



Effects of different nitrogen forms and combination with foliar spraying with 6-benzylaminopurine on growth, transpiration, and water and potassium uptake and flow in tobacco

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Abstract

$\text{NH}_4^+\text{-N}$ can have inhibitory effects on plant growth. However, the mechanisms of these inhibitory effects are still poorly understood. In this study, effects of different N forms and a combination of ammonium + 6-benzylaminopurine (6-BA, a synthetic cytokinin) on growth, transpiration, uptake and flow of water and potassium in 88-days-old tobacco (*Nicotiana tabacum* L. K 326) plants were studied over a period of 12 days. Plants were supplied with equal amounts of N in different forms: NO_3^- , NH_4NO_3 , NH_4^+ or NH_4^+ +6-BA (foliar spraying every 2 days after onset of the treatments). For determining flows and partitioning upper, middle and lower strata of three leaves each were analysed. During the 12 days study period, 50% replacement of $\text{NO}_3^-\text{-N}$ by $\text{NH}_4^+\text{-N}$ (NH_4NO_3) did not change growth, transpiration, uptake and flow of water and K^+ compared with the $\text{NO}_3^-\text{-N}$ treatment. However, $\text{NH}_4^+\text{-N}$ as the sole N-source caused: (i) a substantial decrease in dry weight gain to 42% and 46% of the $\text{NO}_3^-\text{-N}$ and NH_4NO_3 treatments, respectively; (ii) a marked reduction in transpiration rate, due to reduced stomatal conductance, illustrated by more negative leaf carbon-isotope discrimination ($\delta^{13}\text{C}$) compared with the NO_3^- treatment, especially in upper leaves; (iii) a strong reduction both in total water uptake, and in the rate of water uptake by roots, likely due to a decrease in root hydraulic conductivity; (iv) a marked reduction of K^+ uptake to 10%. Under NH_4^+ nutrition the middle leaves accumulated 143%, and together with upper leaves 206% and the stem 227% of the K^+ currently taken up, indicating massive mobilisation of K^+ from lower leaves and even the roots. Phloem retranslocation of K^+ from the shoot and cycling through the root contributed 67% to the xylem transport of K^+ , and this was 2.2 times more than concurrent uptake. Foliar 6-BA application could not suppress or reverse the inhibitory effects on growth, transpiration, uptake and flow of water and ions (K^+) caused by $\text{NH}_4^+\text{-N}$ treatment, although positive effects by 6-BA application were observed, even when 6-BA (10^{-8}M) was supplied in nutrient solution daily with watering. Possible roles of cytokinin to regulate growth and development of $\text{NH}_4^+\text{-fed}$ plants are discussed.

Introduction

Many reports have demonstrated that $\text{NH}_4^+\text{-N}$ has inhibitory effects on plant growth, which is attributed to various factors. Water deficit due to a reduction in water uptake (Quebedeaux and Ozbun, 1973) has been reported as one of the growth-limiting factors

for tomato grown under $\text{NH}_4^+\text{-N}$. However, there are different results in this respect. It has been reported that $\text{NH}_4^+\text{-N}$ increases transpiration rate of alfalfa and tomato (Khan et al., 1994; Lugert et al., 2001) and stomatal conductance of white clover (Høgh-Jensen and Schjoerring, 1997). Other reports show that $\text{NH}_4^+\text{-N}$ results in a reduction in water uptake of muskmelon and sugar beets (Adler et al., 1996; Raab and Terry, 1994) and slightly reduced leaf conductance in castor

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bean (Peuke et al., 1994). Walch-Liu et al. (2000) did not find a reduction in osmotic potential, apparent hydraulic conductivity of roots and the rate of water uptake by NH_4^+ treatment in tobacco plants. Abscissic acid plays an important role when plants are subjected to drought (Hartung and Davies, 1991) and salt stress (Wolf et al., 1990) through its effects on stomatal conductance. Since a threefold increase in the xylem ABA flow and a twofold increase in the phloem ABA flow of NH_4^+ -fed plants were observed compared with NO_3^- -supplied castor bean, ABA is thought to be an important root-to-shoot signal in the regulation of growth and development of NH_4^+ -fed plants (Peuke et al., 1994).

