Absorption and accumulation of nitrate in plants: Influence of environmental factors

Bundana Bose
Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India

and

H S Srivastava*
Department of Plant Science, M.J.P. Rohilkhand University, Bareilly 243006, India

Plants adopt various strategies to fulfill their nitrogen nutrition requirement, the most important being the uptake of nitrate from the soil and its subsequent assimilation into amino acids. The uptake of nitrate is energy dependent and is an active process involving high affinity and low affinity transport systems. The net uptake of the anion depends upon both influx as well as on its passive efflux. When the uptake far exceeds over its assimilation in the plant, there is considerable accumulation of nitrate in the plant parts making them unfit for human and cattle consumption. Various environmental factors affect the uptake and accumulation of nitrate, which along with the genetic component of the plant affecting the net uptake and accumulation of the nitrate, need to be considered and carefully manipulated for effective nitrogen management in the plant, soil and aquatic environment.

Nitrogen is an essential plant nutrient and is of prime importance in the productivity of crops as it exceeds all other elements, as a percentage of plant's dry weight with the exceptions of carbon, hydrogen and oxygen. It is often a limiting nutrient for crop growth and productivity in most agricultural soils. Watson1, as early as in 1947, showed that nitrogen deficiency reduced the plant growth by restricting leaf area development. A number of researches have shown that nitrogen limits seed yield at maturity. Application of nitrogen fertilizer is always found to improve the growth and development of plants which eventually improves the grain/seed yield. Nitrate or ammonium ions are absorbed by the plant roots and their nitrogen is incorporated eventually in to a variety of vital organic molecules such as amino acids, proteins, nucleic acids etc. Thus, total organic nitrogen content and the major nitrogenous metabolites increase quantitatively during NO₃⁻ or ammonium supply in plants growing in nitrogen deficient medium. This is also reflected in increased growth and productivity of the plants with nitrogen supply. Among three forms of inorganic nitrogen viz. nitrate, ammonium and di nitrogen, NO₃⁻ is of the greatest importance to plants raised on arable soil except for legumes and rice. The ammonium and di nitrogen forms of nitrogen are of less importance because their availability and utilization is often restricted by the physico-chemical nature and the microbial population of the soil. In fact, the NO₃⁻ assimilation is estimated to produce 2×10⁴ megatons of organic nitrogen and it is about 100-folds greater than the rate of annual biological nitrogen fixation². Most plants prefer NO₃⁻ over NH₄⁺, as a source of nitrogen. This is in spite of the fact that acquisition and assimilation of NO₃⁻ is more energy demanding than ammonium. Besides, NO₃⁻ assimilation occurs in various micro-organisms also including bacteria, yeast, fungi, algae etc.

In several instances, the stimulatory effect of NO₃⁻ on growth and productivity and some related metabolic aspects could not be directly correlated with the assimilation of NO₃⁻. It appears therefore, that either NO₃⁻ per se, or some secondary metabolites derived from NO₃⁻ assimilation exert some regulatory effects on plants. Several plant biologists have suggested that NO₃⁻ regulated some plant processes, besides acting as a nutrient nitrogen source³. Schlieble et al.⁴ used a tobacco mutant with very low NO₃⁻ reductase activity to demonstrate that NO₃⁻ ion acted as a signal molecule in carbohydrate metabolism. In soybean embryonic axis, the supply of NO₃⁻ to the seedlings increases both NR activity and

*Correspondent author
Nitrate uptake

Nitrate concentration in the soil fluctuates and is affected by several factors, which are both abiotic and biotic. An usual range of soil \( \text{NO}_3^- \) is 0.1 to 5.0 mM\(^8\). However, the \( \text{NO}_3^- \) concentration in the cytoplasm of roots exposed to \( \text{NO}_3^- \) has been estimated in the range of 5 to 30 mM\(^9\). Obviously, the uptake of \( \text{NO}_3^- \) by the roots is an energy dependent active process and is responsive to the soluble carbohydrate content of the roots. Roots absorb \( \text{NO}_3^- \) selectively from the soil through different types of \( \text{NO}_3^- \) transporters present in the plasma membrane. On the basis of various types of physiological and biochemical studies, it has been concluded that plants have a very elaborate system of \( \text{NO}_3^- \) uptake, which involves both a high affinity transport system (HATS) and low affinity transport system (LATS)\(^10\). Further, on the basis of the kinetic experiments involving a wide range of \( \text{NO}_3^- \) concentrations, it has been demonstrated that the high affinity transport system is partly inducible and partly constitutive. Thus, there are three types of \( \text{NO}_3^- \) transport systems:

1. Constitutive low affinity transport system (cLATS), that operates at high \( \text{NO}_3^- \) concentrations (above ~ 0.5 mM)
2. Constitutive high affinity transport system (cHATS) which operates below ~ 0.5 mM \( \text{NO}_3^- \) concentration and
3. Inducible high affinity transport system (iHATS) which also operates at lower concentrations of \( \text{NO}_3^- \) and is inducible by \( \text{NO}_3^- \)

It has been further demonstrated that at low external \( \text{NO}_3^- \) concentration (i.e. less than 1 mM), the uptake kinetics is typical Michaelis-Menten type, while at concentrations above 1 mM the kinetics may be either saturable or linear\(^11\). The \( K_m \) for \( \text{NO}_3^- \) uptake by these two types of transport systems (HATS and LATS) varies according to the species, but is usually in the range of about 0.005 to 0.1 mM for low affinity transport system and in millimolar ranges for high affinity transport system\(^12\). The capacity for \( \text{NO}_3^- \) absorption is higher by LATS than that by the HATS. For example, uptake rate in \textit{Arabidopsis} at 10 mM \( \text{NO}_3^- \) (by LATS) is about 24 mmol h\(^{-1}\) g\(^{-1}\) fresh weight as compared to only about 1 mmol hr\(^{-1}\) g\(^{-1}\) fresh weight by the HATS\(^13\). Thus, while HATS might be important in \( \text{NO}_3^- \) absorption at low soil \( \text{NO}_3^- \), LATS might be responsible for the bulk of the \( \text{NO}_3^- \) acquired by the plants. In fertile soils, the \( \text{NO}_3^- \) concentration is usually in the range of 0.2 to 5 mM but at times may reach 30 mM after the application of nitrogenous fertiliser and then the low affinity transport system seems to be of importance\(^14\).

Both of the \( \text{NO}_3^- \) transport systems seem to operate via a \( 2 \text{H}^+ : 1 \text{NO}_3^- \) symport\(^15\) and thus uptake of \( \text{NO}_3^- \) causes alkalisation of the medium. Recently Poulquin \textit{et al.}\(^16\) have described the presence of a \( \text{NO}_3^- \) unipporter system also in plasma membrane vesicles from maize root cells. The system has an acidic (6.5) optimum pH and is similar to \( \text{H}^+ \)-ATPase in its properties. Attempts have been made to characterise the inducible \( \text{NO}_3^- \) transport proteins in a few systems. A 45 kDa protein has been detected in the plasma membrane of the cyanobacterium \textit{Synechococcus} when fed with \( \text{NO}_3^- \), but this protein is absent in the \( \text{NH}_4^+ \) fed bacterium\(^17\). In maize root cell's plasma membrane, an induced synthesis of 30-31 kDa protein has been demonstrated by Mc Clure \textit{et al.}\(^18\), while Ni and Beevers\(^19\), in the same system demonstrated the \( \text{NO}_3^- \) induced synthesis of 33,38,49 and 50 kDa proteins, of which 33 and 49 kDa proteins were integrated in the plasma membrane. The recognition of \( \text{NO}_3^- \) might involve a guanidine group of arginine residue in the transport protein, because ketones and carbonyl compounds that bind to guanidium groups inhibit \( \text{NO}_3^- \) uptake in maize roots\(^20\).

