Early Effects of Salinity on Nitrate Assimilation in Barley Seedlings

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ABSTRACT

The effect of NaCl and Na2SO4 salinity on NO3- assimilation in young barley (Hordeum vulgare L. var Numar) seedlings was studied. The induction of the NO3- transporter was affected very little; the major effect of the salts was on its activity. Both Cl- and SO42- salts severely inhibited uptake of NO3-. When compared on the basis of osmolality of the uptake solutions, Cl- salts were more inhibitory (15–30%) than SO42- salts. At equal concentrations, SO42- salts inhibited NO3- uptake 30 to 40% more than did Cl- salts. The absolute concentrations of each ion seemed more important as inhibitors of NO3- uptake than did the osmolality of the uptake solutions. Both K+ and Na+ salts inhibited NO3- uptake similarly; hence, the process seemed more sensitive to anion salinity than to cationic salinity.

Unlike NO3- uptake, NO3- reduction was not affected by salinity in short-term studies (12 hours). The rate of reduction of endogenous NO3- in leaves of seedlings grown on NaCl for 8 days decreased only 25%. Nitrate reductase activity in the salt-treated leaves also decreased 20% but its activity, determined either in vitro or by the ‘anaerobic’ in vivo assay, was always greater than the actual in situ rate of NO3- reduction. When salts were added to the assay medium, the in vitro enzymic activity was severely inhibited; whereas the anaerobic in vivo nitrate reductase activity was affected only slightly. These results indicate that in situ nitrate reductase activity is protected from salt injury. The susceptibility to injury of the NO3- transporter, rather than that of the NO3- reduction system, may be a critical factor to plant survival during salt stress.

The assimilation of NO3-, the predominant form of N available in an aerobic environment, is critical if plants are to adapt, grow, and reproduce in saline conditions. Not only is NO3- assimilation required for growth and development, but some of its metabolites accumulate during stress (11, 16, 30). It is well known that both proline (11, 16, 30) and betaine (10) accumulate during stress. Proline apparently originates from recently formed glutamate (5). Methylated quaternary ammonium compounds and possibly some amino acids accumulating in stressed plants could serve as osmoticia for osmoregulation (10, 16, 30). Whether or not the N compounds originating from NO3- accumulate as symptoms of stress or are osmoregulants, they represent a component of the N economy of the plant and emphasize the need to characterize NO3- assimilation during environmental stresses.

Controversial results have been reported for the effects of salinity on N assimilation. Halal et al. (12) observed that in spring barley salinization with NaCl impaired growth and uptake of labeled N. The incorporation of labeled N into the protein fraction, however, was increased by salinity. Langdale et al. (20) also observed that NaCl salinity increased the protein content of Stargrass. In contrast, NaCl salinization had little effect on N uptake in winter barley but impaired its incorporation into the protein fraction (13). In leaf discs of Nicotiana rustica, salt stress reduced both the uptake of L-leucine and its incorporation into proteins (3).

The reported effects of salinity on N assimilation are controversial, because no studies were done that measured all of the processes of NO3- assimilation simultaneously. Measuring only uptake or internal reduced N does not yield a balance sheet needed to determine which processes are affected.

This report describes the effects of salinity on the processes of NO3- assimilation.

MATERIALS AND METHODS

Seedling Growth. Two varieties of barley (Hordeum vulgare L.), Numar and Arivat, were grown. Numar is a salt-tolerant and Arivat is a salt-sensitive variety (7). Seedlings were grown both hydroponically and in vermiculite. For growing seedlings hydroponically, the seeds were germinated in 0.2 mM CaSO4, in darkness at room temperature. After 7 d, the seedlings were transferred to aerated one-quarter strength Hoagland solution lacking N (14) and placed in continuous light for 3 d at 25°C and 60% to 65% RH. Photon flux density (400–700 nm) at the seedling canopy was 400 μE m-2 s-1 and was supplied by incandescent and cool white fluorescent lamps. In some experiments, the seedlings after 2 d in continuous light were transferred to nutrient solutions containing 1 mM KNO3 (presoaked seedlings) or 0.1 to 2 mM NaCl or Na2SO4 solutions (presoaked seedlings) for 24 h.