The occurrence of cytokinins in plants is until now not to be well established (Emery and Atkins, 2002). They can be synthesised not only in roots, but also in aerial parts of a plant (Letham, 1994). It has been reported recently that the reproductive organs of *Lupinus albus* L. cv Kiev mutant is not only a major site of CK synthesis but also a potential source of these substances for the plant (Emery et al., 2000). In the studies on the effects of nitrogen availability and forms on cytokinins relationships and shoot growth and development, it was shown that nitrogen deficiency in growing medium of *Urtica dioica* L. and *Lupinus angustifolius* L. plants could influence xylem delivery of CK and thus shoot growth (Ma et al., 1998; Wagner and Beck, 1993). The replacement of NO_3^- by NH_4^+ as sole nitrogen caused rapid decrease in the concentration of zeatin/zeatin riboside in xylem sap and tissues of tobacco plants (Walch-Liu et al., 2000). Twelve hours after re-application of NO_3^- to NH_4^+ -fed tomato plants, enhanced zeatin/zeatin riboside levels in xylem sap and leaf tissues were observed; these changes coincided with an increase in leaf expansion rate (Rahayu et al., 2001). In spite of these and other results in literature, however, it is questioned recently about role of CK in signaling a root response to different stresses to the shoot. There is no direct connection has been made between xylem-mobile CK and responses in the shoot (detail, see Emery and Atkins, 2002).

Potassium (K) is a macro-element in cells of higher plants and shares with N the property of high phloem mobility. The partitioning and the amount of phloem retranslocation of K^+ from shoot and cycling through the root are quite different in different plants and can be changed, when plants grow under saline conditions (Jeschke et al., 1987; Jeschke and Pate, 1991a), are infected by a parasitic angiosperm (Hibberd et al.,

1999), or decapitated (Jiang et al., 2001). But whether and to what extent uptake, translocation and circulation of K between different plants organs are altered by the supply of different N-forms, is unknown.

If the inhibitory effect of NH_4^+ on plant growth is due to the reduced zeatin/zeatin riboside synthesis in roots and transport to the aerial parts of plants, then exogenous foliar supply of cytokinins might be expected to cause some reversal of NH_4^+ -induced reduction of shoot growth. Therefore, the present experiments were conducted to study the effects of different N forms on growth, transpiration, water and K^+ uptake and flow in tobacco plants, and the possible function of cytokinins to retard or reverse the inhibitory effects on plants caused by NH_4^+ -N supply.

Materials and methods

Plant growth

Seeds of tobacco (*Nicotiana tabacum* L. K 326) were germinated in a mixture consisting of 60% (w/w) peat culture substrate, 20% (w/w) ground maize stalk and 20% (w/w) perlite, and grown in a naturally illuminated glasshouse for 63 days. Afterwards, they were washed with tap water until all substrates were removed from roots, and then transferred into 2.1 L pots (one plant per pot) containing quartz sand (0.25–0.5 mm in diameter). The plants were watered daily, initially with a half strength Hoagland nutrient solution containing (in mM for full strength): 1 KH_2PO_4 , 5 KNO_3 , 5 $\text{Ca}(\text{NO}_3)_2$, 2 MgSO_4 , 4.6×10^{-2} H_3BO_3 , 7.65×10^{-4} $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 3.2×10^{-4} $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 1.6×10^{-5} $(\text{NH}_4)_6\text{MoO}_{24}$, 9×10^{-3} $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 3.7×10^{-2} FeEDTA . After 1 week, full-strength solution was provided. The pots were watered every morning with an excess of the nutrient solution, and the draining solution was removed. Plants were grown in a growth room with a 14-h photoperiod. The photosynthetically active radiation at the surface of the pots was 220–270 $\mu\text{mol m}^{-2}\text{s}^{-1}$ provided by reflector sunlight dysprosium lamps (DDF 400, Nanjing, China). The first harvest was made 88 days after sowing and 25 days after transferring to the constant environmental conditions; the second harvest was performed 12 days later.

Table 1. Macronutrient composition of the solutions with nitrate, ammonium nitrate or ammonium as nitrogen source (in mM)

Composition	Treatments			
	NO ₃ ⁻	NH ₄ NO ₃	NH ₄ ⁺	NH ₄ ⁺ + 6-BA
KH ₂ PO ₄	1	1	1	1
KNO ₃	5			
NH ₄ NO ₃		7.5		
(NH ₄) ₂ SO ₄			7.5	7.5
Ca(NO ₃) ₂	5			
CaCl ₂		5	5	5
KCl		5	5	5
MgSO ₄	2	2	2	2

In addition, the media contained the following micronutrients (mM): 4.6×10^{-2} H₃BO₃; 7.65×10^{-4} ZnSO₄·7H₂O; 3.2×10^{-4} CuSO₄; 1.6×10^{-5} (NH₄)₆MoO₂₄; 9×10^{-3} MnCl₂·4H₂O; 3.7×10^{-2} Fe-EDTA.

Treatment and harvest procedures

For the harvests, plants were separated into five groups of five plants each with similar size and development. One group was used for the first harvest, the remaining four groups for the second harvest. Starting at the day of the first harvest, the latter four groups of plants were treated with different N forms, as shown in Table 1. For the treatment of NH₄⁺+6-BA, 10^{-8} M of 6-benzylaminopurine (6-BA, a synthetic cytokinin, with 0.1% Tween 20) was sprayed to all of the leaves of the tobacco plant every 2 days after the beginning of the treatments. A plastic sheet was placed around the stem base, to avoid 6-BA solution drops falling onto the quartz sand.

