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Effects of NaCl on Flows of N and Mineral Ions and on NO_3^- Reduction Rate within Whole Plants of Salt-Sensitive Bean and Salt-Tolerant Cotton

Houda Gouia*, Mohamed Habib Ghorbal, and Bruno Touraine

Physiologie Végétale, Département des Sciences Biologiques, Faculté des Sciences de Tunis, Campus Universitaire, Tunis, Tunisia (H.G., M.H.G.); and Biochimie et Physiologie Végétales, Ecole Nationale Supérieure Agronomique de Montpellier, Institut National de la Recherche Agronomique, Centre National de la Recherche Scientifique, Unité de Recherche Associée 573, 34060 Montpellier Cedex 1, France (B.T.)

The effects of NaCl on the transport rates of cations, NO_3^-, and reduced N compounds between roots and shoot and on NO_3^- assimilation rate were examined on plants of two species differing in their sensitivity to salinity, bean (*Phaseolus vulgare* L. cv Gabriella) and cotton (*Gossypium hirsutum* L. cv Akala). Biomass production after 20 d in response to 50 and 100 mM NaCl decreased by 48 and 59% in bean, but only 6 and 14% in cotton. The comparison of the flow patterns obtained for control and NaCl-fed plants showed that salinity induced a general decrease in all the fluxes involved in partitioning of N and the various ions. This decrease was markedly higher in bean than in cotton. Within either species, the different flows (uptake, xylem flux, phloem flux) of a given element were affected by NaCl to the same extent with minor exceptions. No specific effect of salinity on any of the components of N partitioning were discerned. The greater sensitivity of nitrate reductase activity to NaCl in bean leaves compared to cotton leaves seems to be due to a decreased compartmentalization of ions rather than to a difference in salt tolerance of the enzyme itself. Overall, our data show that alteration of mineral nutrition is not solely the reflection of a decreased growth rate, but also is a general process that impairs uptake of all the minerals even at mild NaCl salinity.

Higher plant species differ widely in their tolerance of salinity, i.e. high NaCl concentrations in the root environment. On the basis of their tolerance or sensitivity, plants commonly are distinguished as halophytes, which naturally grow on saline soils, and glycophytes or nonhalophytes (Flowers and Yeo, 1988). Although crop species are nonhalophytes and their growth and yield are depressed by salinity, they display a wide range of sensitivity to salinity (Greenway and Munns, 1980; Slama, 1991). The main problem for a plant in saline media is that on the one hand, since external osmotic potential is much lower than in nonsaline soils, osmotica must be accumulated to high levels to create a water potential gradient to facilitate inward water movement, whereas on the other hand increasing concentrations of ions in the symplasm eventually may become toxic (Greenway and Munns, 1980). Most studies on mechanisms of salt tolerance in plants have focused on osmotic adjustment and ion compartmentation at either the whole-plant (roots/shoot) or cellular level (cytoplasm/vacuole) (Greenway and Munns, 1980; Flowers and Yeo, 1988). Furthermore, most investigations on ion compartmentation have concentrated on Na^+, Cl^-, and the major cations (essentially K^+ and to a lesser extent Ca^{2+}) (Yeo, 1983). However, data about salinity effects on N nutrition recently have become available (Aslam et al., 1984; Klobus et al., 1988; Bourgeais-Chaillou et al., 1992; Jeschke et al., 1992; Ourry et al., 1992).

Inhibition of ion uptake by salinity has been reported for several ions, e.g. Ca^{2+} (Cramer et al., 1985), K^+ (Lynch and Läuchli, 1984), and NO_3^- (Aslam et al., 1984; Klobus et al., 1988). The fact that NO_3^- uptake is altered in short-term experiments, in which NaCl was supplied for intervals as short as a few minutes (Klobus et al., 1988), suggests that NaCl directly affects transport of ions across the plasmalemma of root cells. In the case of long-term cultures grown in the presence of NaCl, however, it is not clear whether ion uptake rates are affected directly by NaCl or whether they are affected indirectly as a consequence of growth alteration. Indeed, effects of NaCl on growth rate that do not involve acquisition of mineral ions have been described (Cheeseman, 1988; Brugnoli and Lauteri, 1991; Brugnoli and Björkman, 1992). Furthermore, once absorbed, ions may be metabolized (particularly NO_3^-, NH_4^+, $H_2PO_4^-$, and SO_4^{2-}) and undergo long-distance transport within the plant.

The question of the location of the primary effect of salt, e.g. uptake site, metabolic pathways, or partitioning processes, rarely has been addressed. For this purpose, a quantification of the effects of NaCl on the different flows within the intact whole plant is needed. Most of the work on this topic has been carried out recently by Jeschke and co-workers (Jeschke et al., 1992, and refs. therein). These studies were done with castor bean and white lupin, in which easy access to xylem and phloem saps allows an estimate of mass flows of nutrients within the intact plant by the modeling methods developed by Pate et al. (1979). This method consists of calculating first the C transport rates from measurements of C input into the leaves (net photosynthesis) and output (respiration) from each organ, and then the long-distance

* Corresponding author; fax 216–1–885–480.

Abbreviations: N_r, reduced N; NR, nitrate reductase; NRA, nitrate reductase activity; RGR, relative growth rate.

fluxes of any compound using the [compound]:[C] ratios in xylem and phloem exudates obtained from cut stem or petioles. To estimate uptake rates of ions, net accumulation rates in roots and shoot, transport rates between roots and shoot, and NO_3^- reduction rates in both organs, we applied another modeling technique previously described by Touraine et al. (1988). Briefly, the analytical procedure is as follows: since Ca^{2+} ions are not translocated in the phloem, the xylem flux of Ca^{2+} is estimated by Ca^{2+} accumulation rate in the shoot; the xylem fluxes of other ions are calculated from Ca^{2+} flux in the xylem and [ion]:[Ca^{2+}] ratios in xylem sap; finally, phloem fluxes and NO_3^- reduction rates are calculated from the values for accumulation rates and xylem fluxes by setting the difference between inputs and outputs in a given pool as equal to the increment of change for this pool.

