# Nitrogen transport in plants, with an emphasis on the regulation of fluxes to match plant demand[§]

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## Summary – Zusammenfassung

Physiological methods, especially the use of isotopes of N, have allowed for the detailed characterizations of the several putative transport systems for nitrate and ammonium in roots of higher plants. In the last decade, the cloning of genes that appear to encode both high- and low-affinity transporters represent major advances, as well as substantiating the inferences based on earlier physiological methods. Nevertheless, the unexpected plethora of genes that have been identified now presents even greater challenges, to resolve their individual functions and to attempt to place these functions in a whole plant/environmental context.

**Key words:** nitrate / ammonium / transporter / uptake / demand

## Stickstofftransport in Pflanzen unter besonderer Berücksichtigung der Regulation der am N-Bedarf orientierten Fluxe

Physiologische Methoden, insbesondere der Einsatz von N-Isotopen, haben eine detaillierte Charakterisierung der verschiedenen mutmaßlichen Transportsysteme für Nitrat und Ammonium in Pflanzenwurzeln ermöglicht. Ein besonderer Fortschritt im letzten Jahrzehnt war die Klonierung von Genen, die hoch- und wenig-affine Transportproteine codieren. Hierdurch konnten Schlussfolgerungen aus früheren physiologischen Arbeiten untermauert werden. Trotz dieses Erkenntnisfortschritts stellt die unerwartete genetische Vielfalt die Forschung vor noch größere Herausforderungen bei der Klärung der einzelnen Funktionen und bei dem Bemühen, diese im Gesamtkontext Pflanze/Umwelt zu deuten.

## 1 Introduction

Despite the current massive global N fertilizer inputs (~$10^{11}$ kg $a^{-1}$), plant roots experience enormous soil heterogeneity with respect to N and to other soil nutrients. In seventy seven aerobic agricultural soils in N.Z., Australia, and the USA, mean values (± standard deviations) for soil solution nitrate ($NO_3^-$) and ammonium ($NH_4^+$) were 4.5 ± 9.8 and 0.78 ± 1.5 mM, respectively, ranging across three to four orders of magnitude (*Wolt*, 1994). From a 12 by 10-m grid in a native sagebrush steppe in Utah, *Jackson* and *Caldwell* (1993) analyzed 362 soil samples and reported that $NO_3^-$ and $NH_4^+$ concentrations ranged across three orders of magnitude. In this ecosystem, $NO_3^-$ and $NH_4^+$ concentrations were rather similar. By contrast, in many mature forests $NO_3^-$ may be undetectable and ratios of $NO_3^-$ to $NH_4^+$ may be as low as 0.02 (*Stark* and *Hart*, 1997). Moreover, absolute $NH_4^+$ concentrations ($[NH_4^+]$) in the forest floor can be considerable, ranging from 0.05 to 3.5 mM (*Vitousek* et al., 1979). Likewise in arctic tundra soils $NO_3^-$ may be undetectable and combined amino acid concentrations may exceed those of $NH_4^+$ (*Kielland*, 1994). To add to this heterogeneity there are both seasonal changes, associated with biological activities of soil organisms, as well as the catastrophic disturbances associated with large-scale fires and clearcuts (*Vitousek* et al., 1979). In the context of such heterogeneity, plant roots must respond to local, regional and seasonal changes by means of adaptations that can optimize nutrient capture. This paper will address the physiological and molecular basis of adaptations involving inorganic N transport. While ecologists typically focus on the adaptations to nutrient inadequacy, it is evident that N-starved plants rapidly down-regulate influx when re-supplied with various N forms, perhaps to guard against osmotic damage or the potential ion toxicity associated with excessive accumulation. Previous reviews (e.g. *Haynes* and *Goh*, 1978; *Bloom*, 1988; *Glass* and *Siddiqi*, 1995; *Crawford* and *Glass*, 1998; *Forde* and *Clarkson*, 1999) have do-cumented the earlier physiological literature, therefore the present treatment will be less comprehensive in this area.

## 2 Nitrate transport

### 2.1 High-affinity transporters

Physiological evidence suggests that at least three types of $NO_3^-$ transporter systems co-exist at the plasma membrane (reviewed in *Glass* and *Siddiqi*, 1995; *Crawford* and *Glass*,

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1998). Physiological studies of these transport systems by means of influx versus concentration curves, led to the presumption that the observed kinetics of each of the transport systems resulted from the operation of a single species of transporter. However, molecular data (see below), has established that multiple gene family members are present for both the high-affinity and low-affinity transport systems. If the transporters corresponding to each member of a particular gene family contribute to measured fluxes across the plasma membrane, and if their $K_m$ values for influx are not too dissimilar, the combined kinetics might be indistinguishable from that of a single transporter. Alternatively, if one gene product predominates (see e.g. *Zhuo* et al., 1999; *Touraine* and *Glass*, 1997) the minor contributions of other members of the family might be invisible. Thus each of the physiologically-defined transport systems (CHATS, IHATS and LATS) discussed below, may consist of several genetically distinct transporters.