Leaves were numbered in ascending order, starting with the lowest mature leaf, which was designated as leaf 1. Smaller leaves, which had already senesced, were removed. At the first harvest leaf 3 was the youngest unfolded leaf, and the second harvest, this was leaf 9. At harvest, plants were separated into roots, stem, top (apex and enclosed leaves) and leaves. At the first harvest, the three unfolded leaves were combined as one sample; at the second harvest, six additional leaves had been formed, and the leaves were harvested as the lower, middle, and upper stratum of three leaves each. Roots were washed free of sand with water. All plant parts were weighed (fresh weight), dried (70 °C) and weighed again (dry weight). Ions in different organs were analysed using ICP (Perkin Elmer 3300 DV, USA).

Measurement of transpiration

Whole shoot transpiration was measured on a daily basis by weighing five pots of each of the treatments at the beginning and end of the light period and after the daily addition of nutrient solution and draining. Corrections were applied for the water loss from pots without plants. The partitioning of transpiration between various plant parts was determined gravimetrically at harvest. This was done by first measuring the water loss of a whole potted plant and then that of its separate, excised organs by a series of consecutive weighing over a 6-min period immediately following detachment of each organ. The procedure was the same as described by Jeschke and Pate (1991b) and this gravimetric measurement was in good agreement with the values of leaf transpiration using a portable open gas exchange system.

Collection of xylem sap

For collection of xylem sap, plants were grown in special pots (Seel and Jeschke, 1999) for the application of pressure to the root system, but otherwise treated in the same way as the plants for the harvests. The procedure for collection of xylem sap was the same as described by Jeschke and Pate (1991a). In brief, xylem sap was collected by pressurising the moist quartz sand substrate and the root system contained in a pressure vessel. At about midway along the length of leaves an incision was made into the midrib of leaves number 2, 5 and 8 from the base. The cut surface was carefully washed and a Teflon tube attached. After slowly applying pressure, xylem sap started to exude from the midribs after reaching a balancing pressure (Seel and Jeschke, 1999), and sap was collected 50 kPa above this pressure. The first exudate was discarded to avoid contamination from cut cells. Xylem sap was also collected from the stem base of the pressurised plants either from an incision into the stem (Hibberd et al., 1999) or by inserting a syringe needle into the stem until it reached the xylem vessels. Xylem sap was kept on ice during collection and stored at -20 °C before analyses. Ions in the sap were analysed directly after appropriate dilution using ICP (Perkin Elmer 3300 DV, USA).

Measurement of carbon isotope discrimination ($\delta^{13}C$)

Leaves were dried in an oven at 70 °C and then ground into fine powder. The samples were then treated and

analysed for carbon-isotope composition using a mass spectrometer (Delta S, produced by Finnigan MAT, Germany) according to Farquhar et al. (1989). The ratios of $^{13}\text{C}/^{12}\text{C}$ of the samples were expressed as $\delta^{13}\text{C}$ relative to the isotope composition of the fossil belemnite standard from the Pee Dee Formation (PDB).

Estimation of the net flows of water and K^+ through xylem and phloem in the whole plant

Net flows of water and K^+ in plants were estimated using the method described by Hibberd et al. (1999). Based on water loss by the plants and the gains in tissue water the total water uptake was calculated. Using the partitioning of transpiration and the individual gains in water, the xylem water intake into each organ was obtained and these data were then used to construct the net water flows into each of the organs studied during the experimental period (see Figure 3).

Based on the assumption that mass flow occurred in the xylem, the net xylem flows of K^+ , $J_{\text{K},\text{x}}$, towards each organ was calculated from the net water flows, $J_{\text{H}_2\text{O},\text{x}}$, and the measured concentration of K^+ , $[\text{K}^+]_{\text{x}}$, in the xylem sap:

$$J_{\text{K},\text{x}} = J_{\text{H}_2\text{O},\text{x}} \times [\text{K}^+]_{\text{x}} \quad (1)$$

The net flow of K^+ in the phloem, $J_{\text{K},\text{p}}$ was then calculated from the difference between the measured K^+ increment ΔK in each organ and the net xylem import.

$$J_{\text{K},\text{p}} = \Delta K - J_{\text{K},\text{x}} \quad (2)$$

A positive difference indicated net phloem import, while a negative difference implied net phloem export from an organ. Working progressively along the plant from the root to the leaves and top, the net flows of K^+ within the whole plant as shown in Figure 4 were obtained; the differences between the quantities of K^+ translocated in the phloem and in the xylem then also allowed to estimate transfer processes between these translocation streams.

Statistical treatment

Dry weight increments were obtained from five replicates of each of the treatments at the first and the second harvest. All further analyses were made with five individual samples for each organ. Where appropriate, data are presented using LSD test.

