The characterization of inhibition of net nitrate uptake by salt in salt-tolerant barley (Hordeum vulgare L. cv. California Mariout)

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Abstract

Barley seedlings (Hordeum vulgare L. cv. California Mariout) grown hydroponically for 14–19 d without addition of NaCl were used for describing the effects of salt application on net nitrate uptake and for the calculation of kinetic parameters. The addition of NaCl, KCl, CaCl₂, and Na₂SO₄ to the uptake solution in the experiments led to similar inhibition of nitrate uptake, only at low and very high salt concentrations were ion-specific effects found. The same decrease in nitrate uptake can also be achieved by sorbitol or betaine at corresponding osmolalities. Thus, it was concluded that the inhibition of uptake was caused mainly by the osmotic effects of salts. Differences in the mechanisms of inhibition were detected between the two systems of nitrate uptake (high affinity system: HATS, and low affinity system: LATS). The HATS was inhibited non-competitively by NaCl, an apparent Ki of 60 mol m⁻³ was calculated using a Dixon-plot. Fitting an equation assuming a non-competitively inhibited HATS by computer program to the raw data resulted in an apparent Ki of about 37 mol m⁻³. In contrast, the LATS was affected in a complex way: up to 60 mol m⁻³ NaCl the affinity was increased, which led to a stimulation of nitrate uptake at low nitrate concentrations (< 2 mol m⁻³). An inhibition of the LATS became obvious at concentrations above 3 mol m⁻³ nitrate (for all applied salt concentrations) or with 100 mol m⁻³ NaCl (throughout the whole nitrate range). Related plots of the data pointed to a competitive effect.

Key words: Barley, Hordeum vulgare L., net nitrate uptake, high affinity transport system (HATS), low affinity transport system (LATS), salt, inhibition, apparent kinetic parameters.

Introduction

The growth and physiological reactions of higher plants are affected by salinity in many ways. One effect is that in a salinized rhizosphere the uptake of mineral nutrients is inhibited, including uptake of nitrogen which is quantitatively the most important mineral for plants. The rate of nitrate uptake was shown to be lowered by both NaCl and Na₂SO₄, but nitrate reduction was affected less in barley (Aslam et al., 1984). The inhibitory effect of salinity was diminished by adding calcium to the solution (Ward et al., 1986). The different effects of osmotic stress caused by NaCl or polyethylene glycol on nitrate metabolism in ryegrass has been investigated (Ourry et al., 1992). The kinetics of nitrate uptake in non-salinized and salinized wheat seedlings have been compared (Hawkins and Lewis, 1993; Botella et al., 1994). In these papers, however, nitrate uptake was treated as a single mechanism over a wide range of nitrate concentration (up to 10 or 1 mol m⁻³ nitrate, respectively). However, the uptake of nitrate into the roots of plants is mediated by at least a biphasic system (Rao and Rains, 1976; Doddema and Telkamp, 1979; Siddiqi et al., 1990; Aslam et al., 1992; Peuke and Jeschke, 1998; for a review see Peuke and Kaiser, 1996). In the low nitrate concentration range one system with a high affinity following Michaelis–Menten characteristics operates. With increasing external nitrate concentrations a second system contributes to uptake, but the nature of this system is
still under debate. This low affinity system has been described as a linear component of uptake (Siddiqi et al., 1990; Aslam et al., 1992), but it has also been described as a further saturable one (Doddema and Telkamp, 1979; Peuke and Jeschke, 1998). For a kinetic description of the inhibition of nitrate uptake it is necessary to distinguish between the two uptake systems. Since HATS and LATS are independent uptake systems it is possible that salt acts in different ways and to a different degree on both.

In this paper the effect of different salts and osmotica on net nitrate uptake in intact barley seedlings which were not acclimated to salinity during precultivation is described. The data of net uptake were also used to evaluate the type of inhibition and to estimate the apparent kinetic parameters of the two nitrate uptake systems. To avoid long-term effects of salt on growth, distribution of transporters in the root surface, accumulation of solutes, N-status, and other effects, plants were used which were previously cultivated without salt (NaCl). The salt treatment started at the beginning of the uptake experiments and the depletion of nitrate from the medium was monitored. The aim is to demonstrate the effect and the kinetics in situ in the whole intact plant.