The present paper reports on the effects of mild NaCl stress on flows of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and N (NO_3^- and N_r) in whole plants of bean (*Phaseolus vulgaris* L. cv Gabriella) and cotton (*Gossypium hirsutum* L. cv Akala), which were chosen for their difference in sensitivity to NaCl salinity (Brugnoli and Lauteri, 1991; Slama, 1991). Both of these species reduce NO_3^- predominantly in the shoot (Andrews, 1986), which is contrary to white lupin and castor bean, for which data on the effects of NaCl on partitioning of ions and N are available (Jeschke et al., 1992, and refs. therein). Moreover, to complete the study of the effect of salinity on N nutrition, we present data on the effect of NaCl on nitrate reductase activities.

MATERIALS AND METHODS

Plant Material

The seeds of bean (*Phaseolus vulgaris* L. cv Gabriella) and cotton (*Gossypium hirsutum* L. cv Akala) were surface sterilized in 10% H_2O_2, thoroughly rinsed with distilled water, and germinated between wet paper towels at 25°C in the dark. After 3 d the seedlings were transferred to aerated nutrient solutions and grown in a culture chamber for up to 28 d. The day period was 16 h with a light irradiance of about 150 μmol m^{-2} s^{-1} at the canopy level. Temperature was maintained at 26°C during the day and 20°C during the night, and RH (±10%) was 70% during the day and 90% during the night. The basal nutrient solution contained 2 mM KNO_3, 2.5 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 1 mM KH_2PO_4, 100 μM FeKEDTA, 50 μM KCl, 30 μM H_3BO_3, 5 μM $MnSO_4$, 1 μM $CuSO_4$, 1 μM $ZnSO_4$, and 0.1 μM $(NH_4)_6Mo_7O_{24}$. Saline treatments were imposed by adding 0, 50, or 100 mM NaCl to this solution as soon as the seedlings were transferred to the growth chamber (time taken as time zero). Solutions were renewed twice to three times a week to minimize pH shift and nutrient depletion.

Collection of Xylem Sap

Xylem sap was collected using a hand-made pressure bomb by the method of Touraine et al. (1988). The decapitated root system was sealed into a pot filled with fresh nutrient solution identical to the culture medium (with or without NaCl). The pot was pressurized using a cylinder of 20:80% (v/v) O_2:N_2 gas mixture to 100 kPa with roots from control plants and 300 kPa with roots from NaCl-treated plants. Sap exuding from the cut section of the stem emerging out from the pressure bomb was collected in a hemolysis tube through Tygon tubing.

Analytical Methods

At harvest, plants were divided into roots and shoot. Depending on the age of the plant (between 12 and 28 d), blades of individual leaves were separated from the stem plus petioles. Fresh and dry weights of each part were determined before chemical analyses.

Inorganic ions were extracted from dry matter with 0.1 N H_2SO_4 at room temperature for 48 h. Diluted xylem exudates were directly analyzed. K^+ and Na^+ were analyzed by flame emission using an Eppendorf spectrophotometer. Ca^{2+} and Mg^{2+} were determined by atomic absorption using a Perkin Elmer 2380 spectrophotometer. Cl^- was determined by a colorimetric method using a Büchler-Cotlove chloridometer. NO_3^- was colorimetrically determined on an automatic analyzer following diazotation of the nitrite obtained by reduction on a Cd column.

Reduced N was determined after Kjeldahl digestion of the fine powder obtained using a Dangoumau grinder. NH_4^+ content of the digest was titrimetrically determined with 0.05 N HCl after distillation in the presence of NaOH and a catalyst containing Se, $CuSO_4$, and K_2SO_4.

NRA

In vitro NRA was determined according to the method described by Robin (1979). Samples were ground at 4°C in 0.1 M potassium phosphate buffer, pH 7.4 (8 mL g^{-1} fresh weight), containing 7.5 mM Cys, 1 mM EDTA, and 1.5% (w/v) casein. After filtration on a Vileda-mop towel, 0.1 mL of the extract was incubated in a solution 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 reaction was stopped by adding 0.1 mL of 1 M zinc acetate. The nitrite ions produced were assayed after diazotation with 1 mL of 1 g L^{-1} sulfanilamide, 1.5 N HCl, and 1 mL of 0.2 g L^{-1} *N*-naphtyl-ethylene-diamine-dichloride. The solution was centrifuged at 12,000*g* for 5 min and the A_{540} of the supernatant was determined.

In situ NRA was determined according to the method described by Robin et al. (1983). Whole leaves (0.5–1.5 g fresh weight) were excised 6 h after the beginning of the light period and weighed, and the petioles were immersed in 250-mL glass vials containing 5 mL of an incubation medium. This solution was either pure distilled water or 0.1 M KNO_3. The vials were then tightly sealed with rubber caps, flushed with N_2 (150 kPa) for 60 s via hypodermic needles inserted in the cap to ensure anoxia, and covered with aluminum foil for darkness. After a 1-h incubation period, reaction was stopped by adding 20 mL of boiling, distilled water. Extraction of the nitrite ions accumulated in tissues was carried out in a boiling-water bath for 10 min. The nitrite ions were then determined following the same procedure used for in vitro NRA assay (see above). A blank made with a similar sample was kept in air and light before extraction.

Six to 10 replicates were used in each in vitro or in situ

NRA assay. The activity is expressed as μmol NO_2^- produced h^{-1} g^{-1} fresh weight.

Calculations of the Flows of N_r and Mineral Ions between Roots and Shoot

Net transport rates of N_r and mineral ions were determined using the modeling technique developed by Touraine et al. (1988). Since the natural logarithms of the amounts of N_r and mineral ions in roots and shoot increased linearly with time (see "Results"), net gains of N_r and mineral ions in roots and shoot were calculated using the following formula:

$$dQ/dt = a \cdot \exp(a \cdot t + b)$$

where Q is the amount expressed in μmol per plant part, t is the time in days, and a and b are the slope and y intercept of the regression lines, respectively. Because of the nonmobility of Ca^{2+} ions in phloem (Allen and Raven, 1987; Munns et al., 1988), xylem flux of Ca^{2+} is equal to net accumulation rate of Ca^{2+} in shoot. Xylem fluxes of the other compounds were taken as the product of Ca^{2+} xylem flux by the [compound]:[Ca^{2+}] ratio in xylem sap. Phloem fluxes and NO_3^- reduction rates in roots and shoot were then calculated simply by setting the net increase within a pool of a given compound as equal to the balance between inputs and outputs. For nonmetabolized ions (cations and Cl^-), phloem fluxes were taken as the differences between xylem fluxes and net accumulation rates in the shoot. Since NO_3^- translocation in sieve sap is trivial (Fellows et al., 1978; Allen and Raven, 1987; Jeschke and Pate, 1992; Schobert and Komor, 1992), the phloem flux of N_r was calculated as the difference between total N (NO_3^- plus N_r) xylem flux and total N accumulation in shoot. NO_3^- reduction rate in shoot was estimated from the difference between NO_3^- xylem flux and net NO_3^- accumulation in shoot. Finally, NO_3^- reduction rate in roots was obtained by subtracting NO_3^- reduction rate in shoot from the total accumulation of N_r in the whole plant. All the values obtained for the different fluxes (uptake rates, accumulation rates, translocation rates in xylem or phloem, NR rates) were divided by the dry weight of the whole plant, which was calculated from the regression lines between their natural logarithms and the time in days.

RESULTS

Effect of NaCl on Growth Rate

In both control and NaCl-treated plants, the biomass production as a function of time from d 12 to d 28 was exponential. RGR values were 0.092 and 0.136 g dry weight g^{-1} dry weight d^{-1} for control plants of bean and cotton, respectively. The growth rate of bean between 12 and 28 d was markedly decreased to 0.069 g dry weight g^{-1} dry weight d^{-1} by addition of 50 mM NaCl to the culture solution (i.e. 25% lower than RGR of control plants), whereas that of cotton plants was reduced only slightly to 0.126 g dry weight g^{-1} dry weight d^{-1} (i.e. 7% lower than RGR of control plants). Moreover, the y intercept (y value for time = 0 d) of the regression lines between the logarithm of plant dry weight and time was lowered by 7% for bean but was not changed for cotton. This indicates that growth of bean but not cotton was reduced by salinity within the initial 12 d.

In 20-d-old bean the fourth leaf had not emerged on NaCl-treated plants, whereas it had reached about half its maximum expansion on control plants. Growth of roots and shoot, as well as of stem + petioles and total leaves, was affected to the same extent by NaCl treatments (Table I). For bean, the reduction in dry matter accumulation in the leaves comprised a lesser effect of NaCl on the growth of the oldest leaves and a greater effect on the growth of the youngest ones. For cotton plants, however, there was no difference in the effects of salinity on biomass accumulation in leaves of different ages (Table I). The presence of 50 or 100 mM NaCl did not significantly affect the water content of the different organs for either bean or cotton plants (data not shown).

Effect of NaCl on Accumulation of Nutrients

Logarithms of the amounts of K^+, Ca^{2+}, Mg^{2+}, Na^+, Cl^-, NO_3^-, and N_r in roots and shoot for bean and cotton growing in the culture solution with or without 50 mM NaCl were linearly related to days of culture (Tables II and III for bean and cotton, respectively). Accumulation rates for these nutrients were calculated using the regression coefficients according to the equation in "Materials and Methods."

Sodium contents were negligible in control plants but increased in both species with external NaCl concentration. The amounts of Na^+ accumulated in bean and cotton plants,

Table I. *Effect of salinity on dry weight accumulation in the different organs of bean and cotton plants*

Plants were grown for 20 d on a nutrient medium containing 0, 50, or 100 mM NaCl. Values given are the averages of 10 observations ± SE.

Species	Treatment	Dry Weight				
		Roots	Stem + petioles	Leaves 1 and 2	Leaf 3	Leaf 4
	mM NaCl			*mg*		
Bean	0	189 ± 21	135 ± 25	324 ± 66	191 ± 58	124 ± 36
Bean	50	104 ± 20	70 ± 17	262 ± 56	64 ± 19	
Bean	100	74 ± 14	47 ± 7	258 ± 54	19 ± 3	
Cotton	0	86 ± 17	85 ± 9	362 ± 24	47 ± 12	
Cotton	50	82 ± 29	103 ± 28	312 ± 46	49 ± 15	
Cotton	100	74 ± 20	80 ± 11	310 ± 28	35 ± 7	

Table II. *Coefficients of the linear regressions of the natural logarithms of the amounts of K^+, Ca^{2+}, Mg^{2+}, NO_3^-, N_r, Na^+, or Cl^- in shoot and roots of bean as a function of days in culture*

Bean and cotton plants, grown on a nutrient medium containing 0 or 50 mM NaCl, were harvested on d 12, 17, 21, and 28. On each harvest, six individual plants for each species and each treatment were analyzed for cations, Cl^-, NO_3^-, and N_r. The amounts of the compounds in shoot or roots, expressed in μmol plant^{-1}, were plotted against the culture time in days. The parameters of each regression line, together with the corresponding correlation coefficient (r^2), are shown.

Parameter	K^+	Ca^{2+}	Mg^{2+}	NO_3^-	N_r	Na^+	Cl^-	K^+	Ca^{2+}	Mg^{2+}	NO_3^-	N_r	Na^+	Cl^-
	Medium without NaCl							*Medium with 50 mM NaCl*						
Shoot														
Slope	0.111	0.114	0.110	0.086	0.050			0.067	0.051	0.049	0.055	0.034	0.102	0.089
y intercept	5.23	4.63	3.76	5.14	6.74			5.18	5.49	4.11	4.28	5.96	3.40	5.03
r^2	1.00	1.00	1.00	1.00	1.00			1.00	0.94	0.94	0.99	0.99	0.99	1.00
Roots														
Slope	0.072	0.098	0.054	0.070	0.048			0.067	0.092	0.019	0.036	0.059	0.095	0.102
y intercept	4.45	2.36	3.25	4.17	5.20			3.60	1.97	3.42	4.03	4.38	4.14	3.16
r^2	0.99	0.99	0.99	0.98	0.97			0.95	0.99	0.99	0.98	1.00	1.00	1.00

however, differed markedly. Thus, sodium accounted for only about 10% of the cationic charges accumulated in bean shoots (Fig. 1B), whereas it became the predominant cation in shoots of 50 mM NaCl-fed cotton plants (Fig. 2B). In bean plants fed with 50 mM NaCl, about 60% of the total amount of Na^+ was accumulated in roots, whereas about 95% of the Na^+ present in cotton plants was distributed in shoots (Figs. 1B and 2B). In bean plants, the 50-mM NaCl treatment resulted in a marked decrease in accumulation rate of other major cations (K^+, Ca^{2+}, Mg^{2+}) in shoot but not in roots (Fig. 1). On the contrary, in cotton plants the salt treatment did not affect the accumulation rate of other cations in shoot or roots.

