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The regulation of ammonium translocation in plants

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Abstract

Much controversy exists about whether or not NH_4^+ is translocated in the xylem from roots to shoots. In this paper it is shown that such translocation can indeed take place, but that interference from other metabolites such as amino acids and amines may give rise to large uncertainties about the magnitude of xylem NH_4^+ concentrations. Elimination of interference requires sample stabilization by, for instance, formic acid or methanol. Subsequent quantification of NH_4^+ should be done by the OPA-fluorometric method at neutral pH with 2-mercaptoethanol as the reducing agent since this method is sensitive and reliable. Colorimetric methods based on the Berthelot reaction should never be used, as they are prone to give erroneous results. Significant concentrations of NH_4^+, exceeding 1 mM, were measured in both xylem sap and leaf apoplastic solution of oilseed rape and tomato plants growing with NO_3^- as the sole N source. When NO_3^- was replaced by NH_4^+, xylem sap NH_4^+ concentrations increased with increasing external concentrations and with time of exposure to NH_4^+. Up to 11% of the translocated N was constituted by NH_4^+. Glutamine synthetase (GS) incorporates NH_4^+ into glutamine, but root GS activity and expression were repressed when high levels of NH_4^+ were supplied. Ammonium concentrations measured in xylem sap sampled just above the stem base were highly correlated with NH_4^+ concentrations in apoplastic solution from the leaves. Young leaves tended to have higher apoplastic NH_4^+ concentrations than older non-senescing leaves. The flux of NH_4^+ (concentration multiplied by transpirational water flow) increased with temperature despite a decline in xylem NH_4^+ concentration. Retrieval of leaf apoplastic NH_4^+ involves both high and low affinity transporters in the plasma membrane of mesophyll cells. Current knowledge about these transporters and their regulation is discussed.

Key words: Ammonium, apoplast, translocation, uptake, xylem.

Introduction

In permanent forest and grassland ecosystems ammonium (NH_4^+) is usually the dominating source of inorganic nitrogen available to plant roots. Also agricultural soils contain some NH_4^+ although the actual concentration at the root surface must be expected to be low due to diffusional limitations. In plants, NH_4^+ is a central intermediate generated in processes such as nitrate reduction, photorespiration, phenyl propanoid metabolism, degradation of transport amides, and protein catabolism (Joy, 1988).

Despite the importance of NH_4^+ in plant nitrogen metabolism it is generally believed that NH_4^+ concentrations in healthy plant tissues always remain low (Howitt and Udvardi, 2000). Evidence against this assumption was recently summarized (Britto *et al*., 2001*a*). Another dogma concerning NH_4^+ is that NH_4^+ absorbed by or generated in roots becomes assimilated there and is not translocated to the shoot (Tobin and Yamaya, 2001). It is the objective of the present paper to show that root-to-shoot NH_4^+ translocation does indeed occur in plants.

One reason for the contrasting opinions on NH_4^+ transport and content in plants could be analytical problems. It has, for example, been claimed that the occurrence of high NH_4^+ concentrations in plant tissues are artefacts produced by the degradation of N metabolites during extraction and analysis or by interference from other metabolites in analytical methods with poor selectivity for NH_4^+ (Oaks, 1994; Kafkafi and Ganmore-Neumann, 1997). Clearly, in order to obtain a true

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picture of NH_4^+ distribution in plants it is a fundamental requirement to obtain stable plant extracts and to use precise, sensitive and selective analytical procedures. The present paper will therefore also focus on methods to analyse NH_4^+ in plant tissues.

Analysis of NH_4^+ in plant tissues

Without stabilization, amino acids and other labile N metabolites in leaf tissue extracts, xylem sap and apoplastic fluid can indeed be degraded to NH_4^+ during extraction and subsequent instrumental analysis even at low temperatures (<4 °C). Thus, during a 10 h period the NH_4^+ concentration in unstabilized xylem sap from oilseed rape increased more than 30% (Fig. 1). A simple and efficient stabilization of the samples by the addition of 10 mM ice-cold HCOOH prevented the NH_4^+ concentration from increasing.

