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Is the sensitivity to ammonium nutrition related to nitrogen accumulation?

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ABSTRACT

Nitrate and ammonium can be used as nitrogen sources by most plant species although plant response to continuous ammonium nutrition is species dependent. In the present study, the effect of the nitrogen source (nitrate and ammonium) on growth, photosynthetic parameters, nitrogen content and nitrogen assimilating-enzymes (nitrate reductase, glutamine synthetase and glutamate dehydrogenase) was investigated in wheat (*Triticum aestivum* L.), tomato (*Solanum lycopersicum* L.) and lucerne (*Medicago truncatula* L.). Obtained results showed that these plant species vary in their sensitivity to NH_4^+ nutrition, with wheat to be highly sensitive, tomato moderately sensitive and lucerne tolerant to ammonium nutrition. For the three plant species, the growth reduction was correlated closely to ammonium accumulation in leaves. Moreover, contrary to that was observed for wheat plants, glutamine synthetase and glutamate dehydrogenase activities were higher in roots than in leaves, for tomato and lucerne plants. Taken together, these data suggest that the site of ammonium assimilation is a key factor controlling tolerance to ammonium nutrition in the different plant species, with plants being more tolerant when ammonium is assimilated in roots.

Introduction

Plants can absorb and use various forms of nitrogen (N) from soils, most importantly the inorganic ions ammonium (NH_4^+) and nitrate (NO_3^-), and in legumes, N can be obtained by N_2 fixation by means of a symbiotic relationship of rhizobial species (Munoz and Weaver, 1999). The N source affects numerous physiological processes including not only N-assimilation, but also other processes such as root respiration (Matsumoto and Tamura, 1981), water relations (Ragab, 1980), photosynthesis (Shelp and Taylor, 1990), and secondary metabolism (Wang and Below, 1996). Although most plants use either or both forms as a source of N, NO_3^- is generally the preferred source for crop growth (Britto and Kronzucker, 2002). However, excessive NO_3^- application can have detrimental effects such as contamination of ground water via NO_3^- leaching and gaseous losses of N as N_2O, a factor leading to deterioration of ozone layer (Barker and Mills, 1980). Considering the high ability to accumulate NO_3^- in leaves (Santamaria *et al.*, 1998) and the high toxicity of NO_3^- to human (Gangolli *et al.,* 1994) and animal health (Bruning-Fun and Kaneene, 1993), NH_4^+ fertilization can be a desirable source of N nutrition under certain conditions (Britto and Kronzucker, 2002). Furthermore, NH_4^+ application would seem to be a factor in establishing best management practices since the NH_4^+ ion is not readily subject to leaching and denitrification losses (Xiaoyang and Jinfeng, 2007).

Despite the fact that NO_3^- assimilation consumes more energy than NH_4^+ assimilation, only a few species perform well when NH_4^+ is the sole N source (Marschner, 1995). Indeed, many plant species develop symptoms of toxicity when subjected to high concentrations of NH_4^+, which are not detected when plants are grown with the same concentration of NO_3^- or in mixed N nutrition (Britto and Kronzucker, 2002). Although NH_4^+ is an important intermediate in many metabolic reactions, it has been reported that high concentrations of NH_4^+ in the soil or in the nutrient solution may lead to an "NH_4^+ syndrome", which may include leaf chlorosis, lower plant yield production and root/shoot ratio, lower cation content, acidification of the rhizosphere, and changes on several metabolite levels such as amino acids or organic acids (Britto and Kronzucker, 2002). Since NH_4^+ is a photophosphorylation uncoupler (Peltier and Thibault, 1983), its accumulation can decrease net photosynthesis and therefore reduces plant growth (Goyal *et al.,* 1982; Britto *et al.,* 2001).

Plant response to NH_4^+ nutrition varies according to growth conditions and plant species (Britto and Kronzucker, 2002). For example, species such as carob prefer NH_4^+ as the N-source (Cruz *et al.,* 1993), whereas wheat is tolerant only to low NH_4^+ concentrations (Cox and Reisenauer, 1973). NO_3^- is reduced to NH_4^+ which, like root-absorbed NH_4^+, is used in amino acid synthesis. This reduction can occur in roots as well as in shoots of higher plants depending on the species and on the growth conditions (Britto *et al.,* 2001). If the supply of a particular N form results in more uptake than that is needed for optimum growth, the accumulation of amino-containing compounds will occur (Millard, 1988). It is well known that NH_4^+ accumulation can produce toxic effects and reduce growth rate, whereas, in contrast, most plants tolerate large excesses of NO_3^- and accumulate it within their tissues (Britto *et al.,* 2001; Britto and Kronzucker, 2002).