Saline treatments led to an accumulation of Cl^- in all parts of the plants. The distributions of Na^+ and Cl^- within the plants, however, differed with the plant species. In bean Cl^- accumulated at a higher rate than Na^+ in shoot, but the reverse occurred in roots. In cotton plants, the two ions were accumulated at comparable rates in both roots and shoots. NO_3^- accumulation rates decreased in all organs of bean and in roots of cotton plants in response to 50-mM NaCl treatment.

The accumulation rates of N_r were lowered, especially in bean and cotton shoots, by feeding plants with 50 mM NaCl. This effect, however, was more pronounced in bean (Fig. 1) than in cotton (Fig. 2).

Effect of NaCl on Xylem Transports

For control plants (grown in NaCl-free medium), a xylem exudation rate similar to the transpiration rate was obtained when 200 kPa pressure was applied; however, the exudation rate from root systems of plants grown with 50 mM NaCl remained below the transpiration rate measured on intact plants even when pressures of 500 kPa were applied. For control plants, increasing the pressure (in the range 50–300 kPa) increased the volumic exudation rate and decreased the ionic concentrations in the exudates. Thus, the composition of xylem sap in situ was not directly measurable using this procedure. However, in these experiments the ratios between concentrations of the different ions in the xylem exudates remained constant over the pressure range applied (results not shown). A similar behavior was recently observed by Duarte and Larsson (1993) in nonnodulated pea. Since the estimation of the upward transport of a given compound between roots and shoot requires only the knowledge of these ratios in the xylem sap (see "Materials and Methods"), we considered that the method used for collecting xylem sap was suitable. Table IV gives the concentrations of N_r and major ions in the xylem exudates of control and NaCl-fed plants when pressures of 100 and 300 kPa, respectively, were applied. The excess of cationic charges in xylem sap of bean plants presumably was balanced by carboxylic anions.

Table III. *Coefficients of the linear regressions of the natural logarithms of the amounts of K^+, Ca^{2+}, Mg^{2+}, NO_3^-, N_r, Na^+, or Cl^- in shoot and roots of cotton as a function of days in culture*

Same legend as Table II.

Parameter	K^+	Ca^{2+}	Mg^{2+}	NO_3^-	N_r	Na^+	Cl^-	K^+	Ca^{2+}	Mg^{2+}	NO_3^-	N_r	Na^+	Cl^-
	Medium without NaCl							*Medium with 50 mM NaCl*						
Shoot														
Slope	0.112	0.127	0.105	0.098	0.120			0.115	0.143	0.106	0.117	0.101	0.166	0.149
y intercept	4.18	4.02	2.35	4.53	4.96			3.89	3.36	1.99	3.54	4.85	3.37	3.72
r^2	0.98	0.98	0.96	0.99	1.00			1.00	0.94	0.94	0.99	0.99	0.99	1.00
Roots														
Slope	0.174	0.164	0.089	0.170	0.155			0.098	0.133	0.087	0.094	0.114	0.117	0.071
y intercept	1.44	0.20	1.53	0.81	1.87			2.69	0.29	1.47	1.24	2.64	1.35	3.23
r^2	0.99	0.99	1.00	1.00	1.00			0.99	0.99	0.98	0.98	0.97	1.00	1.96