The molecular genetics of \( \text{NO}_3^- \) uptake has been examined in several recent studies. Genes coding for
NO₃⁻ transporter in root cells have been isolated and characterised. Two families of genes encoding NO₃⁻ uptake systems - the Nrt1 and Nrt2 (NRT2:1) - have been identified. Both these genes code for carrier proteins that drive NO₃⁻ uptake by cotransporting at least two protons for every one NO₃⁻ (ref. 23). Experiments involving expression and functional studies indicate that NRT1 is a low affinity and NRT2 is a high affinity transporter, although the CHLJ (AtNRT1) is a double affinity NO₃⁻ transporter. Huang et al. have isolated a AtNRT1:2 gene for constitutive low affinity NO₃⁻ transporter from Arabidopsis. This is in addition to the inducible transporter gene CHLJ (NRT1) reported earlier. When the gene was injected to Xenopus oocytes, it yielded a Km for NO₃⁻ of 5.9 mM. A NO₃⁻ transporter gene called OssNRT1 has been cloned from rice also, which displayed a low affinity transport activity with a Km value of 9.0 mM in Xenopus oocytes. Such molecular studies might be helpful in genetic engineering of the crop plants for regulated NO₃⁻ uptake with a view to increase nitrogen use efficiency and/or to restrict the NO₃⁻ content of the edible parts of the plant.

The net uptake of NO₃⁻ in plants is equal to the difference between NO₃⁻ influx and NO₃⁻ efflux. Therefore, the uptake of NO₃⁻ is regulated either by NO₃⁻ influx or by NO₃⁻ efflux, both of which are substantial and probably regulated independently. As mentioned earlier influx is a carrier mediated active process. However, the efflux appears to be a channel mediated passive process.

**Nitrate accumulation**

The accumulation of NO₃⁻ in the plant tissues results from the difference in the absorption and the assimilation of NO₃⁻. The amount of NO₃⁻ accumulated depends upon several factors such as (a) the plant species (b) soil NO₃⁻ content (c) NO₃⁻ assimilating potential of the plant (d) presence of other nutrients and (e) environmental factors influencing the uptake and assimilation of NO₃⁻ (Table 1). Species variation in NO₃⁻ accumulation has been demonstrated by Nambiar et al. who compared the NO₃⁻ content in the leaves of groundnut, cowpea, soybean, maize, and Sorghum at 66 to 94 days after sowing the seeds and at 0, 100 or 200 kg ha⁻¹ externally applied nitrogen as urea. At almost all sampling dates, groundnut (non-nodulating) and cow pea had high NO₃⁻ content and maize had lower NO₃⁻ content. On percent basis, tomato may contain about 20% of its total nitrogen in the form of NO₃⁻. Usually there is a positive correlation between the soil NO₃⁻ content and the plant NO₃⁻ content as has been observed in a variety of species including tomato and beans even though the relationship may not be exactly linear. However, many reports have shown a negative relationship between NO₃⁻ uptake rate and NO₃⁻ accumulation in plants. This obviously reflects a complex mechanism of NO₃⁻ uptake which may not be related to the external NO₃⁻ concentrations.

Usually roots accumulate more NO₃⁻ than the shoots (Table 1). Cardenas-Navarro et al. have demonstrated that in tomato and lettuce, the NO₃⁻ content is directly related with the water content on g⁻¹ dry weight basis under different light intensities and day/night conditions. They have suggested that changes in tissue NO₃⁻ content are due to changes in NO₃⁻ content of a water reservoir of variable size.

The intracellular compartmentation of stored NO₃⁻ has been examined in a few studies and it has been suggested that the major pool of NO₃⁻ is vacuolar. With the help of nuclear magnetic resonance (NMR) spectroscopy, Belton et al. demonstrated that the cytoplasmic pool of NO₃⁻ was small as compared to the vacuolar pool. Many studies have indicated that the range of vacuolar pool of NO₃⁻ is between 58% and 99% of total NO₃⁻ (ref. 36) in the protoplast of the different species. In fact the cytoplasmic NO₃⁻ pool is to be transient because of the activity of nitrate reductase (NR, E.C. 1.6.6.1-2), the enzyme reducing NO₃⁻ to nitrite. Any significant accumulation of NO₃⁻ in the cytoplasm may be either due to activated uptake of the anion from the medium or due to the diminished activity of the enzyme.