Constitutive transporters (CHATS) which operate at low capacity and with high affinity, facilitate the entry of $NO_3^-$ into roots, from low external [$NO_3^-$], allowing for the induction of high-affinity and high-capacity transporters (IHATS). CHATS activity is expressed simultaneously with IHATS and also upregulated roughly three-fold by exposure to $NO_3^-$ in roots of barley and white spruce (*Aslam* et al., 1992; *Kronzucker* et al., 1995a). An *Arabidopsis* mutant (*chl8*) with defective CHATS activity, was isolated by screening for chlorate ($ClO_3^-$) resistance at low external $ClO_3^-$ concentration in the absence of $NO_3^-$ (*Wang* and *Crawford*, 1996). The gene corresponding to this mutation has not yet been cloned (Crawford, personal communication), but RT-PCR indicates that several of the *AtNRT2* family of genes are constitutively expressed and upregulated by exposure to $NO_3^-$ (Okamoto et al., unpublished, see Figure 1). These might be candidates for the CHATS function.

The IHATS transport function may be induced by $NO_3^-$ or $NO_2^-$ (*Aslam* et al., 1992; *Siddiqi* et al., 1992). In barley roots, depending on external $NO_3^-$ concentration ([$NO_3^-$]) , IHATS activity may increase to as high as 30 times the CHATS activity at roughly 6 to 12 hours after provision of $NO_3^-$. By contrast, in white spruce and in lodgepole pine the increase was modest and required 3 days to achieve maximum induction (*Kronzucker* et al., 1995a; *Min* et al., 1998). After peak induction has been achieved, $NO_3^-$ influx is downregulated to a steady-state level. Although pulses of extremely low [$NO_3^-$] (e.g. 20 µM) are sufficient to induce IHATS activity in various species (*MacKown* and *McClure*, 1988; *Tischner* et al., 1993; *Laine* et al., 1995), maximum IHATS activity in barley roots was achieved more rapidly, and downregulation initiated earlier, when IHATS was induced at higher [$NO_3^-$] (*Siddiqi* et al., 1989). For example, plants pretreated with 10 mM $NO_3^-$ reached peak values for $^{13}NO_3^-$ influx at 12 h and then declined to approximately 20% of peak influx values. By contrast, $^{13}NO_3^-$ influx in plants pretreated with 10 µM $NO_3^-$ increased to approximately 60% of the value of 10 mM $NO_3^-$-treated plants by 24 h, and failed to change significantly during the next 72 h. Overall, $NO_3^-$ influx was negatively correlated with root [$NO_3^-$] during the period of downregulation that followed

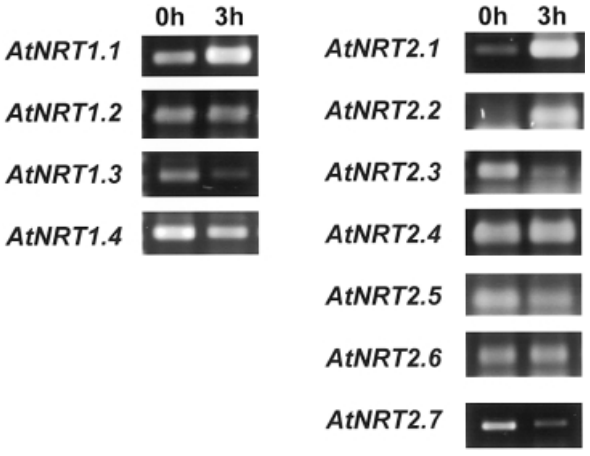


**Figure 1:** Transcript abundance of *AtNRT1* (A) and *AtNRT2* (B) genes in roots of *Arabidopsis thaliana* determined by RT-PCR at 0 h (after 7 days without $NO_3^-$) and 3 h after provision of 1 mM $KNO_3$. (Okamoto et al., unpublished).
**Abbildung 1:** Transkript-Häufigkeit der Nitrattransporter-Gene *AtNRT1* (A) und *AtNRT2* (B) in Wurzeln von *Arabidopsis thaliana*, bestimmt mittels RT-PCR im Zeitpunkt 0 (7 Tage ohne $NO_3^-$-Angebot) und nach 3-stündigem Angebot von 1 mM $KNO_3^-$.

peak induction. These and other similar observations have led various groups to propose that $NO_3^-$ influx may be downregulated by tissue $NO_3^-$ or some product of nitrate assimilation.

Using 300 µM tungstate ($WO_4^{2-}$) to block the enzyme nitrate reductase (NR) during a 24 h pretreatment in the presence of 1 mM $KNO_3$, *Ingemarsson* et al. (1987) demonstrated that, in *Lemna gibba*, tissue $NO_3^-$ rose to 31 µmol $g^{-1}$ and $^{13}NO_3^-$ influx was reduced to zero. The same $WO_4^{2-}$ treatment during a 24 h pretreatment with 10 µM $NO_3^-$ resulted in tissue [$NO_3^-$] of only 2.8 µmol $g^{-1}$, and $^{13}NO_3^-$ influx was measured to be 1.07 µmol $g^{-1}$ $h^{-1}$. The authors concluded that $NO_3^-$ itself was responsible for the observed down-regulation of $^{13}NO_3^-$ influx. This effect of $WO_4^{2-}$ on nitrate influx was confirmed by *Mattson* et al. (1991) and by *Wieneke* (1994) using barley seedlings. By contrast, in roots of bean and corn, methionine sulfoximine (MSX) which blocks the conversion of $NH_4^+$ to glutamine by inhibiting the enzyme glutamine synthetase (GS), actually increased $NO_3^-$ influx, suggesting that down-stream metabolites of $NO_3^-$ were responsible for the observed downregulation (*Breteler* and *Siegerist*, 1984; *Lee* et al., 1992). Yet, in a study of the downregulation of $NO_3^-$ influx in barley roots, MSX failed to relieve the characteristic reduction of influx associated with continued $NO_3^-$ provision (*King* et al., 1993). Using NR mutants of barley, *Warner* and *Huffaker* (1989) and *King* et al. (1993) observed that induction and downregulation of $NO_3^-$ uptake was normal despite the block of nitrate reduction. As a consequence of these contradictory results, a consensus on the role of tissue $NO_3^-$ versus down-stream metabolites in downregulating $NO_3^-$ influx has not been achieved.