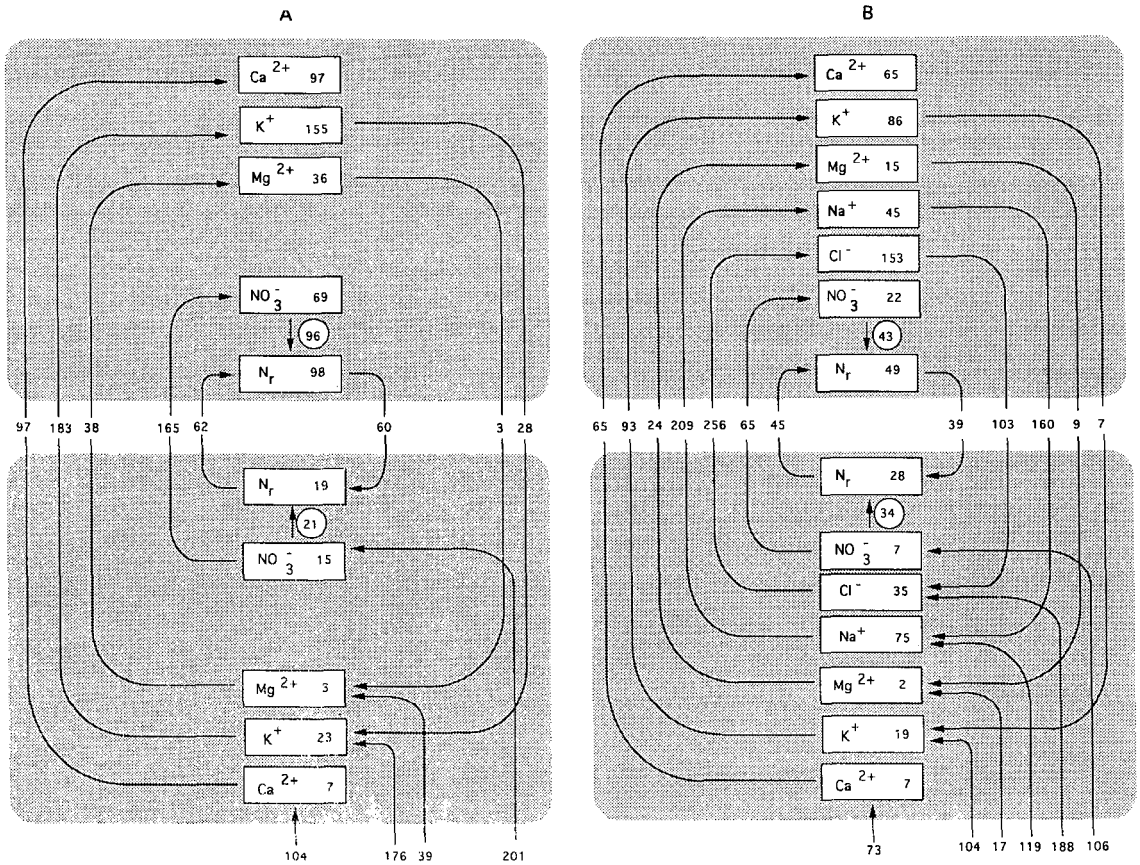

Figure 1. Flow patterns within 20-d-old plants of bean grown in the absence (A) or presence (B) of 50 mM NaCl in the nutrient medium. The lower shaded compartments represent the roots, and the upper ones, the shoot. The accumulation rate of each element in shoot or roots are indicated in white boxes. The arrows represent uptake (below the root compartment), upward flows in the xylem (on the left), downward flows in the phloem (on the right), and NO_3^- reduction (NO_3^- reduction rates given in the white circles). The values, expressed in μmol g^{-1} dry weight d^{-1}, were calculated using the data from Tables II and IV as explained in "Materials and Methods."

Effect of NaCl on Flow Patterns within the Plant

Flow patterns within the plants for nutrient elements are presented in Figures 1 (bean) and 2 (cotton). Transport between medium and roots and between roots and shoot was impaired much more in bean plants than in cotton plants. The flux of every mineral nutrient appears to be about equally sensitive to the presence of NaCl in the nutrient medium.

Effect of NaCl on NRA

The presence of NaCl in the culture solution resulted in a decrease of in vitro NRA in bean leaves, and this effect became more pronounced as NaCl concentration increased (Table V). Conversely, in vitro NRA of cotton leaves was not affected by NaCl treatments (Table V). The presence of increasing NaCl concentrations in the incubation medium during the enzymatic assay resulted in a decrease of in vitro NRA measured in roots and leaves of both species (Fig. 3). On the other hand, decreasing the osmotic potential of the incubation medium with mannitol had no effect on NRA, whether in roots or shoot, for bean or cotton (Fig. 3).

The levels of in situ NRA were 4 to 20 times lower than those of in vitro NRA (Table V). The in situ NRA values were lower on leaves of NaCl-fed plants than on those of control plants, and the effect of NaCl treatments was more pronounced for bean than for cotton plants (Table V). When 100 mM KNO_3 was present in incubation medium of the in situ assay, NRA values measured in leaves of control plants increased slightly (Fig. 4). For plants fed with 50 mM NaCl, the increase in in situ NRA caused by the presence of exogenous KNO_3 in the incubation medium was greater for bean leaves than for cotton leaves (Fig. 4).

DISCUSSION

The presence of 50 to 100 mM NaCl in the nutrient medium altered the growth rate of bean cv Gabriella markedly but had only a slight effect on that of cotton cv Acala (Table I). This result concurs with the reported sensitivity of bean and tolerance of cotton to salinity (Greenway and Munns, 1980; Brugnoli and Lauteri, 1991). At 50 mM NaCl the dry matter production in 20 d was reduced by 55 and 18% for bean and cotton, respectively. These values are close to those obtained

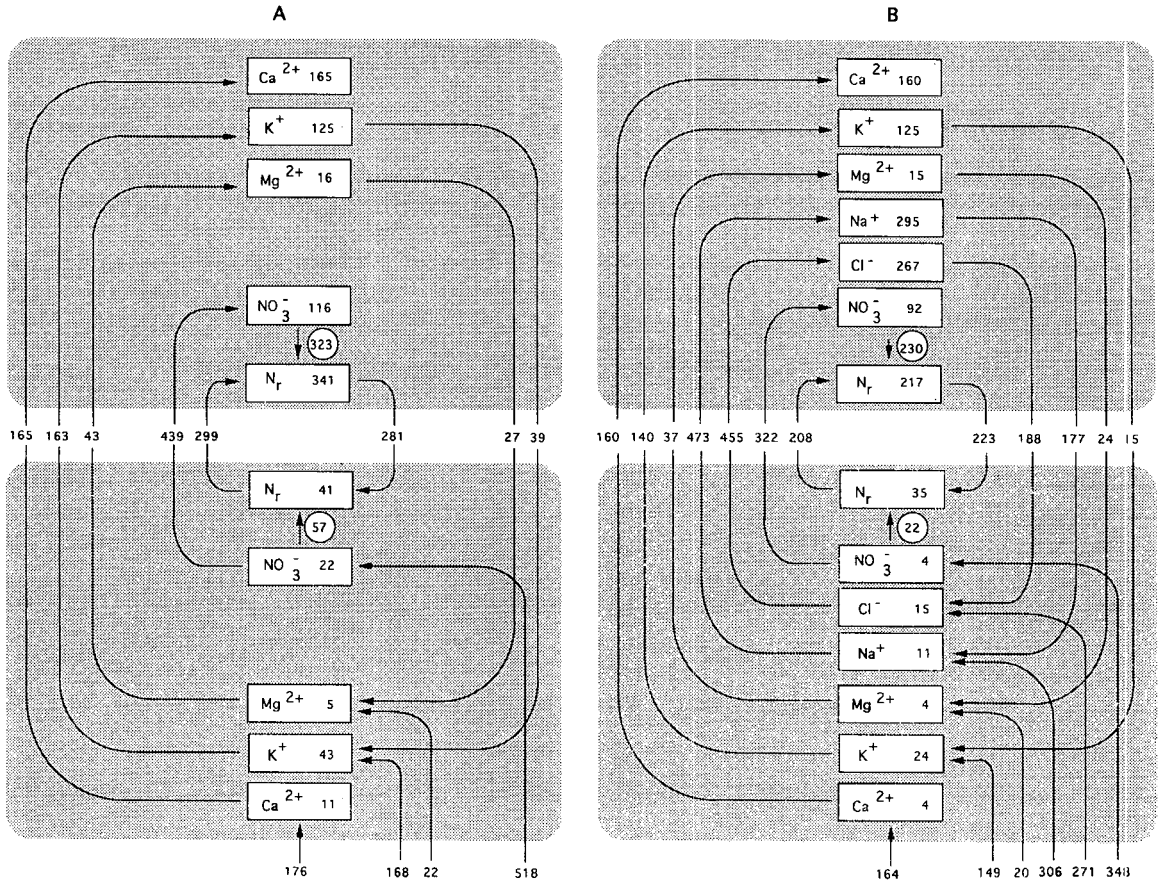

Figure 2. Flow patterns within 20-d-old plants of cotton grown in the absence (A) or presence (B) of 50 mM NaCl in the nutrient medium. See Figure 1 for legend (except that Table III was used in calculations instead of Table II).