Materials and methods

Plant culture

For the experiments, seedlings of the salt-tolerant barley (Hordeum vulgare L.) variety ‘California Mariout’ (Epstein et al., 1980) were used. Seeds were germinated for 4 d on moistened filter paper (0.5 mol m⁻³ CaSO₄) and then 12 seedlings were transferred to hydroponic pots with 4 dm⁻³ nutrient solution. The medium was based on ‘Long Ashton solution’ (Hewitt, 1966) and contained in mmol m⁻³: KNO₃ 333, Ca(NO₃)₂ 333, MgSO₄ 150, MnSO₄ 2, H₃BO₃ 10, Na₂MoO₄ 0.20, CoSO₄ 0.08, ZnSO₄ 0.19, CuSO₄ 0.13, FeCl₃ 10, Na₂EDTA 10, NaH₂PO₄ 150. The continuously aerated nutrient solutions were renewed after 1 week and subsequently every 3 d (nitrate depletion less than 25%). For the next 10–15 d the plants were grown in a greenhouse with an artificial light period (16 h, 300–500 μmol photon m⁻² s⁻¹, Osmar HQI 400). The conditions during the cultivation were 15–25 °C and 45–75% relative humidity. During this time the plants showed no significant changes with respect to internal nitrate concentration or nitrate uptake rates (related to a fresh weight basis). The relative growth rate was about 8% per day (Peuke and Jeschke, 1998).

Uptake experiments

The uptake experiments were carried out with an automated computer-controlled HPLC-system (according to Goyal and Huffaker, 1986) and described previously in detail (Peuke and Jeschke, 1998). With this system 7 or 15 treatments were tested in parallel. In defined periods from the start of each treatment a sample was taken automatically from the nutrient solution and injected into the HPLC-system for determination of the nitrate concentration. In this way the depletion of nitrate from the nutrient solution was monitored. The HPLC-system (according to Thayer and Huffaker, 1980) allowed the determination of nitrate in the presence of high NaCl concentrations, for example, 1 mol m⁻³ nitrate was detected without interference in a sample with 300 mol m⁻³ NaCl.

For acclimation to the experimental conditions, plants selected for uniformity were placed in fresh nutrient solution in an experimental chamber 2–4 h before the start of the experiments. For starting the experiments, 2–15 plants were transferred to 0.5 or 1.0 dm⁻³ of new solution after rinsing the roots with corresponding solution. In a single experiment 7 or 15 treatments were tested in parallel. Every 4.2–7.5 min a sample was collected automatically, the nitrate concentration was determined and in this way every nutrient pot (treatment) was tested periodically within 34–60 min. The experimental periods were 10 ± 2 h, unless indicated otherwise, so that 10–20 measurements to determine uptake rates were performed for each treatment. The experimental conditions during uptake were: 25–30 °C temperature, 35–60% relative humidity, and 500 μmol photon m⁻² s⁻¹ (Osmar 400 W HQI) photon flux density. Uptake rates were related to the fresh weight of the roots at the end of the experiments and expressed as μmol NO₃ g⁻¹ FW h⁻¹.

The standard uptake solution corresponded to the nutrient solution during the precultivation (1 mol m⁻³ nitrate as N-source). When lower nitrate concentrations were needed the amount of nitrate salts was reduced and balance of ionic strength was achieved by adding KCl and CaCl₂. For higher nitrate concentrations more KNO₃ and Ca(NO₃)₂ were added.

Effects of different salts and osmotica

To investigate the effect of different salts and ions, NaCl, KCl, CaCl₂, or Na₂SO₄ were added to the uptake medium at 20, 40, 60, 80, 100 or 150 mol m⁻³ Na⁺ or Cl⁻, respectively. For testing osmotic effects the osmolality of the solution was adjusted with betaine or sorbitol to 120 and 200 μmol kg⁻¹ and compared with NaCl at corresponding osmolality. To prevent microbial growth penicillin G (100 KU dm⁻³) was added to these solutions and the experimental time was restricted to 8 h.

Type of inhibition and kinetic parameters

To evaluate the type of inhibition and apparent Kᵣ values, two different strategies were followed which resulted in two different series of experiments. In the first series, data were evaluated to estimate the kinetic parameters in the classical way by plots. In the other way, the kinetic parameters were estimated by fitting different model equations to the data.