In this paper, we compare the sensitivity to NH_4^+ nutrition of three plant species (wheat, tomato, and lucerne) with the purpose of establishing a possible relationship between NH_4^+ sensitivity and N-accumulation and partitioning in different plant organs.

Material and Methods

Plant materials and growth conditions

Seeds of lucerne (*Medicago truncatula* L. cv. Jemalong), tomato (*Solanum lycopersicum* L. cv. Micro-Tom), and wheat

(*Triticum aestivum* L. cv. Salambo) were germinated on filter paper moistened with distilled water for 1 week at 23 °C in the dark, and then grown hydroponically in growth chambers (16 h light at 23°C/8 h dark at 18°C with an irradiance of 350 µmol m^{-2} s^{-1}, and 75-80 % relative humidity). Each seedling was placed in a vermiculite plug on a polystyrene tray floating on the nutrient solution (Desbien *et al.,* 2004), with 6 plants per 7-L tank. At this time, N treatments were initiated. Plants were fed with either 2.5 mM NO_3^- applied as $Ca(NO_3)_2$ or with 2.5 mM NH_4^+ provided as $(NH_4)_2SO_4$. The macro and microelement solution compositions were as described in Horchani *et al.* (2010a). The nutrient solutions, continuously aerated, were renewed every 4 days to restore nutrients to their original concentrations and pH was controlled two times per day and restored to 5.8 as in Horchani *et al.* (2010a).

Vegetative growth and photosynthesis parameters analysis

At harvest (3 weeks after transplanting), six plants for each species and N treatment were separated into roots and shoots. Dry weights (DW) were obtained by weighing the plant material after drying at 80 °C until a constant mass was reached. Tissue water content (WC) and stomatal conductance were determined as in Horchani *et al.* (2008). Photosynthetis was measured as described in Horchani *et al.* (2010a).

Nitrogen compounds and enzyme activities measurement

For organic N analyses, dried samples were ground and sieved through a screen with 0.8-mm pores, and total N was determined by the Kjeldahl method (*AOAC*, 1990). Nitrogen productivity was calculated as described by Ingestad (1981). Nitrate, ammonium and soluble proteins were extracted in fresh samples and assayed according to Horchani *et al.* (2010b).

Nitrate reductase (NR), glutamine synthetase (GS), and glutamate dehydrogenase (GDH) were extracted and assayed as described in Horchani *et al.* (2010a).

Statistics

Statistical data analysis was made using the Student's t-test. The results are given as means with standard errors of at least six replicates per treatment. The significance of differences between the control and the treatment mean values was determined at the significance level of $p<0.05$. Experiments were replicated two to three times.

Results

Vegetative growth and photosynthesis parameters analysis

Dry matter production was significantly lower in NH_4^+-fed plants than in NO_3^--fed plants. However, reduction in the total biomass (root + shoot) was different for the three species, being 54, 29, and 27% for wheat, tomato and lucerne, respectively, relative to the NO_3^- treatment (Table 1). The shoot/root ratio was not affected by the N source, except a slight decrease in wheat under NH_4^+-based nutrition. Root water content was similar in the three species regardless of N-nutrition. Wheat plants grown with NO_3^- had higher leaf water content than those grown with NH_4^+, whereas N-source did not affect leaf water content in tomato or lucerne (Table 1).

Net photosynthetic rate of wheat or tomato was significantly higher with NO_3^- than with NH_4^+ nutrition. However, net photosynthetic rate of lucerne was not affected by N-source (Table 2). Stomatal conductance and transpiration rate were not affected by N-nutrition in wheat or lucerne plants. In tomato, however, both parameters increased slightly with NH_4^+ as compared to NO_3^- nutrition (Table 2).