by Slama (1991) for the same cultivars. The growth of bean was more depressed by exogenous NaCl than the growth of cotton during the exponential growth phase between 12 and 28 d, as well as during the initial, nonexponential growth phase, since both slope and y intercept for the linear regression of the logarithm of plant dry weight versus time were decreased more by the presence of 50 mM NaCl for bean than for cotton. Salinity did not modify the dry matter allocation between roots and shoot. Irrespective of NaCl treatments (0, 50, or 100 mM), the shoot:root weight ratio remained in the 4 to 4.3 range for bean and close to 5.7 for cotton (calculated using data from Table I).

As stated above, the different flows in plants are expressed on a whole plant dry weight basis. This allows comparison of flows in plants of different sizes and the opportunity to highlight the specific effects of NaCl salinity on these flows that cannot be accounted for by changes in growth rate. The calculation procedure consisted simply of dividing the values for accumulation rates, translocation rates, uptake rates, and nitrate reduction rates in μmol h^{-1} $plant^{-1}$ obtained for 20-d-old plants by the dry weight of these same plants. This is equivalent to use of the equation given by Trought and Drew (1980), in which the flows per plant were divided by the ratio $dW:dlnW$ (where W is the dry weight), since this ratio in

Table IV. *Concentrations of N_r and dominant ions in the xylem exudates of 20-d-old plants of bean or cotton grown with or without NaCl*

The plants were grown for 20 d on a complete nutrient medium in the absence or presence of 50 mM NaCl. Root systems were decapitated and inserted into sealed pots with their excised surface emerging. Xylem exudates were collected under 100 kPa (0 mM NaCl) or 300 kPa (50 mM NaCl) for 0.5 h. Values are the averages of six observations. SE did not exceed 15% of the mean values.

Plant	Treatment	K^+	Ca^{2+}	Mg^{2+}	NO_3^-	Na^+	Cl^-	N_r
	mM NaCl				*mM*			
Bean	0	10.45	5.62	2.06	9.43	0	0	3.59
Bean	50	10.12	7.02	2.56	7.02	22.56	27.50	4.84
Cotton	0	4.25	4.29	1.11	11.49	0	0	7.80
Cotton	50	4.29	4.89	1.15	9.94	14.50	13.90	6.35

Table V. *Effect of NaCl on NRA of the third leaf of 20-d-old bean and cotton*

Plants were fed with standard culture solutions containing 7 mM (S) or 1 mM (S') NO_3^-, or the same solutions also containing 50 mM (+50) or 100 mM (+100) NaCl. Values are the averages of 6 (in vitro NRA) or 10 (in situ NRA) observations ± SE.

Species	NRA Assay	Culture Solution				
		S	S'	S + 50	S' + 50	S + 100
		μmol NO_2^- h^{-1} g^{-1} fresh weight				
Bean	In vitro	6.11 ± 0.73	3.88 ± 0.56	4.58 ± 0.69	2.14 ± 0.41	1.97 ± 0.36
Bean	In situ	1.46 ± 0.41	0.40 ± 0.06	0.73 ± 0.21	0.12 ± 0.03	0.36 ± 0.12
Cotton	In vitro	12.45 ± 1.86		14.14 ± 4.86		12.67 ± 3.38
Cotton	In situ	1.54 ± 0.21		1.00 ± 0.17		0.98 ± 0.18

fact is equal to W at time t. In the circulation patterns of Figures 1 and 2, all the flows are expressed on a same whole plant dry weight basis rather than a shoot or root dry weight basis to make values of different flows comparable. Furthermore, since the presence of 50 mM NaCl did not change the shoot:root dry weight ratios in our plants, this choice does not affect the discussion on the effects of salinity on circulation patterns.

Although there were some differences in amount and distribution of ions in bean and cotton with addition of NaCl to the nutrient solution, both species accumulated high amounts of Na^+ and Cl^-, whereas contents of other minerals decreased (Figs. 1 and 2). Such behavior has been observed in a variety of glycophytes by many authors (e.g. Jeschke and Wolf, 1988; Bogemans et al., 1990; Jeschke et al., 1992). Among several glycophytes, high recirculation rates of Na^+ occur in the salt-sensitive species lupin (Munns et al., 1988) but not in the more tolerant species barley (Munns et al., 1986) and tomato (Grunberg and Taleisnik, 1991). Furthermore, the sieve saps of the halophytes *Aster tripolium* (Downing, 1980) and *Avicennia marina* (Munns et al., 1986) contain very low Na^+ concentrations. Although phloem fluxes of Na^+ in bean and cotton had similar values (Figs. 1B and 2B), Na^+ recirculation was markedly higher in bean, which is the more sensitive species. In bean, 77% of xylem-transported Na^+ was re-exported to roots, whereas in cotton this proportion was only 37%. These proportions are associated with a relatively low Na^+ accumulation rate in bean shoot, which was about half the K^+ accumulation rate, and a high rate in cotton shoot, which was about 2.5 times the K^+ accumulation rate (Figs. 1B and 2B). Hence, it appears that the accumulation rate of Na^+ in shoots is not determined by the xylem transport rate of Na^+. Rather, it is the phloem translocation rate that is determined by the balance between xylem supply of Na^+ and accumulation by tissues. The net uptake rates of Na^+, especially the net accumulation rate of this ion in leaves, thus differed greatly between the two species, whereas the net export rates from shoots were of similar magnitude. Generally, the net fluxes of Cl^- in the plant were higher in cotton than in bean except for the accumulation rate in roots (Figs. 1B and 2B). Contrary to the behavior of Na^+, Cl^- imported to shoots through xylem sap was similarly distributed between shoot accumulation (about 60%) and phloem translocation (about 40%) in the two species.