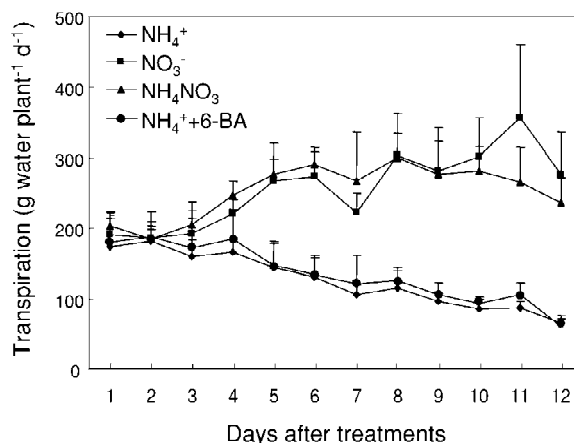


Figure 1. Time-course of transpiration of whole shoots of tobacco plants as dependent on N-form treatments for 12 days. The bars denote standard errors of the mean, $n = 5$.

Results

Plant growth

The net increase in dry weight of both of NO_3^- - and NH_4NO_3 -fed plants was greater than that of NH_4^+ and NH_4^+ +6-BA treated plants (Table 2). The difference in net dry weight gain was smaller for older leaves, since these leaves had essentially completed growth by the first harvest. In the NO_3^- - and NH_4NO_3 -fed plants, the upper and middle leaves were the largest sinks for assimilate deposition at this stage of development, while in the NH_4^+ - or NH_4^+ +6-BA-treated plants the middle leaves instead of upper leaves constituted the largest sink, suggesting a retardation on upper leaf growth in these plants. A negative net increase in dry weight of the top (Table 2) indicated that the dry weight of the apex at the second harvest was smaller than that at the first harvest. Foliar spraying with 6-BA to the NH_4^+ -fed plants stimulated growth, and the total net dry weight increased by 15%. However, this stimulatory effect was only observed in the shoots of the plants.

Total water transpiration and leaf $\delta^{13}\text{C}$

There was a steady increase in transpiration in the NO_3^- - and NH_4NO_3 -fed plants because of the expansion of the total leaf area, while that in NH_4^+ -fed plants decreased soon after the second day, a tendency that despite some leaf expansion continued until the end of the experiment (Figure 1). At the 6th day after the beginning of the treatments the transpiration of

Table 2. Initial values and net changes in dry weights of different organs and of whole tobacco plants over a 12 day study period. Within treatments and for each organ, values in row followed by a different letter are significantly different (LSD Test, $P < 0.05$)

Position	Initial DW (g DW per organ)	Net increase in DW (g DW per organ)			
		NO_3^-	NH_4NO_3	NH_4^+	$\text{NH}_4^+ + 6\text{-BA}$
Top	0.75	0.76 a	0.89 a	-0.44 b	-0.29 b
Upper leaves		5.21 a	4.30 a	1.47 b	1.84 b
Middle leaves		4.62 a	4.44 a	2.99 b	3.32 b
Lower leaves	2.10	0.58 a	0.29 a	0.22 a	0.25 a
Stem	0.52	2.88 a	3.07 a	1.64b	1.75 b
Roots	0.81	1.54 a	1.43 a	0.71 b	0.73 b
Whole plant	4.18	15.59 a	14.42 a	6.59 b	7.60 b

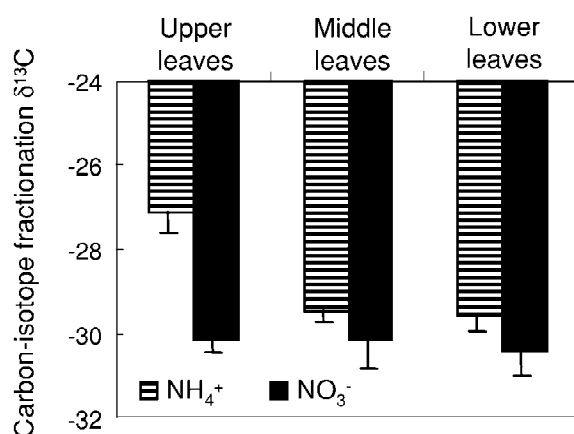


Figure 2. Carbon-isotope fractionation ($\delta^{13}\text{C}$) of different leaves of NH_4^+ - and NO_3^- -fed plants after a 12 day study period. The bars denote standard errors of the mean, $n = 5$.

the NO_3^- -fed plants was twice that of the NH_4^+ -fed plants, and 4.2 times greater at day 12. The application of 6-BA to the NH_4^+ -fed plants promoted transpiration slightly, but the effect was not significant (Figure 1).

At the end of the experiment, in the leaves of the NH_4^+ -treated plants the value $\delta^{13}\text{C}$ was significantly more negative than in the NO_3^- treatment, especially in the upper leaves (Figure 2).