In support of downregulation of $NO_3^-$ influx by down-stream metabolites, pretreatments with various amino acids, applied either directly via exogenous application or indirectly via cotyledonary or stem-flap feeding, have been shown to

inhibit $NO_3^-$ influx into roots of various plants (*Doddema* and *Otten*, 1979; *Breteler* and *Arnozis*, 1985; *Lee* et al., 1992; *Muller* and *Touraine*, 1992; *Gessler* et al., 1998). Unfortunately, many of these studies involved extremely long periods of pretreatment (from 15 to 48 h) with specific amino acids. As emphasized by *Lee* et al. (1992) and *Vidmar* et al. (2000b), the exogenous application of individual amino acids, even when they are the very amino acids normally cycled back to the roots within the phloem, inevitably results in their conversion to other amino acids. Nevertheless, using several different methods of reducing or increasing root glutamine (gln) or asparagine (asn), *Lee* et al. (1992) concluded that these were the most likely candidates responsible for down-regulating $NO_3^-$ and $NH_4^+$ influx in maize roots. In *Ricinus communis, Tillard* et al. (1998) demonstrated that when N was withheld completely, glutamine and serine concentrations of the phloem were rapidly lowered by 50%. By contrast, in split-root experiments, there was no change in amino acid composition of the fed root, despite a significant increase of $^{15}NO_3^-$ influx, and only the starved portion of the root exhibited a response similar to that of the completely starved plant. The authors suggested that the shoot-borne signals responsible for regulating $NO_3^-$ influx were not quantitatively related to changes in phloem amino acids. Also using split roots, *Laine* et al. (1995), concluded that pools of free amino acids in roots were not involved in the control of root $NO_3^-$ uptake. In summary, notwithstanding these two papers, physiological studies have tended to favor amino acids as the agents responsible for the down-regulation of $NO_3^-$ influx, but a number of other studies claim that $NO_3^-$ itself may participate. Molecular data (see below) may suggest that both amino acids and inorganic N ($NO_3^-$ and $NH_4^+$) may regulate $NO_3^-$ influx through effects operating at different levels (transcriptional and posttranscriptional).

## 2.2 Developments in molecular biology

### 2.2.1 The *NRT2* family

By use of degenerate primers to conserved sequence motifs of the high-affinity nitrate transporter gene of *Aspergillus nidulans* (*CRNA*), *Trueman* et al. (1996) were able to isolate two full length cDNA clones (originally *BCH1* and *BCH2*, now renamed *HvNRT2.1* and *HvNRT2.2*, respectively) from barley. The polypeptides corresponding to these genes shared 41–43% sequence identity with the CRNA polypeptide and 56–57% identity with NAR-3, the corresponding *Chlamydomonas* polypeptide. Southern blots indicated that there might be up to 10 related genes in the barley genome. mRNA corresponding to *HvNRT2.1* was rapidly induced by exposure to $NO_3^-$, but not $NH_4^+$. *Vidmar* et al. (2000a) isolated two more members of this barley family (*HvNRT2.3* and *HvNRT2.4*) and demonstrated that following peak induction, there was a gradual reduction in expression levels of *HvNRT2.1, HvNRT2.2* and *HvNRT2.3*. This corresponds to the reduction of $^{13}NO_3^-$ influx under similar conditions. By contrast, *HvNRT2.4* expression was not subject to downregulation. *NRT2* homologues have also been cloned from several other plant species including *Arabidopsis* (*Filleur* and *Daniel-Vedele*, 1999; *Zhuo* et al., 1999), *Lycopersicon esculentum* (*Ono* et al., 2000), *Nicotiana plumbaginifolia* (*Quesada* et al., 1997) and *Glycine max* (*Amarasinghe* et al., 1998). In total, 7 members of the *NRT2* family have been identified in *Arabidopsis*. Expression patterns of *AtNRT2.1, AtNRT2.2, AtNRT2.3, AtNRT2.4, AtNRT2.5, AtNRT2.6*, and *AtNRT2.7*, following provision of $NO_3^-$ are shown in Fig. 1. *AtNRT2.1* and *AtNRT2.2* appear to require provision of $NO_3^-$ for expression, and show the strongest differential induction. By contrast, *AtNRT2.3*, *AtNRT2.4, AtNRT2.5, AtNRT2.6*, and *AtNRT2.7* are expressed even without exposure to $NO_3^-$, and transcript abundance of these genes fails to increase substantially following exposure to $NO_3^-$. Thus, by reference to the physiological characteristics of the CHATS and IHATS transporters, we speculate that *AtNRT2.1* and *AtNRT2.2* correspond most closely to *IHATS* whereas *AtNRT2.4, AtNRT2.5*, and *AtNRT2.6* most resemble the CHATS function. Clearly, it will be necessary to determine the tissue-specific localization of these genes before any more definitive assignment to physiological function can be achieved.