In control plants, the substantial recirculation of K^+ that occurred in the phloem (16 and 24% of the xylem-borne K^+ for bean and cotton, respectively; Figs. 1A and 2A) was less than the 80% reported for pea (Duarte and Larsson, 1993),

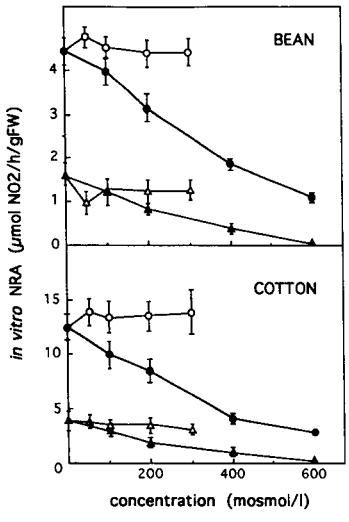

Figure 3. Effect of increasing osmotic potential in the incubation medium on in vitro NRA. The in vitro NRA assay was performed on extracts from third leaf (○, ●) and root (△, ▲) and with an incubation medium containing either NaCl (closed symbols) or mannitol (open symbols). Values are the averages of six observations ± SE.

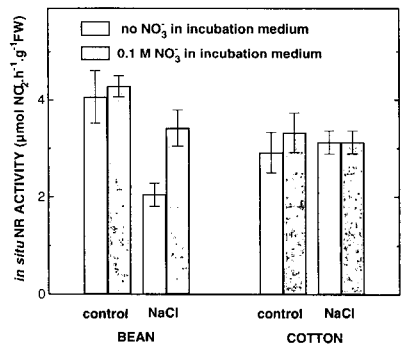

Figure 4. Effect of the presence of 100 mM KNO_3 in the incubation medium on in situ NRA of the third leaf of bean and cotton. The plants were grown for 20 d on a NaCl-free nutrient solution (control) or on the same solution to which 50 mM NaCl was added (NaCl). Values are averages of 10 observations ± SE.

the 68% for soybean (Touraine et al., 1988), or the 33% for lupin (Jeschke et al., 1992), but was in the range of the 20% for tomato (Armstrong and Kirkby, 1979). The presence of 50 mM NaCl in the culture solution similarly reduced K^+ uptake (by 41 and 12% in bean and cotton, respectively) and xylem transport from roots to shoot (by 49 and 14% in bean and cotton, respectively); however, NaCl reduced phloem translocation of K^+ (by 74 and 61% in bean and cotton, respectively) to a greater extent than uptake or xylem transport (Figs. 1 and 2). Thus, although NaCl salinity depressed the uptake rate of K^+, it impaired K^+ partitioning within the plant to a greater extent. This concurs with the findings of Jeschke et al. (1992). The uptake and xylem transport rates of Ca^{2+} and Mg^{2+} were altered by exogenous NaCl in the same manner and to the same extent as those of K^+. In contrast to K^+, however, phloem translocation of Mg^{2+} was affected by exogenous NaCl to about the same extent as uptake and xylem transport (Figs. 1 and 2).

The overall pattern of N flows within bean and cotton (Figs. 1A and 2A) resembles that of other species when grown with NO_3^- as N source (Simpson et al., 1982; Van Beusichem et al., 1987; Touraine et al., 1988). An extensive root-to-shoot cycling occurred. For control plants, 38% of xylem-borne N (xylem-translocated N_r plus reduction of xylem-translocated NO_3^-) in bean and 46% in cotton was translocated back to roots. This is comparable to 37% for soybean (Touraine et al., 1988), 24% for lupin (Jeschke et al., 1992), and 23% for wheat (Larsson, 1992). Duarte and Larsson (1993) found an even higher figure (64%) for pea plants growing with an RGR of 0.12 g dry weight g^{-1} dry weight d^{-1}. For both bean and cotton plants, NO_3^- was the predominant form of N in the xylem sap (73 and 60% in bean and cotton, respectively; Table IV), which indicates that the shoot is the main site for NO_3^- reduction in these species. This conclusion is in accordance with previous estimates of the distribution of NO_3^- reduction within whole plants using measurements of NO_3^-:N_r ratios in xylem sap or NRA assays (Andrews, 1986, and refs. therein). The proportion of NO_3^- in the xylem sap underestimates the actual contribution of the shoot in NO_3^- reduction (82 and 85% for bean and cotton, respectively) due to the high recirculation rate of N_r through roots. Indeed, a great fraction of N_r that was transported in xylem originated from the shoot rather than from NO_3^- reduction in roots (Figs. 1A and 2A), as has been demonstrated in experiments using ^{15}N (Rufty et al., 1982; Cooper and Clarkson, 1989; Larsson et al., 1991).

As was the case for the cations, NO_3^- uptake and N flows within the plants were negatively altered by salinity (Figs. 1 and 2). This effect was more pronounced for bean, in which NO_3^- uptake was inhibited by 47%, than for cotton, in which it was inhibited by 33%. In both species, salinity decreased NO_3^- xylem transport rate, NO_3^- reduction rate in shoot, and N_r xylem and phloem transport rates. The question then is which of these steps of N nutrition is specifically affected by salinity. It has been shown that NO_3^- uptake by roots of barley seedlings was decreased by the addition of salt to the nutrient solution within 1 to 5 min (Klobus et al., 1988), which can hardly be explained by an endogenous feedback control of the NO_3^- uptake system. Furthermore, recovery of NO_3^- uptake after NaCl removal seems to require a resynthesis of NO_3^- transporters (Klobus et al., 1988). Nevertheless, it should be noted that this inhibition of NO_3^- uptake, observed in short-term experiments, was obtained for 150 mM NaCl or more, whereas 50 or 100 mM had no effect (Klobus et al., 1988).