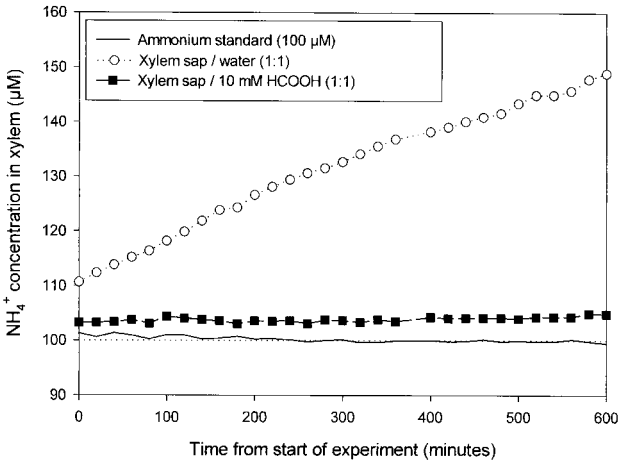

Fig. 1. Stability of the NH_4^+ concentration in xylem sap diluted with water and in xylem sap stabilized with 10 mM formic acid from oilseed rape plants at 4 °C. NH_4^+ was measured three times every hour in a 10 h time series. To correct for a possible drift in the signal from the NH_4^+ measurement an external standard of 100 µM NH_4^+ was analysed simultaneously (solid line). The 100 µM reference line is shown as a broken line (from Husted *et al*., 2000).

Equally important is to use an analytical method for NH_4^+ which is free from interferences from other metabolites. The widely used colorimetric methods based on the Berthelot reaction suffer severely from interference caused by amino acids, amines, amides, and proteins (Table 1). These methods should never be used unless careful sample clean-up has been carried out. However, purification steps based on micro-diffusion, gas dialysis or ion exchange cannot easily be applied to small sample volumes as obtained from extraction of apoplastic fluid and xylem sap. In addition, the first two methods require a high sample pH which creates risk for the degradation of amino acids and amides liberating NH_4^+. Another frequently used method for analysing NH_4^+ together with other ions in plant tissue is ion chromatography. Results obtained by this method may, however, severely overestimate the NH_4^+ concentration due to co-elution of NH_4^+ with amines like methylamine, ethanolamine and the non-protein amino acid γ-amino butyric acid (Table 1).

The most selective and sensitive method, which is also applicable to small sample volumes, is based on derivatization of NH_4^+ with o-phthaldehyde (OPA) at pH 6.8 and with 2-mercaptoethanol as a reductant (Table 1). Ammonium is subsequently quantified by fluorescence spectroscopy. Derivatization and detection can be performed on-line using a column-less HPLC system, enabling rapid quantification of NH_4^+ in a few min. With this method the detection limit for NH_4^+ can be as low as 3.3 µM in a 10 µl sample volume. These qualities make this method suitable for analysing various samples from plant tissues.

Table 1. *Comparison of three different methods for measuring NH_4^+*

(i) The OPA-fluorometric method at neutral pH with 2-mercaptoethanol as reducing agent, (ii) a salicylate-type colorimetric method based on the Berthelot reaction, and (iii) ion chromatography. The three methods were applied on solutions containing 100 µM NH_4^+ together with different amino acids or amines in concentrations of 100 µM (ratio NH_4^+ : metabolite 1 : 1) or 1000 µM (ratio NH_4^+ : metabolite 1 : 10).

Metabolite	Fluorometric method 1 : 10	Colorimetric method		Ion chromatography 1 : 10
	µM NH_4^+	1 : 1	1 : 10	µM NH_4^+
		µM NH_4^+	µM NH_4^+	
Glycine	99	88	0	102
Glutamine	102	90	5	105
Alanine	105	86	0	105
Arginine	101	84	2	94
Methylamine	99	77	0	996
Ethylamine	101	75	0	88
Ethanolamine	100	69	0	994
Putrescine	102	64	0	81
GABA	100	81	0	1002

Ammonium concentrations in xylem sap

Fulfilling all analytical requirements, Husted *et al.* were able to measure significant NH_4^+ concentrations in the xylem sap as well as in apoplast and leaf tissue water of both oilseed rape and tomato plants growing with NO_3^- as the only N-source (Husted *et al.*, 2000) (Fig. 2). Furthermore, the NH_4^+ concentration in the xylem increased with external NO_3^- concentration, particularly in tomato where the concentration increased from 0.2 to 1.4 mM when external concentrations were raised from 1.5 to 6 mM. The increase in xylem NH_4^+ concentration was less dramatic in oilseed rape possibly due to a higher capacity to assimilate NH_4^+ in the root. The high xylem concentration of tomato plants did not, however, cause any toxicity symptoms or growth reduction.