For the classical evaluation one set of experiments was carried out in order to obtain a Dixon-plot. In the low nitrate concentration range (30, 100 and 300 mmol m⁻³) 0 (control), 1, 10, 20 or 40 mol m⁻³ NaCl were added, and in a high nitrate concentration range (1, 2 and 4 mol m⁻³), 0 (control), 40, 60, 80, and 100 mol m⁻³ NaCl, respectively. The uptake rates were calculated from solutions with less than a 30% depletion in nitrate from the initial solutions, since in this concentration range, the influence of external nitrate concentration on uptake rate is low in barley (HATS apparent Kᵣ 8–12 mmol m⁻³, Peuke and Jeschke, 1998). The reciprocal means of uptake rates were plotted as a function of the NaCl concentrations in the uptake solutions (Dixon-plot).

For estimating kinetic parameters by fitting model equations to a high number of data, a second set of experiments was performed. For 0 (control), 1, 10, 60, and 100 mol m⁻³ NaCl nitrate uptake rates for the whole range of nitrate concentration up to 4 mol m⁻³ were assessed. The nitrate concentrations were divided once again into a low and a high range (30, 100 and
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300 mmol m⁻³ or 1, 2 or 4 mol m⁻³ initial nitrate concentration, respectively). This time the depletion of nitrate from the solution was followed. For each measured nitrate concentration, one nitrate uptake rate was calculated. The apparent kinetic parameters of HATS and LATS (i.e. \( K_m \) and \( V_{max} \)) were calculated for each salt concentration using non-linear curve fitting programs. The resulting models were plotted in hyperbolic and Lineweaver-Burk plots.

To calculate apparent \( K_m \) values from the two experimental sets were combined and were calculated by non-linear curve fitting assuming the inhibition types evaluated by the different plots. In all kinetic calculations a biphasic nitrate uptake system even a net loss of nitrate was detected in the presence of 

\[ \text{Hordeum vulgare} \]

saturable uptake systems (HATS and LATS) by a non-linear curve fitting assumption (see above). The uptake data were fitted to an equation assuming two saturable systems was assumed as detected before in barley (Peuke and Jeschke, 1998). The use of other model equations resulted, to a certain extent, in unphysiological parameters.

**Statistical treatment and calculation**

All experiments (different salts, osmotica, different nitrate plus salt concentrations) were repeated at least four times. Standard errors and confidence limits are given in the text as \( \pm \) and by bars in the figures. Apparent kinetic parameters of uptake systems were calculated after fitting the data to an equation with two saturable uptake systems using the ‘non-linear curve-fitting’ option of SigmaPlot 4.1 (Jandel Scientific, Corte Madera, CA USA). Standard errors (\( P=0.95 \)) calculated by Sigmaplot 4.1 are given as \( \pm \) in Table 1.

**Results**

**Effects of different ions and osmotica**

After application of salt to non-salinized cultivated barley seedlings effects on nitrate uptake were detected immediately without a delay. These effects lasted several hours (8–10 h) and the net-uptake rates were constant (data of time-courses not shown). Over a wide range of Na⁺ or Cl⁻ concentrations the effect of the different salts on nitrate uptake were similar; only at the lowest and the highest salt application were there significant differences (Fig. 1). At 20 mol m⁻³ Na⁺ or Cl⁻ a slight stimulation of uptake was observed for KCl and Na₂SO₄ (139% and 140% of control, respectively). A clear inhibition was first seen at 60 mol m⁻³ for all applied salts (50–85% of control). At the highest concentration (150 mol m⁻³ Na⁺ or Cl⁻) with Na₂SO₄ or CaCl₂ only low uptake rates were found (12% or 15% of control, respectively) and even a net loss of nitrate was detected in the presence of NaCl and KCl.

When the non-ionic osmotic betaine or sorbitol were added to the uptake solution uptake was inhibited to the same extent as that with NaCl at corresponding osmolalities (Fig. 2). Sorbitol seemed to be slightly more effective in inhibiting net nitrate uptake than betaine was (68 and 51% or 79 and 70%, respectively, at 120 and 200 mOsmol kg⁻¹ compared to controls).