**Factors affecting nitrate uptake and accumulation**

Plants growing in the natural environment have to experience varied environmental conditions, which directly or indirectly may affect the uptake and accumulation of NO₃⁻ also. These factors have to be identified and understood in order to regulate the uptake, assimilation and accumulation of NO₃⁻ in plants. Some prominent factors are described in the following paragraphs.
Plant factors

The rate of nitrate absorption differs according to plant species, age, nutrition and growth conditions. The kinetic parameters of $\text{NO}_3^-$ absorption also differ according to species. For example, while $K_m$ for active absorption is 9.5 $\mu\text{mol}$ in *Lactuca sativa* \(^{37}\), it is 60 $\mu\text{mol}$ in *Vicia faba* \(^{38}\). The uptake rate also varies according to the age of the plant and again, the period or age of maximum rate of $\text{NO}_3^-$ absorption differs according to species \(^{39}\). The differences in $\text{NO}_3^-$ absorption among species and also according to the plant age are perhaps due to differences in the nature

<table>
<thead>
<tr>
<th>Species &amp; Plant parts</th>
<th>Nutrient $\text{NO}_3^-$ status</th>
<th>Tissue $\text{NO}_3^-$ N $\mu\text{g} \text{g}^{-1} \text{Dry wt}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arachis hypogaea</em></td>
<td>Field grown, 200 kg ha$^{-1}$ urea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- non nodulating/Leaves</td>
<td>823</td>
<td>1194</td>
<td>29</td>
</tr>
<tr>
<td>- nodulating/Leaves</td>
<td>Hydroponically grown for 66 days in 9 mM KNO$_3$ +50 mM NaCl</td>
<td>96.2</td>
<td>98</td>
</tr>
<tr>
<td>- Shoots</td>
<td>--do--</td>
<td>19.6</td>
<td>98</td>
</tr>
<tr>
<td><em>Glycine max</em> Leaves</td>
<td>Field grown, 200 kg ha$^{-1}$ urea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- nonodulating</td>
<td>267</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>- nodulating</td>
<td>512</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td><em>Hordeum vulgare</em></td>
<td>whole plant 15 mM NO$_3^-$</td>
<td>~4000-4200</td>
<td>99</td>
</tr>
<tr>
<td>- Shoots</td>
<td>10 mM NO$_3^-$</td>
<td>1300</td>
<td>100</td>
</tr>
<tr>
<td>- Roots</td>
<td>10 mM NO$_3^-$</td>
<td>1200</td>
<td>100</td>
</tr>
<tr>
<td><em>Phaseolus vulgaris</em></td>
<td>Shoots 8 mM NO$_3^-$</td>
<td>800-3800</td>
<td>89</td>
</tr>
<tr>
<td>- Roots</td>
<td>--do--</td>
<td>3300-11470</td>
<td>89</td>
</tr>
<tr>
<td>- Leaves</td>
<td>10 mM NO$_3^-$ for 24 hr</td>
<td>140</td>
<td>56</td>
</tr>
<tr>
<td>10 mM NO$_3^-$ for 7 days</td>
<td></td>
<td>1500</td>
<td>1500</td>
</tr>
<tr>
<td><em>Pinus lambertiana</em></td>
<td>Needles Field grown</td>
<td>15-80</td>
<td>101</td>
</tr>
<tr>
<td><em>Pisum sativum</em></td>
<td>Whole plant 2 mM NO$_3^-$, hydroponic culture</td>
<td>1400</td>
<td>102</td>
</tr>
<tr>
<td><em>Pieridium aquilinum</em></td>
<td>Leaves --do--</td>
<td>20-100</td>
<td>101</td>
</tr>
<tr>
<td><em>Ricinus communis</em></td>
<td>Whole plant Nutrient solution containing 5 mM NO$_3^-$</td>
<td>597</td>
<td>103</td>
</tr>
<tr>
<td><em>Quercus kelloggii</em></td>
<td>Leaves Field grown</td>
<td>100-500</td>
<td>101</td>
</tr>
<tr>
<td><em>Sisymbrium officinale</em></td>
<td>Seeds --do--</td>
<td>26.6</td>
<td>104</td>
</tr>
<tr>
<td><em>Sorghum bicolor</em></td>
<td>Leaves Field grown, 200 kg ha$^{-1}$ urea</td>
<td>198</td>
<td>29</td>
</tr>
<tr>
<td><em>Zea mays</em></td>
<td>Roots 2 mM NO$_3^-$, hydroponic or sand culture for 17 d</td>
<td>9000-12000</td>
<td>102</td>
</tr>
<tr>
<td>- Shoots</td>
<td>--do--</td>
<td>8000</td>
<td>102</td>
</tr>
</tbody>
</table>
and relative abundance of NO$_3^-$ transporters$^{40}$. 