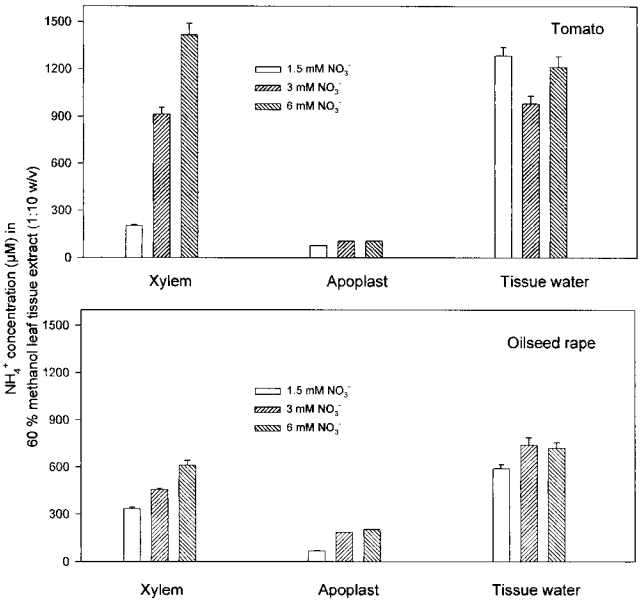

Fig. 2. The NH_4^+ concentration in leaf tissue water, xylem sap and apoplastic fluid analysed by fluorometry at neutral pH with 2-mercaptoethanol as reducing agent. Values are means ± SE; $n = 4$.

Replacement of NO_3^- with NH_4^+ to oilseed rape plants growing in nutrient solution resulted in a linear increase in xylem NH_4^+ concentration both with external concentration and with time of exposure to NH_4^+ (Fig. 3A). Fifty hours after exposure to NH_4^+, xylem NH_4^+ concentrations had increased to 5 mM NH_4^+ in plants receiving 10 mM NH_4^+ in the root medium, while plants deprived of external N for the same period still contained 0.5 mM NH_4^+ in the xylem sap (Fig. 3B). Starvation prior to the addition of 10 mM NH_4^+ did not seem to delay the appearance of NH_4^+ in the xylem sap since, after 12 h, the concentration was 2 mM both with and without prior starvation (Fig. 3A, B). In the plants receiving 10 mM NH_4^+ for 50 h, NH_4^+ constituted 11% of the nitrogen translocated in the xylem and 19% of all cationic charge equivalents (Table 2). With NH_4^+ supplied to the roots,

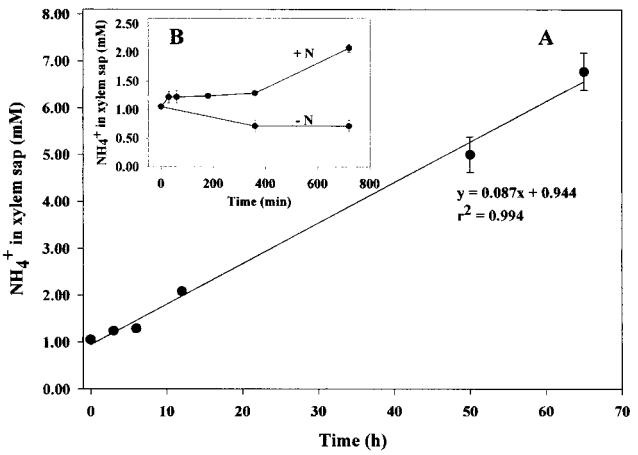

Fig. 3. Time-dependent NH_4^+ content in stem xylem sap of oilseed rape. Plants were grown hydroponically in a complete nutrient solution containing 3 mM NO_3^-. At the start of the experiment, the nitrogen supply was changed as indicated and xylem sap was sampled after 50 h. Stem xylem sap was sampled just above the 2nd oldest leaf of plants exposed up to 65 h to 10 mM NH_4^+ as sole nitrogen supply (A) or N-starved for 72 h followed by 0 or 10 mM NH_4^+ (−N or +N, respectively) in the nutrient solution (B). Values are means ± SE; $n = 4$–6 (from Finnemann and Schjoerring, 1999).

Table 2. *Anion and cation composition of stem xylem sap from oilseed rape*

Plants were grown hydroponically and supplied with a complete nutrient solution containing 3 mM NO_3^-. At the start of the experiment, the nitrogen supply was changed as indicated and stem xylem sap sampled after 50 h. Sampling of stem xylem sap was done just above the 2nd oldest leaf ($n = 4$, mean ± SE).