For growing seedlings in vermiculite, about 15 g of seeds were planted at a depth of 2 cm in plastic pots (13.5 cm in diameter × 15.0 cm tall) containing vermiculite. The pots were subirrigated with a modified Hoagland solution containing 10 mM KNO3 or 20 mM KNO3 and 0.2 mM NaCl. In the salt treatment, the seedlings were soaked in H2O for 24 h prior to planting in vermiculite in order to enhance the germination process. The seedlings were grown for 7 to 8 d in a controlled environment growth chamber under a 16-h photoperiod at 65% to 70% RH and 25°C/15°C light/dark temperature.

Nitrate Uptake. Uptake of NO3- by the whole seedlings grown hydroponically was measured by following its disappearance from the uptake solutions as described before (1, 6). Eight seedlings, weighing about 2 g/treatment, were placed in 140 ml of the uptake solutions. The uptake solutions contained 1 mM KNO3 and 0 to 0.2 mM of either NaCl or Na2SO4 in one-quarter strength Hoagland solution. The seedlings presoaked with salts were placed in uptake solutions containing 1 mM KNO3 only.

Cumulative NO3- uptake was determined at 2-h intervals in light

1 Supported in part by a grant from the Kearney Foundation of Soil Science.
(400 μM m⁻² s⁻¹) at 25°C over a 12-h time course. Rates of NO₃⁻ uptake were calculated from a linear regression of these curves. At the end of the uptake period, the seedlings were weighed, and analyzed for NO₃⁻ and NO₂⁻. All the data are presented on a g fresh weight basis of plant material.

**In Vitro Reduction of Absorbed NO₃⁻.** In vivo reduction of absorbed NO₃⁻ was determined simultaneously along with uptake. The difference between the total amount of NO₃⁻ absorbed and that accumulated in the seedlings was considered to be reduced in vivo (1, 6). Rates of NO₃⁻ reduction were calculated from a linear regression of the 12-h time course curves after the linearity was attained.

**In Vivo Reduction of Endogenous NO₃⁻.** The apical 8 cm of leaves from 8-d-old seedlings grown in vermiculite and irrigated with nutrient solutions containing NO₃⁻ were excised and placed base down in glass vials containing 10 ml of deionized H₂O. The depth of the water in the vials was such that only basal 1.5 cm of the leaves was in water, and the remainder of the leaves were exposed to the atmosphere. The vials containing the leaves were then placed in light (400 μM m⁻² s⁻¹) at 25°C. The leaves were removed at intervals and analyzed for NO₃⁻. No NO₂⁻ leaked into the water from the leaves during the experimental period. The disappearance of NO₃⁻ from the leaves is designated as in vivo reduction of endogenous NO₃⁻.

**NR² Assay.** Nitrate reductase was assayed by both in vitro and 'anaerobic' in vivo assay methods. For the in vitro assay, one g of leaves was homogenized in 4 ml of 0.1 M K-phosphate (pH 7.5) containing 1 mM EDTA, 1 mM cysteine, and 3% (w/v) casein. The homogenate was centrifuged at 30,000g for 15 min. The supernatant was used for the enzyme assay. The reaction mixture contained 30 μmol K-phosphate (pH 7.5), 20 μmol KNO₃, 0.5 μmol NADH, 0.1 ml of the supernatant, and various concentrations of NaCl in a total volume of 2 ml. The reaction was carried out at 28°C for 15 min and was stopped by boiling the mixture for 3 min. Excess NADH was oxidized by adding phenazine methosulfate (15 μmol/ml) to the assay mixtures (25). After 20 min, NO₃⁻ content of the assay mixtures was determined.

For the in vivo assay, approximately 0.3 g of leaf sections (2-3 mm wide) were incubated for 1 h in 10 ml of 0.1 M K-phosphate (pH 7.5) containing 50 mM KNO₃ and 1% (v/v) n-propanol under dark anaerobic conditions at 28°C. After incubation, both the assay medium and the tissue were analyzed for NO₃⁻. The enzyme activities were calculated as μmol NO₃⁻ produced/g-fresh weight · h.

**Nitrate and Nitrite Analysis.** The tissue was ground with a mortar and pestle in 10 volumes of deionized H₂O and centrifuged at 30,000g for 15 min. The supernatant was used for NO₃⁻ and NO₂⁻ analysis. Nitrate was determined by measuring its absorption at 210 nm following separation by HPLC on a Partisil-10-SAX anion exchange column (29). Nitrite was determined colorimetrically as described by Sanderson and Cocking (24).