Most of these *NRT2.1* homologues from various plant species demonstrated a rapid induction by $NO_3^-$, but not by $NH_4^+$, and a strong downregulation of expression by treatment with reduced forms of N such as $NH_4^+$ or glutamine (*Krapp* et al., 1998; *Amarasinghe* et al., 1998; *Zhuo* et al., 1999; *Vidmar* et al., 2000a). Using nitrate reductase mutants of *N. plumbaginifolia*, it was demonstrated that *NpNRT2* was over-expressed compared to wild-type plants, again suggesting that downregulation of *NpNRT2* depends upon downstream metabolites of $NO_3^-$ (*Krapp* et al., 1998). However, using exogenous applications of $NH_4^+$ or amino acids, it is impossible to determine which particular compound or compounds are responsible for the observed downregulation, because of rapid interconversions. By applying $NH_4^+$ or $NO_3^-$, together with various metabolic inhibitors to block interconversions of the various intermediates of $NO_3^-$ assimilation, *Zhuo* et al. (1999) demonstrated that the most potent downregulator of *AtNRT2.1* was glutamine. Evidence for effects of $NH_4^+$ itself was only seen when unusually high levels of $NH_4^+$ were accumulated as a result of blocking GS with MSX. Likewise, *Vidmar* et al. (2000b) used 10 mM $KNO_3$ to induce *HvNRT2* expression, with and without various metabolic blockers and amino acid additions. Although aspartate (asp) and glutamate (glu) additions reduced *HvNRT2* transcript abundance and $NO_3^-$ influx to a greater extent than asparagine (asn) or glutamine (gln), when data from effects of amino acids were analyzed together with those from inhibitor studies, the strongest negative correlation (–0.76) obtained was between root glutamine and *HvNRT2* transcript levels (see Fig. 2). Values for the other amino acids were –0.05 (asp), –0.12 (glu), and –0.38 (asn). Two effects may account for this unexpected finding. Firstly, exogenously administered amino acids are subject to extensive interconversion (*Vidmar* et al., 2000b). For example, application of asp and glu resulted in higher root gln concentrations than exogenous application of gln itself. Secondly, the inhibitor azaserine, which blocks the conversion of gln to glu via the enzyme glutamate synthase,

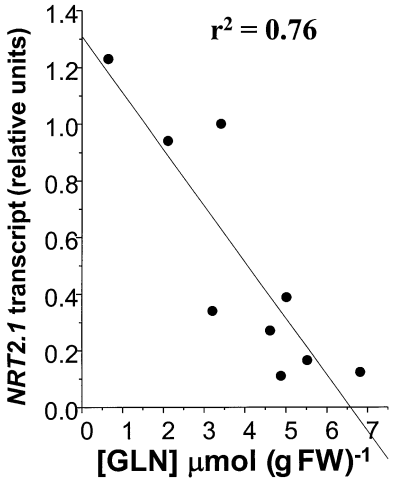


**Figure 2:** Correlation between transcript abundance of the barley *HvNRT2.1* gene and root glutamine concentration after exogenous provision of different amino acids and various inhibitors of $NO_3^-$ assimilation (From *Vidmar* et al., 2000b).
**Abbildung 2:** Korrelation zwischen der Transkript-Häufigkeit des *HvNRT2.1*-Gens in Gerste und der Glutaminkonzentration in der Wurzel nach exogenem Angebot verschiedener Aminosäuren bzw. Inhibitoren der $NO_3^-$-Assimilation.

caused the largest decrease of *HvNRT2* transcript abundance and $NO_3^-$ influx, while strongly reducing tissue concentrations of glu and asp.

It is instructive to compare effects of various inhibitors of $NO_3^-$ assimilation on $NO_3^-$ influx and *HvNRT2* mRNA levels. For example, while $WO_4^{2-}$, an inhibitor of NR, increased *HvNRT2* transcript abundance, confirming the observations of *Krapp* et al. (1998), $^{13}NO_3^-$ influx was reduced to ~55%. This observation may resolve the apparent contradiction between earlier findings concerning effects of accumulated $NO_3^-$ on $NO_3^-$ influx, suggesting that while *HvNRT2* expression is downregulated by tissue gln, $NO_3^-$ itself may exert direct effects upon $NO_3^-$ transporters through mechanisms such as allosteric effects on transporters or protein phosphorylation (*Frazier* et al., 2000; *Vidmar* et al., 2000b). Likewise, while MSX failed to reduce *HvNRT2* expression, there was a significant reduction of $NO_3^-$ influx. Again this may suggest direct effects of accumulated $NH_4^+$ on the $NO_3^-$ transporter. When glu was added exogenously to $WO_4^{2-}$-treated roots, *HvNRT2* transcript abundance declined substantially. Thus downregulation of *HvNRT2* expression can override the anticipated induction associated with high tissue [$NO_3^-$]. In summary, *HvNRT2* mRNA expression depends upon exposure to $NO_3^-$, while downregulation is strongly correlated with tissue gln levels. In addition, there is evidence for direct effects of accumulated $NO_3^-$ and $NH_4^+$ on the $NO_3^-$ transporter.

The above discussion has focussed on $NO_3^-$ influx, but clearly $NO_3^-$ efflux increases with increasing tissue $NO_3^-$ (*Jackson* et al., 1976; *Siddiqi* et al., 1991; *Wieneke*, 1994). Taken together with the reduction of influx, the effects on efflux would inevitably reduce the net uptake of $NO_3^-$ even more. Based upon studies using inhibitors of RNA and protein synthesis, *Aslam* et al. (1996), have suggested that $NO_3^-$ efflux, like influx, is mediated by a $NO_3^-$-inducible transport system.