**Nitrogenous salts and metabolites**

Nitrogen in the root environment has both positive as well as negative effect on NO$_3^-$ uptake depending upon the form of nitrogenous salt. Induction of NO$_3^-$ uptake by external NO$_3^-$ has been demonstrated in many studies$^{41}$. Several studies have demonstrated that this induction is by NO$_3^-$ per se rather than by any assimilatory product of the anion$^{10}$. The time course of the induction process varies according to species, environmental conditions and nutritional history. The induction appears to be from either the derepression of NO$_3^-$ transporters already present in the membrane or from the induction of de novo synthesis (as is the case with iHATS) and subsequent insertion of NO$_3^-$ transporter into the plasmalemma.

In contrast to NO$_3^-$, NO$_2^-$ and NH$_4^+$ inhibit NO$_3^-$ uptake by the plants. In barley, NO$_2^-$ appears to be a competitive inhibitor of NO$_3^-$ uptake. It shares the same transporter and same binding site as NO$_3^-$, however, NO$_2^-$ transport may use different transport systems have also been obtained. For example, Anti-NR immunoglobin G fragments purified from anti-NR serum inhibited NO$_3^-$ uptake but not the NO$_2^-$ uptake, in barley roots$^{42}$. Ammonium ion inhibits the net uptake via stimulating the efflux of NO$_3^-$ in several species$^{43}$. However, evidences for inhibition via NO$_3^-$ influx have been obtained in barley$^{44}$. Aslam et al.$^{45}$ reported that NH$_4^+$ inhibits the high affinity transport system of NO$_3^-$ uptake. Methylamine, an analogue of NH$_4^+$ also inhibits net NO$_3^-$ uptake by increasing NO$_3^-$ efflux$^{46}$. But, methionine sulfoximine, the structural analogue of glutamate and the inhibitor of glutamine synthesis accelerates NO$_3^-$ uptake$^{47}$. This is apparently because under the conditions of inhibited glutamine synthesis, more ATP is spared for the active uptake of NO$_3^-$.

Feedback inhibition of NO$_3^-$ uptake by nitrogenous metabolites which accumulate under conditions of nitrogen sufficiency has also been demonstrated. In Arabidopsis thaliana, it has been shown that this inhibition acts at NO$_3^-$ transporter level, where the expression of Nrt2:1 gene responsible for inwards NO$_3^-$ transport is upregulated by NO$_3^-$ starvation in wild type and by nitrogenous metabolites in NR deficient mutant$^{48}$. Thus, it appears that some product of NO$_3^-$ assimilation rather than the NO$_3^-$ itself, regulates the synthesis of NO$_3^-$ transporter.

Exogenously supplied amino acids and amides inhibit the uptake of NO$_3^-$ in Arabidopsis thaliana$^{49}$ and dwarf bean$^{50}$. Several phloem transported amino acids also reduce NO$_3^-$ uptake in wheat$^{51}$ and in soybean$^{52}$. Rufty et al.$^{33}$ suggested that this type of inhibition of NO$_3^-$ uptake was due to feedback control of NO$_3^-$ transporter.

Chlorate and chlorite, the structural analogues of NO$_3^-$ and NO$_2^-$ also inhibit the NO$_3^-$ uptake and its reduction$^{53}$. The magnitude of inhibition at a given concentration of chlorate is almost species independent. For example, the inhibition of NO$_3^-$ uptake by 5mM chlorate is 25% in barley and about 40% in maize$^{55}$.

Nitrate accumulation in plant parts is also influenced by the nitrogenous salts in the root environment. In most studies, NO$_3^-$ accumulation has been found to increase with the increase in nutrient NO$_3^-$ concentration$^{56}$. However, the amide glutamine inhibits NO$_3^-$ accumulation in excised maize roots$^{57}$, although it has no effect when supplied to the intact seedlings$^{58}$. But the other amide asparagine inhibits NO$_3^-$ accumulation in intact seedlings also$^{59}$. The decreased accumulation in the presence of amides is considered to be the consequence of reduced NO$_3^-$ uptake from the medium.