Ion	Ion concentration in xylem sap (mM)				
	3 mM NO_3^-	10 mM NO_3^-	3 mM NH_4^+	10 mM NH_4^+	−N
NO_3^-	19.81 ± 1.34	23.19 ± 1.46	8.03 ± 0.69	11.44 ± 1.09	9.43 ± 1.51
Cl^-	1.54 ± 0.12	0.70 ± 0.07	6.43 ± 0.70	10.40 ± 0.88	11.95 ± 0.95
$H_2PO_4^-$	3.53 ± 0.08	3.64 ± 0.07	3.52 ± 0.15	4.48 ± 0.12	3.92 ± 0.11
SO_4^{2-}	2.33 ± 0.13	2.24 ± 0.08	3.23 ± 0.26	4.24 ± 0.42	4.13 ± 0.10
NH_4^+	0.70 ± 0.12	0.55 ± 0.03	1.36 ± 0.11	5.00 ± 0.38	0.41 ± 0.02
K^+	16.22 ± 1.44	17.47 ± 0.47	14.18 ± 0.27	13.43 ± 1.19	16.98 ± 0.66
Mg^{2+}	3.79 ± 0.37	4.25 ± 0.13	1.97 ± 0.21	2.02 ± 0.12	3.44 ± 0.22
Ca^{2+}	1.07 ± 0.05	1.29 ± 0.14	1.08 ± 0.05	1.56 ± 0.03	1.59 ± 0.11
Na^+	0.48 ± 0.07	0.92 ± 0.13	0.64 ± 0.06	0.80 ± 0.08	0.76 ± 0.09
NH_4^+-N in % of total-N	2.4	1.8	3.8	10.8	2.5

xylem concentrations of the anions chloride and sulphate increased as a means of obtaining electroneutrality. Previously N-limited barley plants also showed doubled xylem sap NH_4^+ concentrations with 2 mM NH_4^+ compared to 2 mM NO_3^- supplied to the root (Mattsson and Schjoerring, 1996).

Xylem concentrations of NH_4^+ are dependent on the synchronization of NO_3^- or NH_4^+ uptake, NO_3^- reduction and NH_4^+ assimilation. Assimilation of NH_4^+ in the roots is mediated by cytosolic glutamine synthetase (GS1) and glutamate synthase (NADH-GOGAT). The elevated levels of NH_4^+ observed in the xylem upon exposure to NH_4^+ in the root medium may seem surprising, assuming that glutamine synthetase activity is in sufficient excess to incorporate all the NH_4^+ produced in the root into glutamine before translocation to the shoot. However, there exist large differences between plant species in their capacity to assimilate NH_4^+ in the root and the GS activity can also be repressed at high levels of N application. It was shown that after an initial (3 h) small induction both GS isogene expression and activity were repressed upon addition of high levels of NH_4^+ or NO_3^- to the roots of N-replete oilseed rape plants (Finnemann and Schjoerring, 1999). This response may be caused by carbon limitation since a steady supply of photosynthates from the shoot is needed in order to generate sufficient amounts of 2-oxoglutarate needed for GOGAT. Thus, by decreasing GS activity the roots may protect themselves against excessive drain of photosynthates and the NH_4^+ concentration will increase in the xylem.

The above results demonstrate that NH_4^+ may indeed constitute a significant part of the nitrogen translocated from the roots to the shoot in the xylem. The actual amount of NH_4^+ received by the shoot does of course not only depend on the NH_4^+ concentration in the xylem sap but also on the transpirational water flux. In oilseed rape plants growing with NO_3^- as the only N source and exposed to three different air temperatures, namely 15, 20 and 25 °C, the concentration of NH_4^+ in the xylem decreased about 30% (from 1.55 to 1.25 mM) when the temperature was increased from 15 to 25 °C. Nevertheless, due to increased transpiration the corresponding flux of NH_4^+ in the xylem increased about 45% with temperature and reached about 60 nmol NH_4^+ m^{-2} leaf surface s^{-1} at 25 °C (Fig. 4). This increase in NH_4^+ flux was accompanied by an increased total leaf GS activity (not shown).