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Table 1. Effect of nitrogen source: nitrate (2.5 mM) or ammonium (2.5 mM) on biomass production and water content of wheat, tomato and lucerne plants. Samples were taken from plants after three weeks of nitrogen treatment. Values are the mean of six replicates ± S.E. Values denoted by different letters within columns for each parameter are significantly different (P< 0.05)

	N-Source	Wheat	Tomato	Lucerne
Root DW (g $plant^{-1}$)	Nitrate	0.13 ± 0.03 [a]	0.23 ± 0.04 [a]	0.16 ± 0.02 [a]
	Ammonium	0.07 ± 0.02 [b]	0.15 ± 0.02 [b]	0.11 ± 0.02 [b]
Shoot DW (g $plant^{-1}$)	Nitrate	0.71 ± 0.07 [a]	1.42 ± 0.12 [a]	0.85 ± 0.11 [a]
	Ammonium	0.32 ± 0.08 [b]	1.02 ± 0.08 [b]	0.63 ± 0.07 [b]
Shoot/root ratio	Nitrate	5.46 [a]	6.17 [a]	5.31 [a]
	Ammonium	4.57 [b]	6.80 [a]	5.72 [a]
Root water content (ml g^{-1} DW)	Nitrate	13.3 ± 1.5 [a]	16.4 ± 2.3 [a]	14.7 ± 1.1 [a]
	Ammonium	11.4 ± 2.1 [a]	12.5 ± 2.5 [a]	12.2 ± 1.3 [a]
Leaf water content (ml g^{-1} DW)	Nitrate	18.5 ± 2.2 [a]	17.2 ± 3.1 [a]	16.2 ± 1.6 [a]
	Ammonium	13.3 ± 1.4 [b]	15.1 ± 1.4 [a]	13.3 ± 1.4 [a]

Table 2. Effect of nitrogen source: nitrate (2.5 mM) or ammonium (2.5 mM) on gas exchange parameters. Samples were taken from plants after three weeks of nitrogen treatment. Values are the mean of six replicates ± S.E. Values denoted by different letters within columns for each parameter are significantly different (P< 0.05)

	N-Source	Wheat	Tomato	Lucerne
Photosynthesis rate (µmol m^{-2} s^{-1})	Nitrate	11.4 ± 2.1 [a]	14.5 ± 1.4 [a]	9.3 ± 1.6 [a]
	Ammonium	5.5 ± 1.7 [b]	8.7 ± 2.3 [b]	9.7 ± 2.3 [a]
Stomatal conductance (mol m^{-2} s^{-1})	Nitrate	0.16 ± 0.04 [a]	1.21 ± 0.06 [a]	0.13 ± 0.03 [a]
	Ammonium	0.18 ± 0.02 [a]	1.82 ± 0.09 [b]	0.11 ± 0.02 [a]
Transpiration (mmol m^{-2} s^{-1})	Nitrate	3.5 ± 1.1 [a]	9.7 ± 2.0 [a]	2.7 ± 0.5 [a]
	Ammonium	3.8 ± 0.9 [a]	14.1 ± 1.3 [b]	3.1 ± 0.7 [a]

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Table 3. Effect of nitrogen source: nitrate (2.5 mM) or ammonium (2.5 mM) on nitrate, ammonium and protein content in roots and leaves of wheat, tomato and lucerne plants. Samples were taken from plants after three weeks of nitrogen treatment. Values are the mean of six replicates ± S.E. Values denoted by different letters within columns for each parameter are significantly different (P< 0.05). "nd" denotes not detected