**Determination of Soluble Protein, Sugars, and Chl.** Soluble proteins from the plant extract were assayed using the protein-dye binding procedure as described by Bradford (4). Chl was extracted with 80% (v/v) acetone. Absorbance was read at 665 and 645 nm and Chl contents were calculated according to the method of Kirk (19).

Total soluble sugars were extracted by grinding 1 g of the apical 8 cm of leaves in a mortar and pestle with 80% (v/v) ethanol. The extracts were filtered through Whatman filter paper No. 1, heated to 60° to 65°C to evaporate the ethanol, filtered again, and made to 100 ml volume. Total soluble sugars, after acid hydrolysis (28), were determined using the Somogyi method (22) and are reported as glucose equivalents.

**Measurement of Osmolality.** Osmolality of the uptake solutions was measured with a vapor pressure osmometer.

**Transpiration Measurements.** The amount of water transpired by the seedlings was measured gravimetrically and is expressed on the basis of shoot.

### RESULTS

**Effect of NaCl.** The rate of NO₃⁻ uptake by whole seedlings decreased with increasing salt level, most sharply at concentrations greater than 0.1 M (Fig. 1A). A 14, 54, and 83% decrease in the uptake rate occurred at 0.1, 0.15, and 0.2 M NaCl level, respectively. A typical initial lag period of about 4 h occurred before NO₃⁻ uptake rates became constant. The presence of salts in the uptake solutions did not affect the lag period. The effect of salts was seen after 2 h of uptake. Since the reduction of NO₃⁻ is dependent on NO₃⁻ flux (6), the rate of reduction was similarly decreased by salt (Fig. 1B). Salinity had little effect on the percent reduction (61-71%) of the NO₃⁻ that was taken up. Since no NO₂⁻ was detected, it apparently was efficiently assimilated in both stressed and unstressed plants.

Salt had identical effects on short-term NO₃⁻ assimilation in both the salt-sensitive variety (Arivat) and the salt-tolerant variety (Numar); hence, the results for Arivat are not shown.

**Effect of Na₂SO₄.** Increasing Na₂SO₄ concentration in the uptake solutions caused 47% and 73% decrease in uptake rate at 0.1 and 0.15 M, respectively (Fig. 2). The rate of NO₃⁻ reduction also decreased with increasing salt concentration; however, the per cent reduction of the NO₃⁻ that was taken up was not affected.

**Effect of Preinduction with NO₂⁻.** NaCl and Na₂SO₄ inhibited

![Figure 1](image-url)
NO₃⁻ ASSIMILATION AND SALT STRESS

Fig. 2. Effect of Na₂SO₄ on the time course of NO₃⁻ uptake and reduction in uninduced Numar barley seedlings. Experimental details are the same as in Figure 1 except that the uptake solutions contained various concentrations of Na₂SO₄. The rates of NO₃⁻ uptake (μmol/g·h) were 4.06***, 3.75***, 2.16***, and 1.11*** for 0, 0.07, 0.10, and 0.15 M Na₂SO₄, respectively. The rates of NO₃⁻ reduction were 2.69*, 1.41*, and 0.88* for 0, 0.10, and 0.15 M Na₂SO₄, respectively. The correlation coefficients were significant at 0.05 (*) and 0.001 (**) probability.

Table I. Effect of Salts on Rates of Water Transpiration and NO₃⁻ Uptake in Prestressed and Not Prestressed Numar Barley Seedlings

For experimental details, see Figure 4. Correlation coefficients were significant at 0.01 (**) and 0.001 (***) probability.

Table II. Comparative Effect of Cl⁻ and SO₄²⁻ Salts of Na, at Similar Osmolality, on NO₃⁻ Uptake Rates in Numar Barley Seedlings

Experimental details are the same as in Figure 1. Correlation coefficients were significant at 0.001 (***) probability.