Dynamics of leaf apoplastic NH_4^+ concentration

Anatomically, the leaf apoplast is more or less an extension of the xylem. It has recently been recognized that the apoplast plays a major role in both water and nutrient transport (Sattelmacher, 2001). The NH_4^+ concentration in the apoplastic solution is therefore important in relation to NH_4^+ translocation. In barley, a strong relationship between xylem NH_4^+ concentration and apoplastic NH_4^+ concentration was observed (Fig. 5). As the NH_4^+ concentration in the root medium was increased to 5 mM both the xylem NH_4^+ concentration and the apoplastic NH_4^+ concentration increased linearly, but at the highest external concentration of 10 mM the apoplastic NH_4^+ concentration levelled off.

Exposure of oilseed rape plants grown at 3 mM NO_3^- to 10 mM NH_4^+ for 50 h resulted in an apoplastic NH_4^+ concentration in the top and bottom leaves of about 0.4 mM and 0.2 mM, respectively, while the corresponding leaves of plants deprived of external N for the same period only contained about 0.1 and 0.05 mM NH_4^+ (Finnemann and Schjoerring, 1999). Thus, in both the NH_4^+-supplied and the N-deprived plants the top leaves

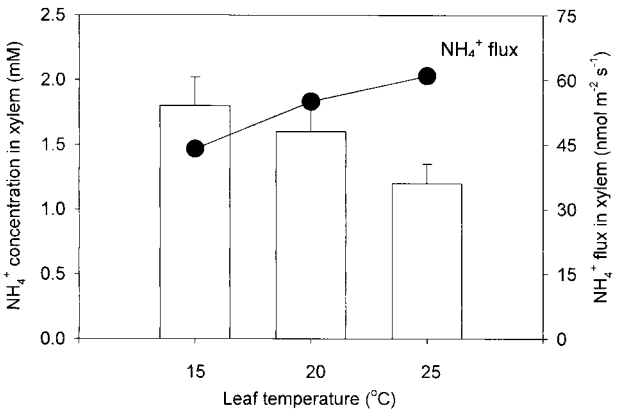

Fig. 4. Effect of temperature (15, 20, 25 °C) on the xylem sap NH_4^+ concentration and the xylem NH_4^+ flux calculated on the basis of NH_4^+ concentrations and the transpiration rate. Plants were adjusted to the actual temperature for 24 h before the experiments were initiated. All experiments were repeated 2–4 times and values are means ± SE.

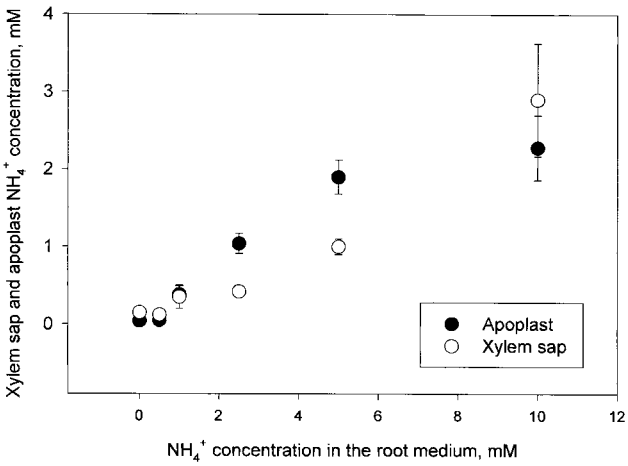

Fig. 5. Xylem sap and apoplast NH_4^+ concentrations in barley leaves in response to increasing NH_4^+ concentrations in the root medium. Values are means ± SE; $n = 3–5$.

had twice as high apoplastic NH_4^+ concentration as the bottom leaves, which is in agreement with a higher transpiration from the top leaves. The NH_4^+ concentration in the leaf tissue water of the top leaves was 0.33 and 1.24 mM in N-deprived and NH_4^+-supplied plants, respectively.

The dynamic changes in apoplastic NH_4^+ concentration in relation to the external N source were further investigated in ryegrass (*Lolium perenne*). A switch of root N-source from NO_3^- to NH_4^+ (3 mM) resulted within 3 h in a 3-fold increase in leaf apoplastic NH_4^+ concentration (Fig. 6A) and a simultaneous decrease in apoplastic pH of about 0.4 pH units (Fig. 6B). The concentration of totally extractable leaf tissue NH_4^+ also doubled within 3 h after the switch. The decrease in apoplastic pH indicates that uptake of NH_4^+ from apoplast to symplast was associated with a net release of H^+ (Hoffmann *et al.*, 1992). Removal of exogenous NH_4^+ caused the apoplastic NH_4^+ concentration to decline back to the original level within 24 h (not shown). This shows that the apoplastic NH_4^+ pool closely reflects changes in the external N supply and