	N-Source	Wheat	Tomato	Lucerne
Root nitrate content (µmol g^{-1} FW)	Nitrate	16.2 ± 3.1 [a]	25.4 ± 2.3 [a]	32.7 ± 3.6 [a]
	Ammonium	nd [b]	nd [b]	nd [b]
Leaf nitrate content (µmol g^{-1} FW)	Nitrate	10.5 ± 2.3 [a]	18.8 ± 1.3 [a]	14.2 ± 3.1 [a]
	Ammonium	nd [b]	nd [b]	nd [b]
Root ammonium content (µmol g^{-1} FW)	Nitrate	0.08 ± 0.02 [a]	0.31 ± 0.03 [a]	1.24 ± 0.12 [a]
	Ammonium	0.28 ± 0.05 [b]	1.05 ± 0.08 [b]	15.80 ± 2.41 [b]
Leaf ammonium content (µmol g^{-1} FW)	Nitrate	0.51 ± 0.12 [a]	0.20 ± 0.05 [a]	0.81 ± 0.15 [a]
	Ammonium	7.12 ± 1.34 [b]	0.45 ± 0.08 [b]	0.94 ± 0.09 [a]
Root organic nitrogen (% DW)	Nitrate	3.62 ± 0.51 [a]	3.56 ± 0.81 [a]	3.12 ± 0.84 [a]
	Ammonium	4.53 ± 1.21 [a]	6.12 ± 1.10 [b]	6.75 ± 1.34 [b]
Leaf organic nitrogen (% DW)	Nitrate	5.37 ± 1.03 [a]	5.76 ± 0.91 [a]	5.32 ± 0.73 [a]
	Ammonium	8.93 ± 1.23 [b]	7.12 ± 0.82 [b]	4.47 ± 0.56 [a]
Root protein content (mg g^{-1} FW)	Nitrate	3.56 ± 0.87 [a]	4.02 ± 0.75 [a]	3.75 ± 1.43 [a]
	Ammonium	7.76 ± 1.02 [b]	6.54 ± 0.43 [b]	6.87 ± 0.98 [b]
Leaf protein content (mg g^{-1} FW)	Nitrate	2.45 ± 0.87 [a]	2.63 ± 0.54 [a]	3.54 ± 0.75 a
	Ammonium	5.03 ± 0.95 [b]	4.56 ± 0.34 [b]	6.04 ± 1.20 [b]
Nitrogen productivity (g DW mol^{-1} N per day)	Nitrate	8.92 ± 1.34 [a]	13.45 ± 0.65 [a]	11.02 ± 1.45 [a]
	Ammonium	4.34 ± 0.87 [b]	10.76 ± 0.98 [b]	7.23 ± 0.61 [b]

Nitrogen compounds analysis

NO_3^- was not detected in tissues of plants grown with NH_4^+. When plants were grown with NO_3^-, the NO_3^- concentration was higher in roots than in leaves for all three species (Table 3). The NH_4^+ concentration in roots was greater when grown with NH_4^+ than with NO_3^-. Irrespective of the form of N supplied, the NH_4^+ concentration was higher in lucerne plants than in tomato plants, and the smallest concentration of NH_4^+ was in wheat roots. Leaf NH_4^+ concentrations in wheat and tomato were significantly greater in NH_4^+-fed plants as compared to NO_3^--fed plants, but in lucerne plants, leaf NH_4^+ content was similar in both N-sources (Table 3). Furthermore, with either N-source, wheat is the only crop in this study that had a greater NH_4^+ concentration in the leaf than in the root (Table 3).

Organic N concentration in roots was significantly greater in tomato and lucerne when these species were grown with NH_4^+ than with NO_3^-, whereas there were no differences in wheat plants grown with the two N-sources (Table 3). Organic N concentrations in leaves were greater in wheat and tomato when these species were grown with NH_4^+ than NO_3^-, whereas no differences occurred in lucerne plants (Table 3). N nutrition differentially affected the organic N partitioning between roots and leaves, depending on the species. Thus, in wheat plants grown with NH_4^+ there was an increase of the organic N concentration in leaves compared to roots, whereas in tomato and lucerne plants grown with NH_4^+, organic N concentration was either the same or higher in roots leading to an increase in root/leaf N content ratio. On the other hand, all studied species showed a similar pattern of organic N accumulation in NO_3^--fed plants (Table 3).

Root and leaf protein content were significantly higher in NH_4^+-fed plants than in NO_3^--fed plants, regardless of species (Table 3). N productivity declined when plants were grown on NH_4^+ as the N-source compared to plants grown on NO_3^-. This decline was more marked in wheat plants (Table 3).

