water absorption. These results suggest that the reduction in growth can be, at least in part, related to nutritional disorders induced by salt.

The observed decrease in NO_3^- content in leaves and roots could affect the subsequent processes involved in NO_3^- reduction and assimilation. In fact, NO_3^- regulates NR and NiR expressions (Wang et al., 2000) and activities (Poonnachit and Darnell, 2004; Takabayashi et al., 2005). The first step in NO_3^- assimilation, the reduction of NO_3^- to NO_2^- by NR, is the main and most limiting step, in addition to being the most prone to regulation (Ruiz et al., 1998). In control tobacco seedlings, more than 70% of NO_3^- reduction occurred in the leaves (Table 2), which is in agreement with the higher biomass production obtained in the leaves relative to roots (Figure 1A). This elevated NO_3^- reduction in leaves relative to roots was related to the higher NR protein content (Abd-El Baki et al., 2000) and sufficient availability of light and reducing power (Lillo et al., 2004). After one week of NaCl treatment, we obtained a similar significant decrease of NR activity in leaves and roots (Table 2). However, NO_3^- content was more decreased in the roots (Table 2). This indicated that NO_3^- content is not a determining parameter in the regulation of NR activity. Strictly speaking, NR activity is controlled by the flow of NO_3^- to the active sites of the enzyme. In our experiments, all salt treatments inhibited NR activity as well as maximum NR activity (NR activity max), whereas NR activation state was almost unaltered and increased only in leaves at high salt concentration (200 mM) (Table 2). This indicated that the decrease of NR activity under salinity may be the result of NR gene expression (Nia) and protein synthesis inhibition, and/or the stimulation of NR protease activity. In fact, toxic reactive oxygen species were generated during NaCl stress and can cause the breakdown of proteins directly by oxidative reaction (Salo et al., 1990) or indirectly by increasing proteolytic activity (Chia et al., 1982).

The next step in NO_3^- assimilation is the conversion of the NO_2^- to NH_4^+ by NiR (Migge and Becker, 1996). For all treatments and in both organs, NiR activity was always

much higher than NR activity (Table 2). This imbalance insures avoidance of toxic accumulation of NO_2^- ions produced by NO_3^- reduction in the cytosol (Debouba et al., 2006; Ezzine and Ghorbel, 2006). According to the higher decrease in the leaf NR activity by salinity, NiR activity was significantly inhibited only in the leaves (Table 2). NiR activity inhibition in tobacco could be the consequence of the decrease in NR activity and in NO_3^- contents (Table 2) as it has been shown that both NiR and NR are induced by NO_3^- (Wang et al., 2000; Guerrero et al., 1981).

Ammonium originating in the plant from NO_3^- reduction is incorporated into an organic form primarily by the GS enzyme (Schjoerring et al., 2006). As for NiR activity, GS activity was significantly inhibited by all NaCl concentrations in the leaves while it was not affected in the roots (Table 2). Chandra et al. (2001) also showed a decrease of GS activity by NaCl in the leaves of maize plants. The GS activity decrease in leaves under stress was generally due to the inhibition of the chloroplastic GS isoform (GS2) activity (Chaffei et al., 2004; Debouba et al., 2006). In addition, the maintenance of a high GS activity in the roots was related to the induction of the cytosolic GS isoform (GS1) protein and transcripts in tomato plants subjected to abiotic stress (Chaffei et al., 2004).

In the contrast, NaCl treatment resulted in a significant increase of NH_4^+ contents in the leaves and in the roots (Table 2). The greater increase in NH_4^+ content in the leaves coincided with the higher decrease in soluble protein content relative to that obtained in the roots (Figure 1E).

These results were in accordance with the higher protease activity in the leaves than in the roots (Figure 2A).

In the same way, salinity provoked a significant increase of aminating and deaminating GDH activities in the leaves (Figure 2B); while in the roots, NADH-GDH activity seemed to be unaffected and NAD-GDH activity was significantly decreased (Figure 2C). The high increase of NH_4^+ content in the leaves may be responsible for the activation of the aminating function of GDH (Ameziane et al., 2000), which could be considered as an alternate pathway of NH_4^+ assimilation when GS activity was inhibited (Table 2). In addition, the enhancement of 2-oxoglutarate synthesis via the deaminating activity of GDH was generally observed under carbon limitation (Aubert et al., 2001), which could have resulted from the reduction of the leaf area (Figure 1B). It has been suggested that plant salt tolerance is improved by the stimulation of aminating GDH activity that catalyzes the reversible amination of 2-oxoglutarate to glutamate and deamination of glutamate to 2-oxoglutarate (Skopelitis et al., 2006; Fontaine et al., 2006).

Conclusion

N. rustica plants showed a moderate tolerance to salt stress and an inclusive behaviour by accumulating high amounts of Na^+ and Cl^-, mainly in the leaves. Accordingly, considerable nutrient and metabolic disorders that were more prevalent in the leaves have been recorded. Down-regulation of nutrient uptake and assimilation were not tightly related to higher accumulation of salt ions in plant tissues. Moreover, as a strategy to ensure nitrogen compounds synthesis in saline conditions, *N. rustica* seedlings maintained adequate nitrogen-assimilating enzymes activities in the roots to overcome their significant decrease in the leaves.

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