**Carbon metabolites**

An increased supply of dissolved inorganic carbon (NaHCO$_3^-$) to root increases 10 fold incorporation of dissolved inorganic carbon and NO$_3^-$ uptake in tomato seedlings$^{59}$. Fixation of inorganic carbon in roots provides skeleton for assimilation of the NH$_4^+$ resulting from the reduction of NO$_3^-$ and this in turn enhances the assimilation of NO$_3^-$ and also increases the uptake of NO$_3^-$ (ref. 59).

Malate appears to be an important metabolite in uptake and translocation of NO$_3^-$ for example, supplying malate to the roots either by addition to the external solution or by increasing artificially the transport from the shoot in the phloem, improves the net NO$_3^-$ uptake rate in soybean seedlings$^{60}$. The uptake of NO$_3^-$ by the root increased when the supply of NO$_3^-$ to the shoot increased and decreased when the activity of nitrate reductase in the shoot was inhibited by tungstate. It was concluded from these studies that the assimilation of NO$_3^-$ in the shoots
controlled NO$_3^-$ uptake by the roots via malate translocation in the phloem.

**Atmospheric pollutants**

The nitrogenous air pollutants specially the NO and NO$_2$ (NOx) are known to affect NO$_3^-$ uptake from the soil as well as the accumulation of NO$_3^-$ in the plant, as they contribute to the NO$_3^-$ pool on their own after their entry to the plant cells. In most experimental studies, it has been difficult to proportionate the NO$_3^-$ absorbed from the soil from that contributed by the dissolution of NOx.

Inhibition of soil NO$_3^-$ uptake by 1.1 ml l$^{-1}$ NO$_2$ for 7 days has been shown in soybean, which is more apparent when the soil NO$_3^-$ concentration is 1 mM than at 5 mM$^{64}$. It has been suggested that this inhibition is due to increased proton concentration in the plant cell due to dissolution of NO$_3^-$ in the cell sap. However, exposure to NO$_2$ causes accumulation of NO$_3^-$ in many species.$^{62}$ A significant portion of this NO$_3^-$ seems to be compartmentalized in the storage pools (vacuoles). In a study with a wild type cv Zephyr and NO$_2$ tolerant mutants (B$_1$ and W$_2$) of barley, it had been found that the inducible activity of nitrate reductase in the leaves was significantly enhanced even after 3 d of the termination of 0.5 ppm NO$_2$ exposure.$^{65}$ It was apparently due to gradual mobilisation of NO$_3^-$ derived NO$_3^-$ from storage pool to the metabolic pool.

Evidences are available for the acquisition and accumulation of nitrogen in the form of NO$_3^-$ and NH$_4^+$ from the atmospheric nitrogenous gases also. Nitrogen deposition from the nitrogenous pollutants in the atmosphere, is considered to be the major factor in forest decline in Europe and other heavily industrialised parts of the world.$^{64}$ The NO$_3^-$ -N is derived from the gases such as NO, NO$_2$, N$_2$O, N$_2$O$_5$, HNO$_3$ vapours and particulate NO$_3^-$. The rate of NO$_3^-$-N deposition has been estimated to be as high as 455 mg m$^{-2}$ hr$^{-1}$ by Ceanothus crassifolius.$^{65}$

**Heavy metals**

Heavy metals, both essential as well as non-essential, are known to affect various plant physiological and biochemical processes. In many instances they have been found to inhibit uptake, even when present in micromolar range.

The presence of 400 mM Al$^{3+}$ has no significant effect on $^{15}$NO$_3^-$ uptake by the tea plants$^{66}$ although in some other species similar or even lower concentrations inhibit NO$_3^-$ uptake.$^{67-69}$ This inhibition may be due to the disruption of the plasma membrane by the heavy metal and hence the disorganisation or blockage of the NO$_3^-$ transport system.$^{70}$ Further, the net uptake may also be reduced due to accelerated efflux of the ion, as has been recorded in wheat roots in response to Al.$^{71}$

Inhibition of NO$_3^-$ uptake by the heavy metal Cd$^{2+}$ has been demonstrated in wheat (Titanium aestivum)$^{72}$, birch (Betula pendula)$^{73}$ and Helianthus annuus$^{74}$. In pea plants, the inhibition of net uptake by 50 mM Cd is complete within 24 hr of heavy metal application, although the inhibition is reversible i.e. when the plants are transferred to Cd free medium, the uptake is normalized.$^{74}$