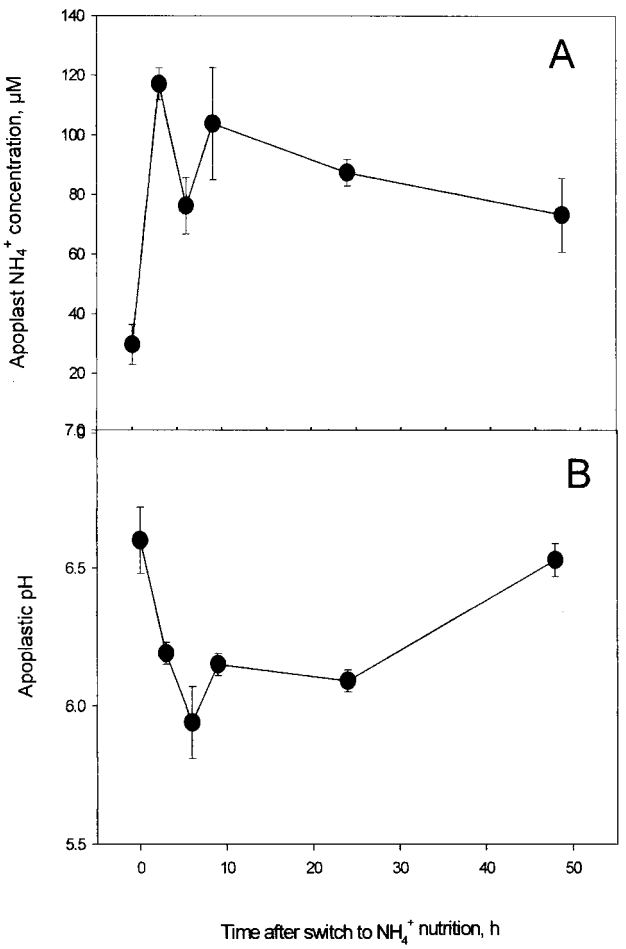

Fig. 6. Apoplastic NH_4^+ concentrations (A) and pH (B) of 11-week-old *Lolium perenne* plants over a 48 h period after a change in N source from 3 mM NO_3^- to 3 mM NH_4^+. Values are means ± SE; $n = 4$.

that the rapid response may constitute a signalling system co-ordinating leaf N metabolism with the actual N uptake by the roots and the external N availability.

Ammonium concentrations in roots and leaves undergo diurnal changes. The diurnal pattern is opposite in these organs: NH_4^+ increases during the daytime in the leaves, but in darkness in the roots, both in NO_3^-- and NH_4^+-grown plants (Kandlbinder *et al.*, 1997). The amplitude of these changes was fairly small in leaves (Matt *et al.*, 2001), but 2-fold in roots (Stöhr and Mäck, 2001) of NO_3^--grown tobacco. A 2–3-fold amplitude was also observed in leaves and roots of NO_3^-- and NH_4^+-grown barley (Kandlbinder *et al.*, 1997).

Molecular basis for ammonium transport

A fundamental requirement for ammonium translocation is the presence of transport systems capable of loading NH_4^+ into the xylem and of subsequently moving NH_4^+ from the leaf apoplastic solution into the leaf cells. Several recent reviews have covered both the physiological and molecular aspects of NH_4^+ transport in roots (Forde and Clarkson, 1999; von Wirén *et al.*, 2000*a*; Glass *et al.*, 2002). However, despite the importance of NH_4^+ as a central intermediate in leaf nitrogen metabolism, kinetic and molecular aspects of NH_4^+ transport in leaf cells have only been investigated to a limited extent.

The first report on ammonium transport was published earlier (Raven and Farquhar, 1981). Methylammonium was used as a transport analogue for ammonium in order to study uptake from a bathing medium into leaf slices. The results obtained showed that methylammonium transport in leaves could not be accounted for by passive diffusion across the plasma membrane, but was mediated by a transport system.