Estimation of transpiration and net water flows within whole plants

The total transpiration (Figure 1) and water uptake (Figure 3) of the NO_3^- - and the NH_4NO_3 -fed plants were much greater than those in both NH_4^+ treatments. The greatest water loss was found for the upper and middle leaves of the NO_3^- - (36% and 31% of

the total uptake, respectively) and NH_4NO_3 -fed plants (33% and 28% of the total uptake, respectively); however, in NH_4^+ - and $\text{NH}_4^+ + 6\text{-BA}$ -treated plants the middle leaves accounted for 38% and 37% of the total uptake, respectively. Of the total amount of water taken up by roots, 14%, 21%, 24% and 29%, was transpired by lower leaves of the NO_3^- -, NH_4NO_3 -, NH_4^+ - and $\text{NH}_4^+ + 6\text{-BA}$ -treated plants, respectively (calculated according to the values in Figure 3), although their net dry weight gains were very low (Table 2).

Contents and changes in K^+

The changes in net K^+ uptake as affected by different N forms were similar but clearly more dramatic than those in net dry weight gain, e.g., net uptake of K^+ in NH_4^+ -fed plants was markedly smaller than in the NO_3^- - or NH_4NO_3 -fed plants, and 6-BA stimulated total K^+ uptake (Table 3). The greatest increments of K^+ were found in the rapidly growing organs, i.e. upper and middle leaves. Substantial increments were also found in the stem and roots of the NO_3^- - and NH_4NO_3 -fed plants. However, a net export of K^+ was found from roots of the NH_4^+ - and $\text{NH}_4^+ + 6\text{-BA}$ -treated plants, and also from the lower leaves of all plants. This loss in K^+ was significantly greater in NH_4^+ -fed plants than in the NO_3^- -fed plants. The K^+ contents in the top of the NH_4^+ - and $\text{NH}_4^+ + 6\text{-BA}$ -treated plants also showed negative values, but this was due to the lower dry weights of the tops (Table 2), and not due to net export from the tops.

Estimation of net flows of K in whole plants

The upper and middle leaves of the NO_3^- -treated

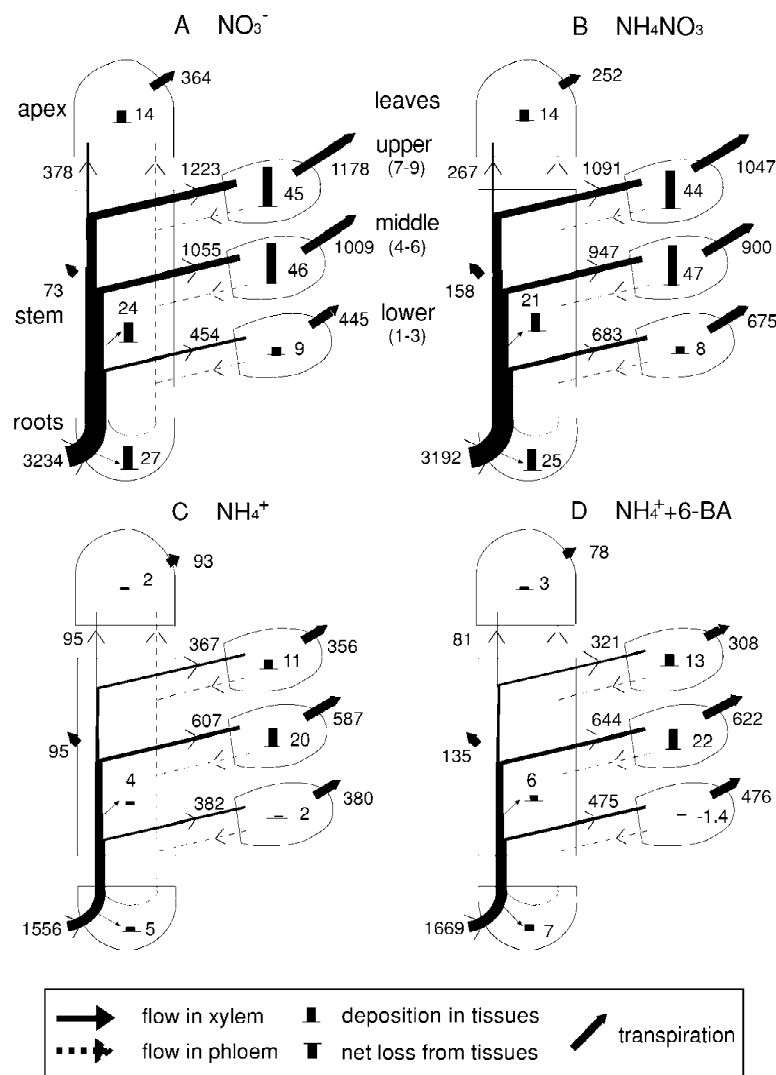


Figure 3. Flow profiles for uptake, transport, utilisation and transpirational loss of H_2O in tobacco plants over a 12 days experimental period, starting 88 days after sowing. The width of the arrows (flow rates), the height of vertical histograms (H_2O incorporation) and the length of oblique histograms with arrows (transpiration) are drawn in proportion to the absolute values. The numbers indicate the values of uptake, transport, utilisation and transpiration [$\text{g H}_2\text{O}$ per plants over the 12 day study period].