### 2.3 Low-affinity transporters

At [$NO_3^-$] beyond those that saturate the IHATS, low-affinity transport systems (LATS) become apparent. In barley roots LATS failed to saturate even at 50 mM external $NO_3^-$ (*Siddiqi* et al., 1990). In corn roots also, a linear response to high external [$NO_3^-$] was observed by *Pace* and *McClure* (1986). Yet, thermodynamic considerations preclude passive transport of $NO_3^-$ even at these high [$NO_3^-$] (*Glass* et al., 1992). Though greater interest has been directed toward the IHATS, under agricultural conditions it is likely that the IHATS is downregulated and that $NO_3^-$ influx may largely be due to LATS activity (see Introduction). Like its HATS counterpart, $NO_3^-$ influx via the LATS is electrically depolarizing and therefore probably occurs via a proton symport (*Glass* et al., 1992). In barley roots, *Siddiqi* et al. (1990) demonstrated that the LATS for $NO_3^-$ influx is constitutive, being present even in $NO_3^-$-deprived plants. Using chlorate ($ClO_3^-$) selection, *Doddema* and *Telkamp* (1979) obtained a mutant (B1) strain of *Arabidopsis* that was defective in LATS activity, but showed normal HATS activity.

#### 2.3.1 The *NRT1* family

Using $ClO_3^-$ selections among T-DNA insertional mutants, *Tsay* et al. (1993) isolated a mutant and cloned the $NO_3^-$-inducible *NRT1* gene corresponding to the B1 mutant of *Doddemma* and *Telkamp* (1979). When expressed in *Xenopus* oocytes, the polypeptide corresponding to the *NRT1* gene behaved as a typical $NO_3^-$ transporter, exhibiting transient membrane depolarization in the presence of $NO_3^-$, and permitting the oocytes to accumulate significantly more $NO_3^-$ than water-injected (control) oocytes. A surprising feature of the T-DNA mutant strain was revealed by detailed measurements of $NO_3^-$ and $ClO_3^-$ accumulation and $^{13}NO_3^-$ influx in the LATS concentration range (*Touraine* and *Glass*, 1997). $ClO_3^-$ accumulation in mutant plants was significantly lower than in wild-type plants, but tissue $NO_3^-$ and $NO_3^-$ fluxes in mutant plants were not different from those of wild-type plants when grown on $KNO_3$. This result suggested that other LATS transporters might contribute to influx in this concentration range. These transporters may also show differential capacity to absorb $ClO_3^-$. The cloning of a second *Arabidopsis NRT1* gene, *AtNRT1:2* (originally named *NTL1*), confirmed this expectation (*Huang* et al., 1999). An earlier report of the presence of two *NRT1* homologues, one constitutive and one inducible, in root hairs of tomato, was reported by *Lauter* et al. (1996). When grown on $NH_4NO_3$, the *Arabidopsis* T-DNA mutants absorbed significantly less $NO_3^-$ than wild-type plants suggesting that the two genes were differentially sensitive to downregulation by $NH_4^+$ or its assimilation products. *In situ* hybridization studies indicate that *AtNRT1.1* is primarily expressed in epidermal cells close to the root tip, but in older roots the

gene is expressed in cells deeper through the cortex, including the endodermis (*Huang* et al., 1996). The constitutive *NRT1.2* gene was primarily expressed in root hairs and the epidermis in both young (root tips) and mature regions of roots (*Huang* et al., 1999). Recently, *Lin* et al. (2000) have cloned a constitutively expressed rice homolog of the *NRT1* family (*OsNRT1*). Since sequencing of the *Arabidopsis* genome is now more than 93% complete, it has become apparent that there are at least 4 members of the *NRT1* family of genes present in the *Arabidopsis* genome. RT-PCR studies indicate that all of these genes are expressed to different extents and they all appear to be expressed without prior exposure to $NO_3^-$ (Okamoto et al., unpublished results, see Fig. 1). The apparent absence of expression of *NRT1.1* in $NO_3^-$-deprived plants in the study by *Tsay* et al. (1993), may have been due to the very high N provision (12.5 mM ammonium succinate) prior to induction.

Recently it was demonstrated that $NO_3^-$ uptake by *AtNRT1*-deletion mutants is also reduced at low [$NO_3^-$], in addition to high external [$NO_3^-$]. It was argued, therefore, that the *NRT1* genes may make a major contribution to both low- and high-affinity transport (*Wang* et al., 1998; *Liu* et al., 1999). This claim was supported by the observed biphasic pattern of $NO_3^-$ uptake in *Xenopus* oocytes injected with *NRT1.1* mRNA (*Liu* et al., 1999). Earlier $^{13}NO_3^-$ influx measurements and electrophysiological studies in barley roots indicated that both IHATS and LATS transport contribute to measured influx at high external [$NO_3^-$] (*Siddiqi* et al., 1990; *Glass* et al., 1992). The present data appear to demonstrate that the same is true at low external $NO_3^-$. It should be noted, however, that mutant plants were grown on 12.5 mM ammonium succinate (equivalent to 25 mM $NH_4^+$) prior to measurements of net $NO_3^-$ uptake at 250 μM $NO_3^-$. These conditions would be anticipated to suppress expression of IHATS (see above). It should also be recalled that the study by *Doddema* and *Telkamp* (1979) reported defective $NO_3^-$- uptake by their B1 mutant only in the LATS range, despite prior growth on 1.5 mM $NH_4NO_3$. Likewise, in the study by *Touraine* and *Glass* (1997), IHATS activity appeared normal in mutant plants grown on 1 mM external $KNO_3$. Therefore, if LATS does contribute to influx at low external $NO_3^-$, its contribution would probably be modest under normal levels of N provision.