Studies of ^{15}N-NH_4^+ uptake into isolated protoplasts from *B. napus* leaves over a range of concentrations up to 5 mM revealed two distinct kinetic components (Pearson *et al.*, 2002). At concentrations greater than 100 µM, linear kinetics was observed, representing a low-affinity, high-capacity transport system, while the transporter dominant at concentrations below 100 µM followed Michaelis–Menten kinetics. Using leaf discs of oilseed rape infiltrated with istonic sorbitol solutions to which increasing concentrations of NH_4^+ were added, Nielsen and Schjoerring observed that the net uptake of NH_4^+ into leaf cells increased linearly with apoplastic NH_4^+ concentration up to 10 mM and could be partially inhibited by the channel inhibitors La^{3+} and tetraethylammonium (Nielsen and Schjoerring, 1998). Increasing temperature increased the rate of NH_4^+ net uptake and reduced the apoplastic steady-state NH_4^+ concentration. These findings strongly indicate the existence of a low affinity NH_4^+ transporter with channel-like properties in

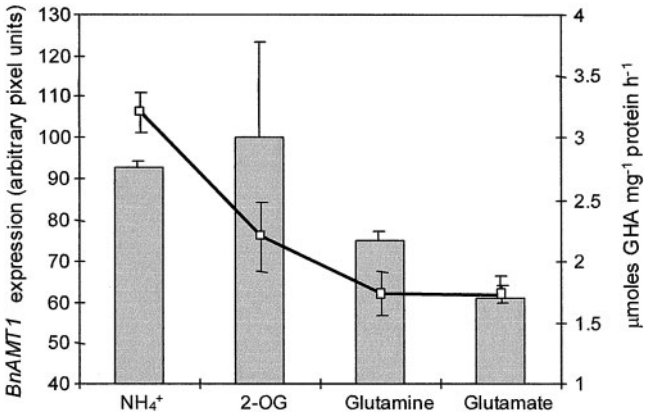

Fig. 7. Relationship between *BnAMT1;2* expression (columns) quantified by RT-PCR and GS2 activity (line). Detached leaves were subjected to 24 h with the following treatment: 1 mM NH_4^+, 1 mM 2-oxoglutarate, 1 mM glutamine or 1 mM glutamate. Primers specific to *Actin1* gene and *BnAMT1;2* were used (from Pearson *et al*., 2002).

the leaf plasma membrane as also observed for root cells (Wang *et al*., 1993). Various reports have suggested that high affinity NH_4^+ transport may occur through a K^+ channel due to the similar chemical characteristics of the two ions. In addition, a broad selection of K^+ channels has been shown to be permeable to NH_4^+ (see Forde and Clarkson, 1999 for review). However, Nielsen and Schjoerring found that K^+ supplied in 10-fold excess to that of NH_4^+ in the apoplastic solution only inhibited NH_4^+ net uptake over a 3 min period by approximately 50% (Nielsen and Schjoerring, 1998). In addition, since the K^+ concentration in leaf apoplastic solution typically seems to be more than 10 times higher than the concentration of NH_4^+, a relatively high affinity for NH_4^+ would be required for efficient NH_4^+ retrieval.

High-affinity transport of NH_4^+ as observed at extracellular NH_4^+ below 100 µM is mediated by the *AMT* gene family (Gazzarrini *et al*., 1999). Two different clones of *AMT*1 have been isolated from oil-seed rape shoot RNA (Pearson *et al*., 2002). BnAMT1;2 was highly expressed in the shoot and was 97% homologous to AMT1;3 from *Arabidopsis* (von Wirén *et al*., 2000*b*). A detached leaf system was used to alter the NH_4^+ concentrations reaching the leaf cells via the xylem and thus to observe differences in the accumulation of mRNA and $^{15}NH_4^+$ transport activity in isolated protoplasts. Gel blot and RT-PCR analysis revealed that *BnAMT1;2* expression was lowest when no or little NH_4^+ was supplied to the leaves for 24 h, but greatly upregulated when NH_4^+ supply exceeded 0.2 mM. Transport of $^{15}NH_4^+$ into protoplasts isolated from the same leaf material closely paralleled mRNA expression. By contrast, long-term exposure of plants to 1.0 mM NH_4^+ resulted in an 80% decrease in mRNA levels or $^{15}NH_4^+$ transport in isolated protoplasts. *BnAMT1;2* expression was related to the activity of the chloroplastic isoform of GS2 (Fig. 7A). Both glutamine and glutamate supply reduced the activity of GS2 and expression of *BnAMT1;2*, while the highest *BnAMT1;2* expression and GS2 activity occurred when the leaves were supplied with either NH_4^+ or 2-oxoglutarate, both of which are primary substrates for amino acid synthesis.