**Dixon-plots**

The Dixon-plot of the uptake at low nitrate concentrations (30, 100 and 300 mmol m⁻³) as affected by NaCl was typical of the type showing classic non-competitive inhibition (Fig. 3a). The plots for each nitrate concentration intersected the x-axis at about 60 mol m⁻³ NaCl (the apparent inhibition constant, \( K_i \)). The plot from the raw data at a high nitrate concentration range showed no clear result (Fig. 3b). However, in the nitrate concentration range from 1–4 mol m⁻³ both uptake systems (HATS and LATS) operate remarkably well. After subtraction of the theoretically calculated uptake rates of a HATS (operating at low nitrate concentrations, \( K_m \); 12.2 mmol m⁻³, \( V_{max} \); 3.4 μmol g⁻¹ h⁻¹ (Peuke and Jeschke, 1998), and inhibited non-competitively by salt, apparent \( K_i \); 60 mol m⁻³; see Fig. 3a) from these data a Dixon-plot resulted which possibly pointed to a competitive inhibition for the low affinity uptake system operating at high nitrate concentrations (Fig. 3c) with an apparent inhibition constant of more than 200 mol m⁻³ NaCl, which is far above from the applied concentrations.

**Kinetic parameters, hyperbolic and Lineweaver-Burk plots at different NaCl concentrations**

The apparent \( V_{max} \) of the HATS decreased with increasing salt concentration; at 100 mol m⁻³ NaCl it was only 25% of the control (Table 1). The effect of salt on the affinity of the HATS was not monotone. The apparent \( K_m \) values seemed to decrease by salt up to 60 mol m⁻³, increasing afterwards, reaching its highest value at 100 mol m⁻³. In conclusion there was no effect of salt on apparent \( K_m \) of HATS taking the error into account. The apparent \( V_{max} \) of the LATS was less and not unidirectionally affected by salt, the values were between 3.7 and 6.8 mmol m⁻³. The apparent \( K_m \) values were more strongly influenced,

### Table 1. Apparent kinetic parameters depending on salt concentration

<table>
<thead>
<tr>
<th>NaCl [mol m⁻³]</th>
<th>0</th>
<th>1</th>
<th>10</th>
<th>60</th>
<th>100</th>
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<tr>
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<tr>
<td>HATS</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>( K_m )</td>
<td>9.7±4.9</td>
<td>7.3±4.0</td>
<td>3.7±5.6</td>
<td>2.2±3.4</td>
<td>18.6±28.9</td>
</tr>
<tr>
<td>( V_{max} )</td>
<td>4.0±0.5</td>
<td>3.3±0.6</td>
<td>2.3±0.5</td>
<td>1.3±0.2</td>
<td>1.0±0.4</td>
</tr>
<tr>
<td>LATS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( K_m )</td>
<td>2450±1800</td>
<td>600±360</td>
<td>440±200</td>
<td>1100±320</td>
<td>1800±860</td>
</tr>
<tr>
<td>( V_{max} )</td>
<td>6.8±1.8</td>
<td>3.7±0.5</td>
<td>4.5±0.3</td>
<td>4.8±0.3</td>
<td>5.1±0.6</td>
</tr>
</tbody>
</table>
Fig. 1. Effects of different salts on net nitrate uptake in barley. Barley seedlings (Hordeum vulgare L. cv. California Mariout) were grown hydroponically for 14–19 d with 1 mol m$^{-3}$ nitrate but without salt. At the beginning of the uptake experiments different salts (NaCl; KCl; CaCl$_2$; and Na$_2$SO$_4$) were applied at several concentrations. Data are shown as the means of net nitrate uptake rates at 1 mol m$^{-3}$ NO$_3^-$ depending on the concentration of Na$^+$ or Cl$^-$ during the first 8–10 h after application. Bars indicate confidence limits (Student’s t-test, $P=0.95$).

Fig. 2. Effects of osmotica on net nitrate uptake in barley. Barley seedlings (Hordeum vulgare L. cv. California Mariout) were grown hydroponically for 14–19 d with 1 mol m$^{-3}$ nitrate, but without salt. At the beginning of the experiments NaCl, sorbitol, or betaine were added to the uptake solution (1 mol m$^{-3}$ NO$_3^-$) at 120 or 200 mOsmol kg$^{-1}$. Bars indicate confidence limits (Student’s t-test, $P=0.95$).