Enzyme activities analysis

Activities of NR, GS, and GDH were assayed in leaves and roots of wheat, tomato and lucerne plants grown for three weeks under two N-nutrition regimes (NO_3^- or NH_4^+). Our results showed that under NO_3^- nutrition, root and leaf NR, GS and GDH activities were almost similar for the three plant species, except a slight increase in root NR, GS and GDH activities for lucerne plants (Fig. 1A, C and E). Under NH_4^+ nutrition, the highest root GS and GDH activities were observed for lucerne plants, whereas wheat plants had the lowest root GS and GDH activities. Lucerne plants had the lowest leaf GS and GDH activities, whereas the highest leaf GS and GDH activities were obtained for wheat plants (Fig. 1 B, D and F).

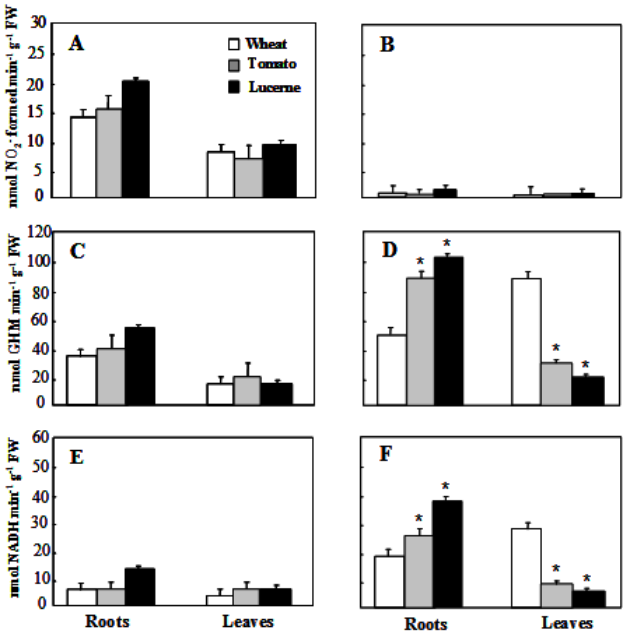

Figure 1. Nitrate reductase (A, B), glutamine synthetase (C, D), and glutamate dehydrogenase (E, F) activities in roots and leaves of wheat (□), tomato ( ) and lucerne (■) plants grown under 2.5 mM nitrate (A, C, E) or 2.5 mM ammonium (B, B, F). Samples were taken from plants after three weeks of nitrogen treatments. Values represent means ± SE (n = 6). *The significance of differences in the enzyme activities between the three plant species was determined by the Student's t-test at the significance level of $p < 0.05$.

Discussion

The sensitivity of plant growth to N fertilization is of great importance in agriculture. The form of N supply (NO_3^- or NH_4^+) influences plant growth and morphology in a distinct manner. The investigations of this effect frequently have led to contradictory results, probably due to differences in experimental conditions or genetic material (Elia *et al.,* 1998; Cruz *et al.,* 2003; Melissa *et al.,* 2007). It is well known that many plants do not tolerate NH_4^+ nutrition (Britto *et al.,* 2001), and vary in their sensitivity to NH_4^+ (Britto and Kronzucker, 2002). In this study, we have compared the effects of NO_3^- and NH_4^+ nutrition in wheat, tomato, and lucerne. These species were chosen for having

a great agronomic interest and displaying some differences in their N and carbon metabolism.

Our results show clear differences between these species in their sensitivity to NH_4^+ nutrition, ranging from high sensitivity in wheat, to medium sensitivity in tomato and virtual tolerance in lucerne. This wide range of response could be an useful tool to study the way in which NH_4^+ can affect plant metabolism.

The sensitivity of many plant species to NH_4^+ nutrition is expressed as growth decrease or suppression (Britto and Kronzucker, 2002). In several cases, this growth inhibition by NH_4^+ nutrition has been related closely to the fall in substrate pH imposed by NH_4^+ uptake (Dijk and Grootjans, 1998). However, in our study, the significant decrease in growth observed in wheat and tomato plants did not seem to be due to the pH of the nutrient solution since it was carefully controlled during the growth of all three species.

One of the physiological processes that can be affected by the N-source is water uptake. As a rule, the presence of NH_4^+ in the nutrient solution as the only source of N inhibits water uptake (Ragab, 1980; Britto *et al.,* 2001), producing imbalances in plant water relations that affect other processes. In our study, we have observed that only wheat plants showed lower leaf water content when grown with NH_4^+ (Table 1), although this reduction was not correlated with differences in stomatal conductance and transpiration (Table 2).