Three different homologues of AMT have been isolated and characterized in tomato leaves (von Wirén *et al*., 2000*a*). The transporters show different expression patterns in response to diurnal rhythm. *LeAMT1;2* and *LeAMT1;3* have a reciprocal diurnal regulation, while *LeAMT1;1* shows constitutive expression. The highest transcript level of *LeAMT1;2* occurs after the onset of light suggesting that there are light-dependent NH_4^+ fluxes in leaves. Such NH_4^+ fluxes can represent the uptake of xylem-derived NH_4^+ via the leaf apoplast or the retrieval of photorespiratory NH_3/NH_4^+ escaping from the cytosol (von Wirén *et al*., 2000*a*). *LeAMT1;3* is mainly expressed during darkness, indicating requirements for transport of NH_4^+ produced by transamination of, for example, asparagine by the dark induced asparagine synthetase (Lam *et al*., 1995) or deamination of glutamate by glutamate dehydrogenase, which is also upregulated during darkness (Melo-Oliveira *et al*., 1996). The constitutive expression of *LeAMT1;1* indicates a household function in leaves (von Wirén *et al*., 2000*a*).

Dealing with net fluxes of ammonium, the passive flux of NH_3 across membranes has to be considered. Biological membranes are expected to be permeable to the uncharged ammonia molecule (Bertl *et al*., 1984; Kleiner, 1981; Raven, 1988; Roberts and Pang, 1992), which makes transport of NH_3 across membranes independent of transport proteins. The direction of flux is mainly determined by the concentration gradient of dissolved NH_3, which depends on ΔNH_4^+ and ΔpH across the membrane. For every one pH unit increase, there is a corresponding 10-fold increase in the NH_3/NH_4^+ ratio. This implies that any acidic compartment can act as an acid trap for NH_3. Although few indications exist that NH_3/NH_4^+ efflux might occur via a transporter (Britto *et al*., 2001*b*; Kronzucker *et al*., 1999), efflux of ammonium is also expected to occur by diffusion of the uncharged NH_3 molecule along the outward directed NH_3 concentration gradient sustained by the pH gradient across the plasma membrane. At the plasma membrane in leaves of oilseed rape plants, a significant and apparently futile NH_3 efflux has been detected (Nielsen and Schjoerring, 1998). This efflux was followed by NH_4^+ retrieval, leading to ammonium recirculation across the plasma membrane ranging up to 35 µmol g^{-1} h^{-1} or approximately 30 times higher than net ammonium uptake (Nielsen and Schjoerring, 1998). Even though the plasma membrane represents the only plant membranes where NH_3 diffusion has been detected (Britto *et al*., 2001*b*; Nielsen and Schjoerring, 1998), indications

of NH_3 diffusion exist for other membranes in the plant cell. By pH-sensitive fluorescence or NMR, Roberts and Pang and Yin *et al.* observed that vacuolar pH increased upon ammonium treatments of maize roots and leaves (Roberts and Pang, 1992; Yin *et al.*, 1996). It was claimed that this was consistent with NH_3 diffusion and could not be accounted for by transport of NH_4^+. In agreement with these findings, Husted and Schjoerring found pH increases in the apoplastic compartment upon treatment with methionine sulfoximine (an inhibitor of GS) strongly suggesting NH_3 diffusion out of the cell (Husted and Schjoerring, 1995). Another potential candidate for significant NH_3 diffusion would be the mitochondrion where a high NH_3 export can be expected upon photorespiration. As far as is known, no studies have been carried out on this aspect. However, since pH in the mitochondrion is higher than the cytosol pH ($\Delta pH \approx 1$) a diffusion of NH_3 out of the mitochondrion into the cytoplasm is to be expected.

Future prospects

Detailed understanding of the processes controlling NH_4^+ transport in plants is essential for future attempts to modify plant metabolism, so that NH_4^+, generated in massive quantities during nitrate reduction, photorespiration and senescence is rapidly and efficiently assimilated without unnecessary energy consumption associated with intracellular transport and pH regulation. Much information is already available, but there are important gaps related to the molecular basis of low-affinity, high-capacity NH_4^+ transport across the plasma membrane and the envelope membranes of plastids. The latter ties in with the general lack of data on NH_4^+ concentrations in different cell compartments. That considerable potential for improvement of plant nitrogen utilization exists is shown by recent data with wheat and oilseed rape plants overexpressing cytosolic GS (Habash, 2001; Schjoerring *et al.*, 2001).

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