however, and the apparent affinity was increased at intermediate salt concentrations (highest at 10 mol m$^{-3}$ NaCl, factor 5.5 compared with the control). The isotherms of the two calculated uptake systems and the total rate of uptake at various NaCl concentrations are given in Fig. 4. The uptake by the HATS is clearly inhibited at all applied salt concentrations within the range of nitrate concentrations investigated and the graph pointed once again to the non-competitive inhibition type (Fig. 4a). For the low affinity system the situation was more complicated; only at the highest salt concentration (100 mol m$^{-3}$ NaCl) was nitrate uptake as mediated by this system inhibited over the whole range of nitrate concentration, while lower NaCl concentrations led to stimulation at intermediate nitrate concentrations, i.e. to lower apparent $K_m$ values (Fig. 4b; see also Table 1). When viewing total nitrate uptake, not separated into the two systems (Fig. 4c), it becomes evident that the low inhibition by 20 and 40 mol m$^{-3}$ NaCl at 1 mol m$^{-3}$ nitrate as used in Fig. 1 was due to an inhibition of the HATS that was partly compensated by the increased uptake by the LATS. The contribution of the HATS to
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Fig. 3. Dixon-plots of net nitrate uptake depending on NaCl concentrations in barley. Barley seedlings (Hordeum vulgare L. cv. California Mariout) were grown hydroponically for 14–19 d with 1 mol m\(^{-3}\) nitrate, but without salt. For the uptake experiments plants were transferred to different nitrate plus salt concentration. The highest value of nitrate depletion from the solution was 30%. (a) Dixon-plot of the mean net uptake rates in the low nitrate concentration range: 30 ( ), 100 ( ), or 300 ( ) mmol m\(^{-3}\) initial nitrate at 0, 1, 10, 20, or 40 mol m\(^{-3}\) NaCl. (b) Dixon-plot of the mean net nitrate uptake rates in the high nitrate concentration range: 1 ( ), 2 ( ), 4 ( ) mol m\(^{-3}\) initial nitrate at 0, 40, 60, 80, or 100 mol m\(^{-3}\) NaCl. (c) Dixon-plot of the values of plot (b) after substraction of the calculated activities of a HATS at corresponding concentrations (\(K_{m1}^\text{HATS}\): 12.2 mmol m\(^{-3}\); \(V_{max1}^\text{HATS}\): 3.4 \(\mu\)mol g\(^{-1}\) h\(^{-1}\); Peuke and Jeschke, 1998, and inhibited non-competitively by salt apparent \(K_i\): 60 mol m\(^{-3}\)).

Fig. 4. Isotherms of HATS, LATS and total uptake at different NaCl concentrations. Barley seedlings (Hordeum vulgare L. cv. California Mariout) were grown hydroponically for 14–19 d with 1 mol m\(^{-3}\) nitrate, but without salt. For uptake measurements plants were transferred to uptake solutions of different initial nitrate concentrations up to 4 mol m\(^{-3}\) and NaCl was added at 0 (control —), 1 (· · ·), 10 ( · · · · ), 60 ( · · · · · · ), or 100 ( · · · · · · · ) mol m\(^{-3}\). The nitrate depletion from the solution was monitored. For each nitrate concentration the uptake rate was determined. The uptake data were fitted to an equation assuming two saturable uptake systems by a non-linear curve fitting program. The corresponding \(K_m\) and \(V_{max}\) values are given in Table 1. (a) high affinity transport system HATS, (b) low affinity transport system LATS, and (c) total nitrate uptake.

total uptake decreased from 67% (control) to 59, 43, 36, and 35% at 1, 10, 60, and 100 mol m\(^{-3}\) NaCl, respectively, with 1 mol m\(^{-3}\) nitrate in the uptake solution. The highest activity of the LATS was found at 10 mol m\(^{-3}\) NaCl, where it was 155% of the control (1 mol m\(^{-3}\) nitrate). The Lineweaver-Burk plot of the LATS showed the type of competitive inhibition (Fig. 5). In contrast to plots typical of competitive inhibition, all plots with NaCl in the solution were below the control plot.

For the computer calculation of the inhibitor constant two saturable nitrate uptake systems (Peuke and Jeschke, 1998) and a non-competitively inhibited HATS, as shown in the Dixon-plot (Fig. 3a), and a competitively inhibited LATS were assumed. For the control (0 mol m\(^{-3}\) NaCl) \(K_m\) and \(V_{max}\) of Table 1 was used. Following these results and assumptions, the following equation was fitted to the data of all NaCl-experiments including the related controls (0 mol m\(^{-3}\) NaCl):

\[
V = (V_{max1}^\text{HATS} + V_{max2}^\text{LATS}) \times \left[\frac{[\text{NO}_3^-]}{(1 + [\text{NaCl}] / [K_{m1}^\text{HATS}])} \times \frac{[\text{NO}_3^-]}{[K_{m2}^\text{LATS}]} \times \frac{[\text{NaCl}]}{([\text{NaCl}] + K_{i1}^\text{HATS})} \times \frac{[\text{NaCl}]}{([\text{NaCl}] + K_{i2}^\text{LATS})}\right]
\]