By comparison to IHATS transport, LATS activity appears to be insensitive to downregulation by accumulated N (*Siddiqi* et al., 1990). Nevertheless, earlier studies by *Clement* et al. (1978), reported that when ryegrass was grown for 8 weeks at constant external [$NO_3^-$] from 14.2 μM to 14.2 mM, N uptake, tissue N, and growth were independent of ambient [$NO_3^-$]. This observation indicates that the LATS is subject to downregulation. While *Tsay* et al. (1993) found no expression of *AtNRT1.1* prior to $NO_3^-$ treatment, their plants had been previously grown on 25 mM $NH_4^+$, a condition likely to suppress constitutive gene expression. By contrast, Okamoto (see Figure 1) found that all four *AtNRT1* genes were expressed in roots prior to $NO_3^-$ exposure in plants previously grown on 0.5 mM $NH_4NO_3$ and then deprived of N for 1 week. Nevertheless *AtNRT1.1* expression levels were strongly increased following $NO_3^-$ treatment.

Considered in the whole-plant context, the cycling and recycling of amino acids, have been suggested as the means for integrating root activity with whole-plant demand for N (*Cooper* and *Clarkson*, 1989; *Marschner* et al., 1997). The demonstrated regulation of HvNRT2 expression by gln, a major component of xylem- and phloem-translocated amino acids, now provides a mechanism whereby translation of this putative root:shoot signal of N status may be achieved. Further work will be required to determine if other amino acids also participate.

## 3 $NH_4^+$ transport

Physiological studies using $^{15}NH_4^+$ or $^{13}NH_4^+$ have established that two distinct categories of transporters (high- and low-affinity) contribute to $NH_4^+$ influx (*Ullrich* et al., 1984; *Wang* et al., 1993a; *Kronzucker* et al., 1996). While the high-affinity transporter displays saturable influx kinetics with respect to external $NH_4^+$, the low-affinity transport system was not saturated even at 40 mM external $NH_4^+$ in rice (*Wang* et al., 1993a) and 50 mM in white spruce (*Kronzucker* et al., 1995b). Despite the common claim that $NH_4^+$ influx is electrogenic, driven by the membrane electrical potential (*Ninnemann* et al., 1994; *Sohlenkamp* et al., 2000), it has been demonstrated in both *Lemna* (*Ullrich* et al., 1984) and in rice (*Wang* et al., 1994), that the electrical potential difference is inadequate to drive $NH_4^+$ influx at [$NH_4^+$] up to 655 μM. This value was found to vary according to prior exposure to $NH_4^+$.

### 3.1 Regulation of $NH_4^+$ influx

Many studies have demonstrated that HATS for $NH_4^+$ influx is downregulated when tissue N is elevated and up-regulated when plants are deprived of sufficient N (*Jackson* et al., 1976; *Wang* et al., 1993a ; *Lee* et al., 1992; *Rawat* et al., 1999). While $NH_4^+$ efflux increases as a % of influx as external [$NH_4^+$] increases, the major effect of increased tissue N is upon $NH_4^+$ influx (*Wang* et al., 1993a, b). Attempts to resolve the mechanism of the down-regulation of influx have consistently made use of MSX to ascertain whether it is tissue $NH_4^+$ or downstream metabolites of $NH_4^+$ that are responsible for the regulation of $NH_4^+$ influx. Unfortunately, there is no consensus on this question; results derived from MSX applications fall into two categories. There are those that provide support for a down-stream regulation (e.g. *Lee* et al., 1992) and (in equal numbers) those that provide evidence for $NH_4^+$ itself as the regulator (*Feng* et al., 1994; see *Glass* et al., 1997 and references therein). The results of recent experiments that combine physiological and biochemical methods with molecular biology (see below) have provided some clarification of this controversy (*Rawat* et al., 1999).

By contrast to the downregulation of the HATS by accumulated N, the LATS for $NH_4^+$ influx appeared to be insensitive to N status in roots of rice and *Arabidopsis* (*Wang* et al., 1993a; *Rawat* et al., 1999). Indeed, this failure to downregulate the LATS may contribute to excessive $NH_4^+$ accumulation and toxic effects that result from exposing plant roots to elevated concentrations of $NH_4^+$.

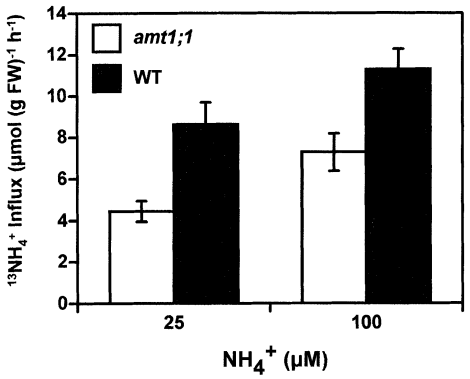


**Figure 3:** $^{13}NH_4^+$ influx into roots of wild-type (WT) and an *AMT1;1* T-DNA insertional mutant of *Arabidopsis thaliana*, measured at 25 and 100 µM $NH_4^+$ after 4 days of N starvation (Kaiser et al., unpublished). **Abbildung 3:** $^{13}NH_4^+$-Influx in die Wurzeln des Wildtyps (WT) und einer *AMT1;1* T-DNA-Mutanten von *Arabidopsis thaliana*, gemessen bei 25 und 100 µM $NH_4^+$ nach 4-tägiger Unterbrechung der N-Versorgung (Kaiser et al., unveröffentlicht).