plants were main sinks for K^+ , 47% and 48% of the total K^+ taken up was translocated to, and 37% and 35% deposited in these leaves, respectively (Figure 4A). Other major sinks were the stem, the roots and the top, which acquired 18%, 9% and 3% of the total K^+ taken up, respectively (calculated according to the values in Figure 4A). 22%, 27% and 103% of the imported K^+ was exported through phloem from the upper, middle and lower leaves, respectively. Phloem retranslocation of K^+ from shoot to root contributed 31% to the xylem transport of K^+ (Figure

4A). In NH_4NO_3 -fed plants, K^+ distribution and flows showed similar patterns as in the NO_3^- controls, but all K^+ flows were clearly somewhat smaller (Figure 4B).

In the NH_4^+ -fed plants, however, the total K^+ uptake was reduced to 1.8 mmol (only 11% of the NO_3^- -fed or 13% of the NH_4NO_3 -fed plants). About 63%, 142% and 21% of the total K^+ taken up was deposited in the upper and middle leaves and in the stem, respectively. Since the sum of these depositions exceeds the total uptake, massive mobilisation of K^+ from lower leaves and even from roots must have oc-

Table 3. Initial values and net changes in K^+ content in different organs and in whole tobacco plants over a 12 day experimental period. Within treatments and for each organ, values in row followed by a different letter are significantly different (LSD Test, $P < 0.05$)

Position	Initial values (mmol per plant)	Net K^+ change (mmol per plant)			
		NO_3^-	NH_4NO_3	NH_4^+	$NH_4^+ + 6\text{-BA}$
Top	1.05	0.43 a	0.64 a	-0.82 b	-0.64 b
Upper leaves		5.78 a	5.09 a	1.12 b	1.42 b
Middle leaves		5.46 a	5.52 a	2.52 b	3.04 b
Lower leaves	3.28	-0.13 a	-0.27 ab	-1.25 b	-0.80 ab
Stem	0.57	2.82 a	2.37 b	0.37 c	0.41 c
Roots	0.63	1.37 a	0.66 b	-0.17 c	-0.11 c
Whole plant	5.53	15.73 a	14.01 b	1.77 c	3.32 c

curred. In the lower leaves, the amount of K^+ exported via the phloem was more than twice that imported via xylem. Phloem retranslocation of K^+ from shoot to root contributed 67% to the xylem transport, 2.2 fold more than in the NO_3^- control (Figure 4C).

Foliar spraying of 6-BA of the NH_4^+ -fed plants enhanced K^+ uptake (by 88%) and xylem transport (by 26%), and stimulated net deposition of K^+ in the upper and middle leaves and in the stem. Phloem retranslocation of K^+ from shoot to root amounted to 53% of the xylem transport of K^+ , while K^+ mobilisation from the lower leaves and roots was reduced (Figure 4D).

Discussion

Effect on plant growth

Growth inhibition in NH_4^+ -fed plants was observed in many plants, such as castor bean, sugar beets, and tobacco (Peuke and Jeschke, 1993; Raab and Terry, 1994; Walch-Liu et al., 2000). In the present study, the middle leaves, top, stem and roots of the NO_3^- - and NH_4NO_3 -fed plants were main sinks for both assimilates and nutrients. In comparison with sole NO_3^- -N supply, 50% replacement of NO_3^- -N by NH_4^+ -N (NH_4NO_3) had little effect on plant growth and development, although there was a small reduction in dry weight gain (Table 2), transpiration (Figure 1), and uptake of water and K^+ (Figures 3 and 4). However, when NH_4^+ -N replaced NO_3^- -N as N-source, plant growth and development were strongly inhibited. Inhibition of shoot growth was stronger than that of roots, resulting in a relative decrease in shoot/total mass DW ratio from 0.88 (NO_3^-) or 0.88 (NH_4NO_3)

to 0.86 (NH_4^+). This shift of biomass partitioning in favour of roots implied enhanced phloem transport of assimilates to roots under ammonium nutrition (Peuke and Jeschke, 1993).