The resulting \(K_{i1}\) for the HATS was 37 ± 3 mol m\(^{-3}\) and the \(K_{i2}\) was 344 ± 135 mol m\(^{-3}\) NaCl for the LATS.
If the equations for the fitting calculation were changed assuming uncompetitively inhibited LATS $K_{i2}$ was $247 \pm 72 \text{ mol m}^{-3}$ and in the case of assuming a non-competitive inhibition of LATS the $K_i$ was $503 \pm 129 \text{ mol m}^{-3}$ $\text{NaCl}$. The $K_i$ for the HATS was less affected in these calculation by these assumptions.

**Discussion**

The effects of the different salts (NaCl, KCl, CaCl$_2$ or Na$_2$SO$_4$) on the net uptake of nitrate in the present experiments were seen immediately after applications and they were relatively similar for all applied salts with the exception of the lower and the higher concentrations within the range used. The effects of different salts were even more compensated when the uptake rates were plotted as a function of the osmolality of the uptake solutions. The hypothesis that the inhibition of nitrate uptake by salts was largely due to an osmotic effect and to a lesser extent to an ionic one, was confirmed by the findings in the experiments with non-ionic osmotica. At the same osmolality nitrate uptake was inhibited by betaine and sorbitol to a similar extent as by the salts used.

A strong inhibition of nitrate uptake by both chloride and sulphate salts has also been found (Aslam *et al.*, 1984). They concluded, however, that the ion itself was more important as an inhibitor than was the osmolality. In agreement with this study’s results (at low and high concentration) it was found that sulphate salts were less inhibitory. The protecting effect of calcium (*Ward et al.*, 1986) was not found in the present experiments when salinity was caused by CaCl$_2$ itself. The lower inhibition by CaCl$_2$ and Na$_2$SO$_4$ at 150 mol m$^{-3}$ Na$^+$ or Cl$^-$ was due to a lower osmolality of the medium. Osmotic stress caused by polyethylene glycol treatment (200 kg m$^{-3}$) inhibited nitrate uptake and reduction in wheat (*Larsson*, 1992). Nitrate uptake, translocation, storage, and reduction in ryegrass under control conditions has been compared with those under osmotic stress caused by the addition of NaCl or polyethylene glycol (*Ourry et al.*, 1992). Only polyethylene glycol caused a decrease in nitrate uptake and reduction. The small effect of NaCl was related to osmoregulation by the uptake of chloride, which was possible in long-term experiments (27 h) but not in these short-term experiments.

In the present paper it has clearly been shown using a Dixon-plot that, in the low nitrate concentration range, nitrate uptake is inhibited non-competitively by salt. At low nitrate concentrations the HATS contributed more to nitrate uptake than did the LATS. At the highest concentration of the low range (300 mmol m$^{-3}$) the contribution of the LATS was only 11% (Peuke and Jeschke, 1998). In the present experiments, two saturable uptake systems for nitrate were found (data not shown), the calculated kinetic parameters were the same or somewhat lower than before (compare Table I and Peuke and Jeschke, 1998). Thus it can be concluded that it is the HATS which was inhibited non-competitively by NaCl with an apparent $K_i$ of about 60 mol m$^{-3}$ NaCl. This conclusion was confirmed by the calculation of the kinetic parameters at different salt concentrations: the apparent $V_{max}$ was clearly and unidirectionally decreased while there was no significant effect on apparent $K_m$. So there is an explanation for the inhibition of uptake by the osmotic environment. At 1 mol m$^{-3}$ nitrate the HATS is the most active nitrate transporter (64% of total uptake;
Peuke and Jeschke, 1998) and, therefore, is more likely to lead to the reaction/inhibition responsible for reduced rates of uptake. This type of inhibition excludes the binding of the inhibitor to the reactive site of the HATS (and therefore the possibility that chloride is transported by the HATS). Therefore effects of sodium chloride, other salts and non-ionic osmotica could be very similar by the osmotic reactions on the protein.

At higher nitrate concentrations the results of the Dixon-plot were not clear, but after substraction of the competition with a stimulation at low salt concentrations osmotic reactions on the protein be explained satisfactorily with the present data of net salts and non-ionic osmotica could be very similar by the salt in di...