Essential heavy metals also inhibit NO$_3^-$ uptake when supplied above sub-optimum level. For example, Zn$^{2+}$ inhibits NO$_3^-$ uptake in Helianthus annuus.$^{69}$

Accumulation of NO$_3^-$ is known to be inhibited by Cd in bean and in tomato, which is apparently due to reduced uptake.$^{75}$

**Osmotic stress**

Osmotic stress caused by either salinity or by withholding water usually inhibits NO$_3^-$ uptake from the medium.$^{76}$ The threshold level of the salinity which causes a significant inhibition of NO$_3^-$ uptake, varies according to the species. Inhibition of NO$_3^-$ uptake has been demonstrated in maize in the presence of 100 mM NaCl$^{77}$ and by 60 mM of the salt in wheat.$^{78}$ In hydroponically grown Leucaena leucocephala, 20 mM NaCl had no effect on NO$_3^-$ uptake by the seedlings.$^{79}$

Salinity has no effect on NO$_3^-$ accumulation in Lolium seedlings, when the nutrient nitrogen is (NH$_4$)$_2$SO$_4$, although it increases accumulation in the roots in the presence of NH$_4$NO$_3$ or NaNO$_3$(ref. 80). On the other hand, increase in salinity up to 150 mM causes a gradual decline in NO$_3^-$ accumulation in wheat.$^{81}$ This is apparently due to decreasing NO$_3^-$ uptake with the increasing salinity. NO$_3^-$ accumulation in chickpea decreases when water stress is created by withholding water.$^{82}$ As describe earlier, this is apparently due to decreased NO uptake during water stress.
External pH

Nitrate uptake is usually favoured by the acidic pH of the medium and it decreases with increasing acidity. Aguera et al. and Ni and Beevers observed that the uptake of NO\textsubscript{3}\textsuperscript{-} drastically decreased at a pH above 5.5 in sunflower and maize respectively. In maize roots, the total NO\textsubscript{3}\textsuperscript{-} uptake after 100 min of incubation in 0.5 mM Ca(NO\textsubscript{3})\textsubscript{2} was about 50\% lower at pH 8.0 than that at pH 5.5

In a very few studies with the effects of external pH on NO\textsubscript{3}\textsuperscript{-} accumulation in plants, it has been found that the accumulation in the roots of the maize seedlings is unaltered at pH 4.5 and 6.5.

Temperature

Nitrate uptake is sensitive to temperature and it depends not only on the temperature of the root environment but also upon the temperature of shoot environment. In barley plants, the uptake is more at low temperature whereas in Pennisetum and maize the uptake is higher at high temperature. In maize, 30\%/30\% day/night temperature appeared to be more suitable for NO\textsubscript{3}\textsuperscript{-} uptake and dry matter accumulation than the lower temperature. In soybean also, the total NO\textsubscript{3}\textsuperscript{-} uptake is higher at 22\°C than at 14\°C. Similarly in beech (Fagus sylvatica L.) and spruce (Picea abies L.) the rate of NO\textsubscript{3}\textsuperscript{-} uptake by the roots at 10-15\°C is substantially lower than that at 25\°C. It appears therefore, that 20-30\°C is the most favourable temperature for NO\textsubscript{3}\textsuperscript{-} uptake in most species.

In bean shoots, accumulation of NO\textsubscript{3}\textsuperscript{-} is substantially higher, when the plants are raised at 27/11\°C day-night temperature than at 19/7\°C (ref. 89). In Lemna fronds also, the accumulation of NO\textsubscript{3}\textsuperscript{-} is higher at higher (23.9\°) than at lower (18.3\°) temperature. This is apparently due to increased NO\textsubscript{3}\textsuperscript{-} uptake at higher temperature.

Irradiance

Under experimental conditions, it has been demonstrated that the increasing irradiance causes a decline in internal NO\textsubscript{3}\textsuperscript{-} concentration. In bean seedlings, NO\textsubscript{3}\textsuperscript{-} accumulation in roots or shoots in NO\textsubscript{2} exposed plants also decreases with the increase in irradiance. This is apparently because the increase in irradiance causes an increased NO\textsubscript{3}\textsuperscript{-} reduction and assimilation.