## 3.2 Developments in molecular biology

### 3.2.1 The *AMT1* family

*Ninneman* et al. (1994) transformed a *Saccharomyces* double mutant (*mep1mep2*) with cDNA from *Arabidopsis*, and successfully cloned the first plant $NH_4^+$ transporter gene *AMT1* (now renamed *AtAMT1.1*). Since then *AtAMT1.2* and *AtAMT1.3*, members of the same family, have been cloned and studied (*Gazzarini* et al., 1999). Using $^{14}$C-methylamine (MA) uptake and the inhibitory effects of $NH_4^+$ on these fluxes, *Gazzarini* et al. concluded that these transporters had $K_m$ values of 0.5 µM (*AtAMT1.1*), and around 40 µM, when expressed in *Saccharomyces* mutants lacking functional high-affinity transport. Given that $NH_4^+$ influx into roots of *Arabidopsis* is characterized by much higher $K_m$ values (*Rawat* et al., 1999), the reported $K_m$ values for the three $NH_4^+$ transporters should be treated with caution, since they were determined in a heterologous expression system using $^{14}$C-MA. In tomato three *AMT1* homologues have been identified (*Lauter* et al., 1996; *von Wiren* et al, 2000). *LeAMT1.1* and *LeAMT1.2* are expressed in the roots while *LeAMT1.3* is preferentially expressed only in the shoots (*von Wiren* et al., 2000). *Rawat* et al. (1999) examined the regulation of the *AtAMT1.1* gene in roots of *Arabidopsis*. By transferring plants to and from solutions that were either sufficient or deficient in N, it was demonstrated that *AtAMT1.1* expression and $^{13}NH_4^+$ influx were strongly correlated, and responded rapidly to changes of external N status. During downregulation of $^{13}NH_4^+$ influx, there was a strong correlation between $^{13}NH_4^+$ influx, *AtAMT1.1* expression, and root [gln]. However, the strongest evidence that gln and not $NH_4^+$ regulates *AtAMT1.1* expression and $NH_4^+$ influx came from the use of MSX. This inhibitor blocked the formation of gln when N-starved roots were resupplied with N, and the roots failed to decrease *AtAMT1.1* transcript levels or $^{13}NH_4^+$ influx, despite a 27-fold increase of root [$NH_4^+$]. In the presence of MSX, roots continued to behave like N-starved roots (*Rawat* et al., 1999). A possible explanation for the apparent failure of MSX to relieve the down-regulation of $NH_4^+$ influx in many physiological experiments is that the associated high $[NH_4^+]_c$ (as high as 80 mM according to *Lee* and *Ratcliffe*, 1991) acts directly on the $NH_4^+$ transporters, inhibiting influx (see *Rawat* et al., 1999, for discussion).

In tomato roots starved of N, *LeAMT1.1*, and *LeAMT1.2* transcript levels increased over time, and this enhancement of *AMT* transcript was correlated with a decrease in pool sizes of gln and $NH_4^+$ (*von Wiren* et al., 2000). However, during re-supply of $NH_4^+$, *LeAMT1.1*, and *LeAMT1.2* expression patterns remained relatively unchanged, only decreasing slightly after a 24-hr period, while gln and $NH_4^+$ pools had dramatically increased from pre N-starved levels. This lack of a strong correlation between gln and *LeAMT1;1* transcript levels in tomato differs from the data obtained by *Rawat* et al., 1999. A possible reason for the differences between *Arabidopsis* and tomato may relate to the different growth conditions used in each set of experiments. In the experiments conducted by *Gazzarrini* et al. (1999) and in particular *Rawat* et al. (1999), plants were cultured within enclosed containers in liquid growth media containing sucrose. Manipulating the carbon pool sizes in tomato by growing plants at elevated $CO_2$ concentrations and at higher light dramatically decreased *LeAMT1.1* transcript levels following $NH_4^+$ re-supply to N-starved plants.

Recently, we have selected a T-DNA insertional mutant that fails to express *AMT1.1* mRNA, yet is able to grow on media containing $NH_4^+$ as sole source of N. This result suggests that compensation for the loss of *AMT1.1* function has occurred. Given that the $K_m$ values for members of the *AMT1* family differed by an order of magnitude, as suggested in *Gazzarini* et al. (1999), it might be anticipated that measured $^{13}NH_4^+$ influx in plant roots would deviate from simple Michaelis Menten kinetics, if multiple transporter systems operate at low $[NH_4^+]_o$. However, the kinetic analysis of $NH_4^+$ influx versus $[NH_4^+]_o$ in roots of rice plants and those of *Arabidopsis* gave Michaelis Menten curves consistent with the operation of a single transporter, suggesting that a single transport protein predominates (*Wang* et al., 1993a; *Rawat* et al., 1999). Fig. 3 shows that $^{13}NH_4^+$ influx into roots of the mutant strain was significantly reduced compared to wild-type plants, but was still substantial, supporting the inference that over-expression of *AMT1.2* or *AMT1.3* may have partially compensated for disruption of *AMT1.1*. It is interesting, that our T-DNA mutants had $K_m$ values that were double those of the wild type plants, consistent with a greater involvement of the lower affinity AMT1.2 or AMT1.3 homologues. The (apparent) redundancy observed within families of N transporters may therefore allow for genetic/physiological compensation in the face of genetic lesions that would otherwise incapacitate mutants.