Transpiration, uptake, partitioning and flow of water in plants

NO_3^- -N and NH_4NO_3 had similar effects on transpiration, uptake and partitioning of water in plants as on plant growth. However, the results indicated marked reduction in transpiration, water uptake and flows in plants receiving NH_4^+ as the sole source of N (Figures 1 and 3), although all of the treated plants were watered daily and showed no signs of water stress. The present results do not accord with the observation that NH_4^+ -N increased leaf transpiration of alfalfa (Khan et al., 1994) and tomato (Lugert et al., 2001) and stomatal conductance of white clover (Høgh-Jensen and Schjoerring, 1997). The strongly reduced transpiration in the present study could not be explained simply by the reduced leaf areas, since, at the second harvest, the NH_4^+ -fed shoot increased its dry weight by 5.88 g, while daily transpiration was 63% reduced compared with the first harvest (Figure 1). Moreover, results on carbon-isotope discrimination of different leaves of the NO_3^- - and NH_4^+ -supplied plants indicated a reduced stomatal conductance, especially in the upper leaves (Figure 2). ABA was probably one of the regulators responsible for the reduced stomatal conductance in leaves of the NH_4^+ -fed plants, since increased biosynthesis of ABA and a marked increase in the xylem and phloem ABA flow of NH_4^+ -fed plants have been observed compared with NO_3^- -supplied plants in *Ricinus communis* (Jeschke and Hartung, 2000; Peuke et al., 1994). It is

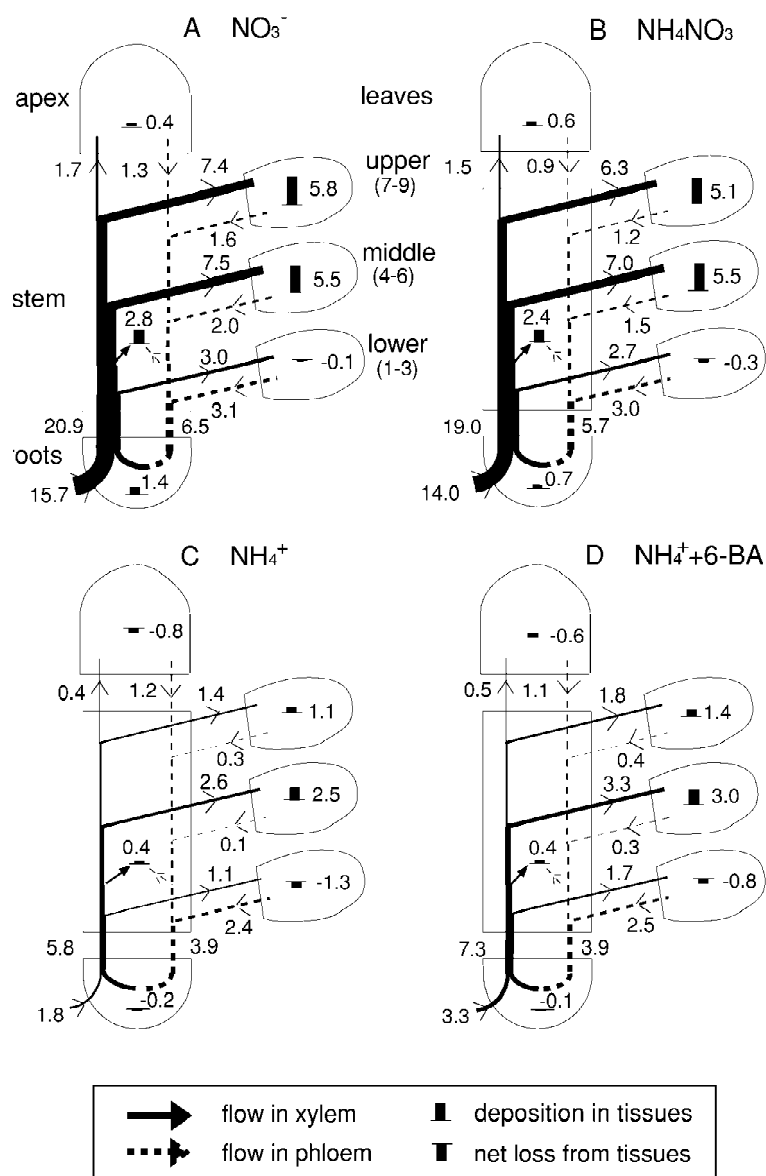


Figure 4. Flow profiles for uptake, transport and utilisation of K^+ in tobacco plants over the 12 days experimental period, starting 88 days after sowing. The values of K^+ deposition and the statistical significance are given in Table 3. The width of arrows and the height of histograms are drawn in proportion to the net flows and deposition of K^+ . The numbers indicate the values of uptake, transport and utilisation [mmol K^+ per plant over the 12 day study period].