Oxygen availability

The requirement of oxygen for NO\textsubscript{3}\textsuperscript{-} uptake indicates that the uptake of NO\textsubscript{3}\textsuperscript{-} depends upon the metabolic energy derived from the root respiration. From an experiment with Lolium, it has been calculated that uptake of 1 mol of NO\textsubscript{3}\textsuperscript{-} from a solution of 1.5 mM NO\textsubscript{3}\textsuperscript{-}, requires about 29 kJ of energy. The energy is expended in the functioning of the NO\textsubscript{3}\textsuperscript{-} transporters. Therefore creating hypoxia or anoxia may cause a decline in NO\textsubscript{3}\textsuperscript{-} uptake. In sunflower, NO\textsubscript{3}\textsuperscript{-} uptake is inhibited drastically with the withdrawal of O\textsubscript{2} from the medium.

Diurnal variations

Diurnal variations in NO\textsubscript{3}\textsuperscript{-} uptake by the plants has also been demonstrated by a few investigators. In soybean, in wheat, in young tomato plants and in hydroponically grown rose plants, it has been demonstrated that the rate of NO\textsubscript{3}\textsuperscript{-} uptake is substantially higher in day time than in night time. This is apparently due to higher soluble sugar content of the roots during day time than in night. However, the accumulation of NO\textsubscript{3}\textsuperscript{-} during light period (or day) is lower than that during dark period, apparently because the reduction and assimilation of NO\textsubscript{3}\textsuperscript{-} is also higher during the day. Thus, it is always advisable to harvest the vegetables, specially the leafy ones, during the day time, because the levels of NO\textsubscript{3} will be low in such harvests.

Conclusion and future prospects

The process of uptake and accumulation of NO\textsubscript{3}\textsuperscript{-} in the plant cells is better understood now than it was about a decade ago, although the processes linked to these aspects are complex and varied. The knowledge of the molecular genetics of NO\textsubscript{3} transporters, specially of the HATS may help in genetic modification of crop plants, for acquisition of NO\textsubscript{3}\textsuperscript{-} from even low NO\textsubscript{3}\textsuperscript{-} soils. The genetically modified plants may also have very little NO\textsubscript{3}\textsuperscript{-} accumulation in their vegetative and reproductive parts, which are often consumed by humans or domesticated cattle. This will not only save a substantial amount of money spent on the manufacture and application of nitrogenous fertilisers, it will also reduce the NO\textsubscript{3}\textsuperscript{-} contamination of soil and water, which is a major environmental problem in areas which have intensive cultivation of crops. However, the nature of the nutritive, osmotic or regulatory role of NO\textsubscript{3} is to be
fully understood, and it has to be demonstrated that even very low levels of NO$_3^-$ are sufficient to perform the non-nutritive roles of the NO$_3^-$. 

References

43 Deane-Drummond C E, Regulation of nitrate uptake into Chrus carollina cells via NH4+ stimulation of NO3- efflux Plant Cell Environ, 8 (1985) 105.
49 Dodderha & Otten, H, Uptake of nitrate by mutants of Arabidopsis thaliana, disturbed in uptake or reduction of nitrate, III. Regulation Physiol Plant, 45 (1979) 339.
51 Cooper H D & Clarkson D T, Cycling of amino nitrogen and other nutrients between shoots and roots in cereals as possible mechanism integrating shoot and root in the regulation of nutrient nitrate J Exp Bot, 40 (1989) 753.
52 Muller B & Touraine B, Inhibition of NO3- uptake by various phloem translocated amino acids in soybean seedings J Exp Bot, 43 (1992) 617.
Ammonium by the roots of spruce (Picea abies) and beech (Fagus sylvatica) trees, New Phytol., 138 (1998) 275.

MacLeod K C & Ornmond D P. Responses of white bean to ammonium or nitrate nutrition at three temperatures Can J Plant Sci, 65 (1985) 201.


Srivastava M & Shankar N, Accumulation of nitrate, nitrite and total nitrogen in response to NO2 exposure or nitrate treatment in bean seedlings at different light intensities in Responses of plant metabolism to air pollution and global change edited by L.J. de Kok and I. Stulen. (Bauhuis Publishers, Leiden, The Netherlands)1998, 453.