Recently another $NH_4^+$ transporter gene, distantly related to the *AtAMT1* family, namely *AtAMT2.1*, has been characterized in *Arabidopsis* (*Sohlenkamp* et al., 2000). This gene appears to be more highly expressed in shoots than in roots, suggesting a possible role in photorespiratory $NH_4^+$ assimilation. In addition there was a small enhancement in

gene transcript abundance after 3 days of N starvation. Functional analysis of this protein in yeast cells deficient in the three MEP/AMT proteins (strain 31019b) demonstrated a limited ability to rescue growth on 1 mM $NH_4^+$, relatively low transport activity at 100 µM $NH_4^+$ and a failure to accumulate MA at high concentrations (*Sohlenkamp* et al., 2000). These characteristics may indicate that *AtAMT2.1* encodes a low-affinity transporter.

A unique $NH_4^+$ transport mechanism has been identified on the peribacteroid membrane which separates the nitrogen fixing bacteria (bacteroids) from the infected host cell cytosol of soybean root nodules (*Tyerman* et al., 1995; *Mouritzen* and *Rosendahl*, 1997). $NH_4^+$ travels across the peribacteroid membrane (PBM) through an $NH_4^+$ selective cation channel (*Tyerman* et al., 1995), and enters the infected cell cytosol following an [$NH_4^+$] gradient established in part by the rapid (plant) assimilation of $NH_4^+$ (*Udvardi* and *Day*, 1997). To clone the gene encoding the PBM $NH_4^+$ channel, the yeast mutant *mep1 mep2* (strain 26972c) was transformed using a nodule cDNA library and screened for restored growth on low $NH_4^+$. This approach identified a novel nodule-specific gene (*GmSAT1*) which rescued *mep1 mep2* growth on 1 mM $NH_4^+$ (*Kaiser* et al., 1998). *GmSAT1* induced $^{14}$C-MA uptake into *mep1mep2* mutants, and when grown on high concentrations of MA (100 mM) resulted in a toxic phenotype.

## 4 Summary and conclusions

With respect to available N, soils are qualitatively and quantitatively heterogeneous, and plants have evolved discrete transport systems capable of absorbing $NO_3^-$, $NH_4^+$ and amino acids. For each form of N, there are both high- and low-affinity transporters that have been characterized physiologically. The high-affinity transport systems, in particular, are rapidly downregulated and upregulated in response to available levels of exogenous N. They are also regulated diurnally, possibly in response to carbohydrate availability (*Lejay* et al., 1999; *Gazzarini* et al., 1999; *von Wiren* et al., 2000; *Ono* et al., 2000). At the genetic level, it has become apparent that each (physiologically-defined) transporter is encoded by multiple members of the corresponding gene families. Evidence presented above and elsewhere, indicates that transcript abundances for both the $NO_3^-$ and $NH_4^+$ transporters is regulated by gln (see Fig. 4). In addition there are strong indications of direct effects of accumulated $NO_3^-$ and $NH_4^+$ and/or reduced N on transporter activity (*Rawat* et al.,1999; *Vidmar* et al., 2000b; *Frazier* et al., 2000). Acknowledging the unexpectedly large number of genes encoding for N transporters in each category, the challenge of the next decade will be to resolve the individual function(s) of these genes and the manner in which their corresponding transporters are integrated to satisfy whole plant demands. In addition, we are still woefully ignorant concerning the physiology and molecular biology of transport to the vacuole, to the stele and into leaf cells. It is to be anticipated that these latter topics might warrant greater attention in the future.

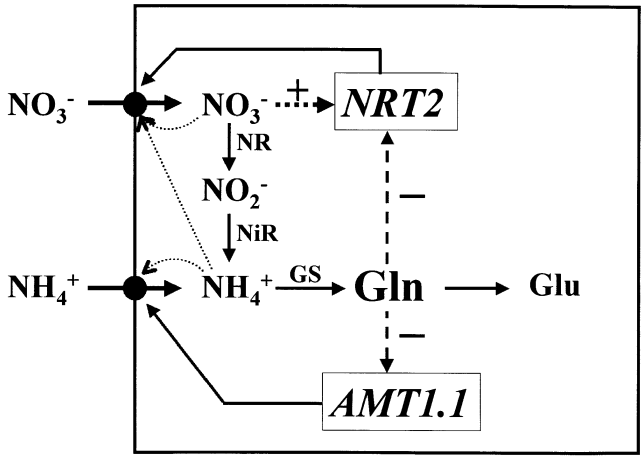


**Figure 4:** A model representing feedback processes involved in the regulation of transcript abundances of *NRT2* and *AMT1* genes by root glutamine concentrations (---) and by direct effects upon the transporters by root $NO_3^-$ and $NH_4^+$ concentrations (.....).
**Abbildung 4:** Modell zu den Feedback-Prozessen in der Regulation der Transkript-Häufigkeiten der *NRT2*- und *AMT1*-Gene durch die Glutamin-Konzentration in der Wurzel (---) sowie durch direkte Effekte der $NO_3^-$- und $NH_4^+$-Konzentration (....) auf die Transporter.

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