noteworthy that increased concentration of ABA and decreased concentration of endogenous cytokinins in the xylem of rice plants occurred simultaneously after root drying (Bano et al., 1993). Cytokinins act as important regulators and have an opposing effect to ABA on stomatal movement (Mansfield and McAinsh, 1995). When cytokinins are supplied together with ABA, they can overcome the strong inhibitory effect

of ABA on stomatal closure of maize and *Commelina* (Blackman and Davies, 1983). It has been reported recently that NH_4^+ supply caused a rapid decrease in endogenous zeatin/zeatin riboside concentration in tissues of tobacco plants (Walch-Liu et al., 2000). Reduced cytokinin synthesis might therefore be another important factor for closure of stomata of the NH_4^+ -fed plants. However, contrary to expectation, foliar

Table 4. The recorded balancing pressures for collecting xylem saps from leaves of different treated plants

	Treatments			
	NO ₃ ⁻	NH ₄ NO ₃	NH ₄ ⁺	NH ₄ ⁺ +6-BA
Balancing pressure (MPa)	0.46	0.57	0.72	0.70

6-BA sprayed onto the NH₄⁺-fed plants, had only a small positive effect on transpiration (Figure 1), water uptake and partitioning (Figure 3).

NH₄⁺-N supply reduced the total water uptake (Figures 1 and 3) as well as uptake rate of the roots. This effect was not due to the reduced root growth, since at the 12th day after onset of the treatments, for example, the water uptake rate on root dry weight basis for NO₃⁻- and NH₄⁺-fed plants was 115 and 86 ml d⁻¹ g⁻¹ DW, respectively. The reduced water uptake rate might be a result of reduced root hydraulic conductivity (Adler et al., 1996; Wilcox et al., 1977). N deficiency markedly decreased hydraulic conductance by reducing the activity or the abundance of Hg-sensitive water channels (Carvajal et al., 1996), which can lower the steady-state water potential of leaves so leaves were unable to maintain adequate turgor for growth (Radin and Boyer, 1982). In the present experiment, hydrostatic pressure was applied to the roots in sand to raise the sand water potential so that xylem sap exuded from small incisions in the leaves (Seel and Jeschke, 1999). The recorded balancing pressures for collecting xylem saps of the NO₃⁻- and NH₄NO₃-treated plants were markedly higher than that of NH₄⁺- and NH₄⁺+6-BA-treated plants (Table 4), which is consistent with a decrease in root hydraulic conductivity caused by NH₄⁺ treatment.

Uptake, flow and partitioning of K⁺ in plants

K⁺ is one of the essential macroelements in plants, and its uptake can be strongly affected by other elements. As shown in Table 3 and Figure 4, NH₄NO₃ compared to NO₃⁻ nutrition had only a small effect on K⁺ uptake and flows, but with a clear tendency to lower flows and uptake. However, when NO₃⁻-N was replaced by NH₄⁺-N, K⁺ uptake was substantially reduced compared with NO₃⁻, and its flow and partitioning patterns in plants changed markedly. Only the middle leaves attracted 143%, together with the upper leaves 206% and together with

the stem 227% of the K⁺ currently taken up, a situation that can be explained only by massive mobilisation and net export of K⁺ from lower leaves and roots (Figure 4). Retranslocation of K⁺ via the phloem from the shoot and cycling through the root contributed 67%, and hence more than concurrent uptake, to the K⁺ transported in the xylem. Thus, relatively, retranslocation of K⁺ in the phloem of NH₄⁺-treated plants was calculated to exceed that of NO₃⁻-fed plants. The results highlight the high degree of reutilisation by retranslocation via phloem, and provide further evidence that the partitioning and retranslocation of K⁺ in plants can be changed, depending on environmental conditions (Hibberd et al., 1999; Jeschke et al., 1987; Jeschke and Pate, 1991a).

The results in the present study demonstrated that total replacement of NO₃⁻-N by NH₄⁺-N caused a substantial decrease in dry weight gain and K⁺ uptake, resulting in a massive mobilisation and net export of K⁺ from lower leaves and even from roots; a strong reduction both in total water uptake, and in the rate of water uptake by roots; and a marked reduction in transpiration rate, apparently due to reduced stomatal conductance. Cytokinins can stimulate both cell division and cell expansion (Colbert and Beever, 1981; Noodén et al., 1990). Good correlations between changes in zeatin/zeatin riboside levels in xylem sap and plant tissues and leaf expansion by supply of different N-forms have been observed (Rahayu et al., 2001; Walch-Liu et al., 2000). In the present study, however, the inhibitory effects on growth, transpiration, uptake and flow of water and ions (K⁺) caused by NH₄⁺-N treatment failed to be reversed by foliar spraying with 6-BA, and even by 6-BA (10⁻⁸ M) supply in nutrient solution daily with watering (results not shown). Although it is possible that exogenously supplied cytokinin can be metabolised rapidly in the shoot (Dieleman et al., 1997), it is still an open question as to whether the xylem translocated cytokinins are responsible for the inhibited shoot growth and development caused by NH₄⁺ supply.

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