Several studies have demonstrated that high concentrations of NO_3^- are accumulated in root and leaf cell vacuoles when the plants are unable to assimilate all the absorbed NO_3^- (Peuke and Jeschke, 1993). In our study, NO_3^- accumulated significantly in roots and leaves of the three plant species (Table 3). In contrast, NH_4^+ assimilation is accomplished quickly, and the N is stored mainly in organic forms (Jackson and Volk, 1995) as indicated by the significant increase in root and leaf soluble proteins (Table 3). Often plants are not able to assimilate all the absorbed NH_4^+, leading to its accumulation in plant tissues (Schjoerring *et al.,* 2002). Numerous authors ascribe NH_4^+ toxicity to its accumulation, especially when it occurs in the photosynthetic tissues, where it can inhibit the photosynthetic processes and consequently, growth and biomass production (Goyal *et al.,* 1982). Our results showed a close relationship between NH_4^+ accumulation in leaves and growth reduction (Tables 1 and 3). Thus, wheat, the species most affected by NH_4^+ nutrition, showed the highest NH_4^+ concentration in photosynthetic tissues, followed by tomato plants. Lucerne plants, however, are virtually tolerant to NH_4^+ nutrition, with the NH_4^+ concentration in photosynthetic tissues being similar in NO_3^- and NH_4^+-fed plants, and with a remarkably high accumulation of NH_4^+ in the roots of NH_4^+-fed plants (Table 3). The present data are compatible with the concept that plants that assimilate N from inorganic NH_4^+ into organic N in the roots have much greater tolerance for NH_4^+ nutrition than plants which translocate NH_4^+ to the shoots (Tobin and Yamaya, 2001), as shown for wheat in our experiments. Our results showed a tight relationship between NH_4^+ accumulation and organic N content (Table 3). Thus, it appears that in each organ the level of accumulated NH_4^+ is related to the organic N concentration and, hence, to the site where NH_4^+ is assimilated. The response of the different species to NH_4^+ nutrition could be governed by the ability of the different plant organs to assimilate NH_4^+. Plants seem to be tolerant to NH_4^+ when NH_4^+ assimilation is located mainly in the roots (Britto and Kronzucker, 2002). The N and carbon metabolism can be different between species and could lead to differences in the site where NH_4^+ is assimilated. NH_4^+ detoxification in the roots is dependent upon the availability of sufficient carbon reserves which provides the necessary energy and carbohydrate skeletons for its assimilation (Claussen and Lenz, 1995). Tomato has a higher photosynthetic efficiency compared to wheat (Table 2), implying a greater supply of carbohydrates. This fact may be related to the lower sensitivity to NH_4^+ of tomato than wheat plants.

Because NH_4^+ is toxic, it needs to be rapidly assimilated, and current evidence indicates that NH_4^+ assimilation is carried out by the GS/GOGAT pathway (Cruz *et al.,* 1993). Some reports have shown little or no effect of the N source available to plant roots on GS activity (Claussen and Lenz, 1999). However, our results are in agreement with those obtained by Lasa *et al.* (2002), showing that GS activity increases in the presence of NH_4^+. Interestingly, GS activity was higher in roots than in leaves, for tomato and lucerne plants, contrary to what has been observed for wheat plants (Fig. 1D) and for many other plant species (Cruz *et al.,* 1993; Lasa *et al.,* 2002). This may allow NH_4^+ assimilation in roots.

Although NH_4^+ assimilation *via* the GS/GOGAT pathway is the major route in higher plants (Lasa *et al.,* 2002), plants are able to use alternate routes at the same time such as those catalyzed by the GDH. Contrary to that was observed for wheat plants, GDH activity was higher in roots than in leaves, for tomato and lucerne plants (Fig. 1F). Such enzyme may have a possible role in NH_4^+ detoxification through its assimilation mainly in roots.

In summary, we found inter-specific differences in the response of plants to NH_4^+ nutrition ranging from a strong sensitivity in wheat to a virtual tolerance in lucerne. The different response to NH_4^+ nutrition could be related to differences on the site of NH_4^+ assimilation and hence, to NH_4^+ accumulation.

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