NO₃⁻ uptake similarly in both preinduced and uninduced plants (compare Fig. 3A with Figs. 1A and 2A). As expected, the uptake rates showed no lag in preinduced seedlings. In preinduced seedlings, the proportion of NO₃⁻ reduced to that taken up...
Table III. Comparative Effect of Na and K Salts of Cl⁻ and SO₄²⁻ on Rates of NO₃⁻ Uptake in Numar Barley Seedlings

| Salt Concentration | NO₃⁻ Uptake Rates (µmol/g fresh wt-h) | SO₄²⁻/Cl⁻
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaCl</td>
<td>KCl</td>
</tr>
<tr>
<td>M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.73***</td>
<td>3.73***</td>
</tr>
<tr>
<td>0.10</td>
<td>3.30***</td>
<td>3.24***</td>
</tr>
<tr>
<td>0.15</td>
<td>1.50***</td>
<td>1.50***</td>
</tr>
</tbody>
</table>

![Figure 5](image)

**FIG. 5.** Effect of NaCl on NRA in primary leaves from salt-sensitive (Arivat) and salt-tolerant (Numar) seedlings. Seedlings were grown for 7 days in vermiculite and irrigated with a full strength Hoagland solution. NRA was assayed in the presence of 0 to 0.4 M NaCl in assay media using both 'anaerobic' in vivo and in vitro assay methods. The in vivo enzyme activities for Numar and Arivat at 100% were 6.8 and 6.2 µmol NO₃⁻/g fresh wt-h, and in vitro enzyme activities were 23.4 and 19.5 µmol NO₃⁻/g fresh wt-h, respectively.

Increased with salt concentration (Fig. 3B). At 0.15 M Na₂SO₄ or 0.2 M NaCl, the rates of reduction were greater than the rates of uptake indicating that the higher rate of reduction relied on stored NO₃⁻.

**Effect of Prestress with Salts.** Figure 4 shows the effect of prestress of whole seedlings for 24 h with various levels of salinity on subsequent uptake of NO₃⁻ from salt-free solutions. Nitrate uptake rates in prestressed seedlings were 35 to 50% lower than those when salts were directly present in the uptake medium (Table I). Prestress of Arivat barley with salt also affected NO₃⁻ assimilation similarly (data not shown).

Transpiration of water decreased similarly with increased salinity in both the prestressed and not prestressed seedlings (Table I); however, the seedlings appeared healthy, and their water content was not affected over the course of the experiment. Seedling viability was also shown by the ability to reduce their internal NO₃⁻ (Figs. 1B, 2B, 3B).

**Comparative Effect of Cl⁻ and SO₄²⁻ Salinity.** At similar osmolarity of uptake solutions, NO₃⁻ uptake by the seedlings was inhibited more by Cl⁻ salts than by SO₄²⁻ salts (Table II). However when compared on the basis of salt concentration in the uptake solution, NO₃⁻ uptake was inhibited more by SO₄²⁻ than by Cl⁻ salts (Table III). At equimolar concentration, the rates of NO₃⁻ uptake were 30 to 40% lower in SO₄²⁻ salts than in Cl⁻ salts. The accompanying cations (Na⁺ or K⁺) had little effect.

**In Vitro Effects of Salinity on NRA.** NaCl in the assay medium differentially affected in vitro and anaerobic in vitro NRA (Fig. 5). Increasing the salinity level in the assay medium decreased the anaerobic in vitro NRA only slightly. In contrast, in vitro NRA was severely inhibited by salinity. At 0.4 M salinity level, the anaerobic in vivo NRA was inhibited only 20% compared with the control, whereas the same level of salinity resulted in an 80% inhibition of in vitro NRA. Salinity had a similar effect on enzyme activities in both varieties.

**In Vivo Effects of Salinity on NO₃⁻ Assimilation.** Table IV shows the effect of growth for 8 d in NaCl on the levels of NRA in leaves of Numar barley seedlings. Both anaerobic in vivo and in vitro enzyme activities were 20% lower in leaves of seedlings grown in 0.2 M NaCl salinity as compared with those grown without salt. The rate of reduction of endogenous NO₃⁻ in stressed leaves also decreased by 25% as compared to that in unstressed leaves (Table IV). The soluble proteins, total soluble sugar, and Chl content of leaves were not affected by salinity (Table IV).

**DISCUSSION**

The results demonstrate that the processes of NO₃⁻ assimilation are affected differently by salinity. Uptake of NO₃⁻ was markedly inhibited, whereas NO₃⁻ and NO₃⁻ reduction were little affected by salts (Figs. 1–3).

Present evidence indicates that the NO₃⁻ transporter is inducible by NO₃⁻ as shown by a lag period before uptake rate becomes constant (6, 15, 23). Since salinity may affect both the induction and activity of the NO₃⁻ transporter, the effect of salt was determined on plants both preinduced and uninduced with NO₃⁻. No effect of salt was detected on induction of the transporter and the presence of salts inhibited subsequent uptake in both induced and uninduced plants (Figs. 1A, 2A, and 3A). Thus, salinity affected the activity of the NO₃⁻ transporter rather than its induction.