Another explanation for how NO_3^- uptake and NO_3^- reduction rates in shoot might be altered to the same extent by salinity would be that NaCl specifically affected NO_3^- reduction in shoot and that the NO_3^- uptake rate was controlled by the NO_3^- reduction rate. It has been proposed that NO_3^- uptake is regulated by the N demand of the whole plant (Touraine et al., 1994). Two possible regulatory systems that have been proposed involve phloem translocation from shoot to roots of organic acids and amino acids as products of NO_3^- reduction (Touraine et al., 1994). Since at least some amino acids repress NO_3^- uptake when supplied to roots through the phloem (Muller and Touraine, 1992), the decrease in phloem translocation rate of N_r caused by NaCl should stimulate NO_3^- uptake rather than inhibit it as was observed. On the other hand, superficially, a decrease in production of organic acids in the shoot (as a result of the lowered NO_3^- reduction rate) should lead to a decrease in the NO_3^- uptake rate by roots (Touraine et al., 1992). The translocation rate of organic acids from shoot to roots, however, is determined not only by their production rate in shoot, but also by their accumulation rate in tissues. Because bean excludes Na^+ but not Cl^- from its leaves (Fig. 1B), the accumulation rate of organic acids in shoot necessary to counterbalance cations accumulated in excess of mineral anions would have been reduced by exogenous NaCl. This release of organic acids for export to roots could compensate for the decrease in the rate of organic acid production in these plants. Conversely, in cotton the electrical charge balance for accumulating inorganic ions was not markedly affected by salinity (Fig. 2B); therefore, the accumulation rate of organic acids should not be altered greatly. Thus, in cotton, the availability of organic acids for potential regulation of the NO_3^- uptake system presumably would have been decreased to the same extent as the NO_3^- reduction in shoot. If the effect of exogenous NaCl on NO_3^- uptake were due to variations in the translocation rate of organic acids, the NO_3^- uptake rate should have been decreased particularly in cotton, but not in bean. Thus, it appears unlikely that phloem translocation of either organic acids or amino acids, which has been proposed as a likely signal for demand-driven regulation of NO_3^- uptake, is involved in the effect of mild salinity on NO_3^- uptake.

In cotton, NO_3^- reduction in roots was lowered by exogenous NaCl in a higher proportion (62%) than were N flows (21–31%). In bean, however, NO_3^- reduction in roots was increased by 61%, whereas other N flows were decreased to a higher extent than in cotton (28–60%). In lupin, for which NO_3^- assimilation occurs predominantly in roots, NO_3^- reduction in roots was especially inhibited by NaCl (Jeschke et al., 1992). In view of the small proportion of NO_3^- reduction that occurs in roots of bean and cotton (Figs. 1 and 2), the observed alterations in rate of NO_3^- reduction in roots should not have had an appreciable effect on N nutrition of the whole plant.

Studies on the effects of exogenous NaCl on NRA have

given contradictory results. Both inhibition (Aslam et al., 1984; Martinez and Cerda, 1989) and stimulation (Misra and Dwiverdi, 1990) have been reported. In soybean, NRA in leaves was not affected by salinity, whereas it was slightly stimulated in roots (Bourgeais-Chaillou et al., 1992). In our study, in vitro NRA was depressed in shoots and roots of both species by the presence of NaCl in the incubating medium (Fig. 3). In vitro NRA seems to be affected by Na^+ or Cl^- ions themselves since decreasing the osmotic potential with mannitol had no effect (Fig. 3). On the other hand, the presence of NaCl in the culture solution did not alter in vitro NRA in cotton leaves but decreased it in bean leaves (Table V). Perhaps differential compartmentalization of ions and NR in the cells protected the enzyme against inhibition when NaCl was added to the assay medium. Thus, one may propose that decreased compartmentalization of ions in bean relative to cotton is the reason for the greater sensitivity of bean NR to NaCl. Comparison of in vitro NRA, however, does not allow evaluation of the relative capacities of the intact tissues of the two species to reduce NO_3^- (Soussana et al., 1989). This is illustrated by the fact that values of in vitro NRA were 1 order of magnitude higher than those of in situ NRA (Table V). Thus, NO_3^- reduction was not limited by the amount of enzyme present in tissues, as estimated by in vitro NRA. It is unlikely, then, that the observed effect of NaCl on in vitro NRA, even if it corresponds to an actual effect on the NR synthesis in situ, had an effect on the actual NO_3^- reduction rate in the intact plants. The in situ NRA was more affected in bean leaves than in cotton leaves (Table V), as was the actual NO_3^- reduction rate in shoot (Figs. 1 and 2).

On the other hand, addition of NO_3^- to the incubation medium during the in situ assay markedly increased NRA measured in NaCl-treated bean plants, whereas it had a slight effect, if any, in NaCl-treated cotton plants or in control bean and cotton plants (Fig. 4). The difference between the in situ NRA values in the presence or absence of NO_3^- in the incubation medium may be interpreted as reflecting the dependence of NO_3^- reduction on NO_3^- import into the leaf, which was interrupted by cutting the leaf from the plant before the NRA assay. If this hypothesis is correct, the main factor that depresses NRA in bean leaves attached to the intact plant should be the effect of the decreased xylem transport rate of NO_3^- to the shoot on decreasing the availability of NO_3^- at the site of reduction rather than a lowering of intrinsic NO_3^- reduction capacities. Finally, our results support the hypothesis of Munns (1993) that the effect of the salts taken up by the plant on plant growth is not a direct effect on the activity of any enzyme. A study of the effects of osmotic stress on NO_3^- uptake and utilization in ryegrass similarly led Ourry et al. (1992) to the conclusion that NR is not affected per se.

Taken as a whole, our results show that for both bean and cotton only slight differences occur for the quantitative effects of NaCl on either the uptake rates of the various ions or the relationship between the uptake rates for each element and its transport rate between roots and shoot. Thus, it is hard to imagine that NaCl specifically affects any one of these rates. On the other hand, inhibition of uptake rates of nutrients was greater than inhibition of growth rate (i.e. flows expressed on a dry weight basis were lower in Figs. 1B and 2B than in Figs. 1A and 2A). This suggests that the effect of NaCl on mineral nutrition may not be regarded simply as the consequence of the operation of a demand-driven control. The main influence of salinity thus appears to be on the flows of mineral ions. Further investigations, however, are needed to elucidate whether one of the ion transport systems of the plasmalemma of root cells is particularly sensitive to salinity or whether the effects on nutrient fluxes are the consequences of other disorders due to salinity. There also is the possibility among species for differential synthesis of H^+-ATPase in response to salinity (Niu et al., 1993) and thus for differential capacity to maintain the H^+ electrochemical potential required for plant ion homeostasis. Clearly, the question of the location of NaCl effect(s) on mineral nutrition must be resolved if the mechanisms involved in the relative tolerance of some species, such as cotton, compared to others, such as bean, are to be elucidated.

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