Both K and Na salts inhibited NO₃⁻ uptake similarly (Table III). These results differ from those of Helal et al. (13) who observed that addition of KCl relieved the inhibitory effect of NaCl salinity on the uptake of labeled N. In Cl salts, both the anion and the accompanying cation are present in equal proportion; whereas in SO₄²⁻ salts the cationic concentration is twice that of SO₄²⁻. The differential effect of Cl⁻ and SO₄²⁻ salts on NO₃⁻ uptake suggests that the process is more sensitive to anionic salinity than to cationic salinity.

At equal osmolality of solutions, Cl⁻ salts inhibited NO₃⁻ uptake from 15% to 30% more than SO₄²⁻ salts (Table II). When compared at the same concentration, SO₄²⁻ salts inhibited NO₃⁻ uptake more than did Cl⁻ salts (Table III); hence, the absolute concentrations of each ion seem more important as inhibitors of NO₃⁻ uptake than does the osmolality of the solutions. Kingsbury et al. (18) observed that specific ion effects were more important than was osmotic stress as factors influencing adaptation to salt.

Transpiration of water by the seedlings was inhibited much less by salinity than was NO₃⁻ uptake; hence, a decreased transpiration rate seemed less responsible for the inhibition of NO₃⁻ uptake than the specific ion effects (Table I). Previous studies have also shown that a 50% reduction in transpiration had only a minor effect (10%) on NO₃⁻ uptake (1). Furthermore, in spite of similar transpiration, NO₃⁻ uptake from nonsaline uptake media by prestressed seedlings was inhibited more than uptake from saline solutions by seedlings not prestressed (Table I; Fig. 4).

When salt was added directly to the assay media, in vitro NRA was inhibited much more than was the NRA determined by the anaerobic in vivo method using leaf slices (Fig. 5). Scharer (26) also observed similar inhibition of in vitro NRA by NH₄⁺, K⁺, and NaCl salts when added to the assay medium. In situ NR in the cytoplasm (27) seems protected against the adverse effects of salinity. Presumably, the salts within the plant cell are compartmentalized into the vacuole (31). There is physiological evidence that in cereals Na⁺ is selectively occluded in the vacuole (17).

Reduction of the endogenous NO₃⁻ in the leaves was decreased
only 25% by salinity. Nitrate reduction was not limited by the potential NRA (in vitro or anaerobic in vivo), which was greater than the rate of endogenous NO$_3^-$ reduction (Table IV). Since the major portion of NO$_3^-$ is stored in the vacuole (9, 21), especially a few hours after the removal of NO$_3^-$ from the nutrient solution (2, 8), the release of NO$_3^-$ from vacuoles might regulate the rate of NO$_3^-$ reduction (2). Thus, the small decrease in the rate of NO$_3^-$ reduction may be partially due to NaCl inhibiting NO$_3^-$ efflux from vacuoles.

**LITERATURE CITED**


14. Hoagland DR, DJ arnon 1950 The water-culture method for growing plants without soil. Calif Agric Exp Station Cir 347


28. Smith D 1969 Removing and analyzing total nonstructural carbohydrates from plant tissue. Univ Wis Res Rep 41


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**Table IV. Effect of NaCl on Endogenous NO$_3^-$ Reduction, NRA, Soluble Protein, Soluble Sugars, and Total Chl Content in Primary Leaves of Numar Barley Seedlings**

<table>
<thead>
<tr>
<th>NaCl Level</th>
<th>NRA</th>
<th>Endogenous NO$_3^-$ Reduction</th>
<th>Soluble Protein</th>
<th>Soluble Sugars (Glucose equiv.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>µmol NO$_3^-$/g.h</td>
<td>µmol/g</td>
<td>µmol/g.h</td>
<td>mg/g</td>
</tr>
<tr>
<td>0</td>
<td>4.7 ± 0.5</td>
<td>18.3 ± 0.8</td>
<td>2.16</td>
<td>9.54 ± 0.66</td>
</tr>
<tr>
<td>0.2</td>
<td>3.7 ± 0.4</td>
<td>14.5 ± 0.6</td>
<td>3.44 ± 3.5</td>
<td>1.63</td>
</tr>
</tbody>
</table>