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The importance of root carbohydrate abundance in ammonium uptake

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Abstract

The influence of carbohydrates on ammonium uptake and ammonium transporter (AMT1) expression was investigated in roots of field pea (*Pisum arvense*) and rutabaga (*Brassica napus var. rapifera*). Ammonium transport into field pea seedlings diminished markedly following cotyledon removal, which indicated that uptake of ammonium was under control of reserves stored in the cotyledons. Excision of cotyledons decreased also the level of some amino acids, glucose and total reducing sugars in field pea roots. To investigate the importance of the sugar supply for the regulation of ammonium uptake at low external NH_4^+ level, 1 mM glucose or sucrose was supplied for several hours to the field pea seedlings deprived cotyledons or to intact rutabaga plants. Supply of both sugars resulted in a substantial increase in ammonium uptake by both plant species and enhanced markedly the expression of AMT1 in rutabaga roots. The results indicate that sugars may regulate ammonium transport at the genetic level.

Introduction

In last decade two kind of ammonium transporters (HATS and LATS) in plant roots were described (Wang *et al.* 1993). A family of AMT1 genes with at least five members encodes high-affinity ammonium transporters in *Arabidopsis thaliana* (Howitt and Udvardi 2000, von Wiren *et al.* 2000). Recently, AtAMT2;1 gene from *Arabidopsis* roots was also cloned and characterized (Sohlenkamp *et al.* 2000). Transcripts of AtAMT1;1 were found in roots, stems and leaves of *Arabidopsis* whereas AtAMT1;3 was expressed only in roots (Gazzarrini *et al.* 1999). The proteins of AMT1 family in different plants show a high homology in their amino acid sequences (Howitt and Udvardi 2000).

The HATS system in *Arabidopsis thaliana* roots requires glucose (Ninnemann *et al.* 1994). Gazzarrini *et al.* (1999) found that ammonium uptake and the expression of three members of AMT1 gene family show diurnal pattern with maximal level of AtAMT1;3 at the end of light period. This suggests that this gene could link ammonium assimilation and carbon provision in root tissues. Previously, it was shown that signals derived from sugar levels in roots are involved in changes in the expression of various genes (Koch 1996, Jang *et al.* 1997). Exogenous sucrose induces or strongly represses the expression of gene encoding some enzymes of nitrogen metabolism (Lam *et al.* 1996, Coschigano *et al.* 1998). Gene expression can be modulated by hexose signalling associated with the transport across plant cell membranes or the action of hexokinase as a sensor (Smeekens 1998, Sheen *et al.* 1999).

In this paper we have studied the extent to which carbohydrate contents in roots may influence the processes that determine the rate of NH_4^+ uptake by

roots of young field pea and rutabaga plants. We reported the effect of carbohydrate limitation and recovery, as well as the carbohydrate excess on the ammonium uptake and AMT1 gene expression. The experiments show that signals derived from carbohydrate abundance are involved in changes in AMT1 gene expression.

Materials and methods

After 3 days of germination in darkness and 27 °C, field pea (*Pisum arvense* L. var. Nieznaniecki) seedlings were grown 5 days in the nutrient solution deprived nitrogen (Kubik-Dobosz *et al.* 1999) at 16-h photoperiod. After nitrogen-deprivation period the seedlings were divided into two groups. One group of plants was intact and from the second group the cotyledons were excised. The uptake experiments were performed on seedlings placed in ammonium nutrient solutions (pH 6.5) containing the same macroelements and 0.1 mM or 0.5 mM NH_4^+ added as $(NH_4)_2SO_4$. The rutabaga (*Brassica napus var. rapifera*) seeds were germinated 1 day in darkness and 27 °C in ammonium nutrient solution pH 6.5, containing 0.1 mM NH_4^+. Then rutabaga seedlings were grown in the same ammonium solution for 7 days. In uptake experiments concerning both plant species 1 mM sucrose or 1 mM glucose was added to the ammonium nutrient solution. The uptake solutions were changed every hour. In these conditions the possibility of bacterial or fungal infection of solutions was insignificant. Determination of net ammonium uptake rate was based on NH_4^+ disappearance from the ammonium nutrient solutions. Ammonium was assayed according to the method of Hecht and Mohr (1990). Free amino acids were extracted according to Perez-Soba *et al.* (1994) with some modifications. Roots (1 g) were frozen in liquid nitrogen and ground in mortar. The powdered roots were mixed with 5 ml 0.5 M $HClO_4$ and centrifuged at 4 °C for 10 min at 10 000 g. The supernatant (1 ml) was neutralised with 0.2 ml 2 mM $KHCO_3$ and incubated for 15 min in ice-bath. The suspension was next centrifuged for 10 min at 10 000 g and the supernatant was stored at -70 °C. For amino acid analysis the amino acids were derivatived with o-phthalaldehyde and separated by reversed phase high-performance liquid chromatography according to Schuster (1988). Roots (1 g) of plants used for measurement of sugar contents were frozen in liquid nitrogen, powdered and extracted with 5 ml 0.1 M tris-HCl pH 8.0. After centrifugation for 15 min at 10 000 g the supernatant was deproteinized with 2 M $HClO_4$ to the final concentration of 0.5 M $HClO_4$. The samples were centrifuged again and neutralised with 2 M KOH. The concentration of glucose in roots was determined by the enzymatic method according to Slein (1965) and total reducing sugar levels were measured by Somogyi (1952) method. Poly(A+) mRNA was isolated from plant roots with superparamagnetic, polystyrene beads - Dynabeads Oligo $(dT)_{25}$. Plant tissues were harvested, immediately frozen in liquid nitrogen and homogenised quickly in glass homogenizer with lysis/binding buffer obtained with Dynabeads mRNA Direct kit. Isolation protocol was the same as described by the manufacturer. The cDNA syntheses with reverse transcriptase AMV as well as the PCR were performed with a commercial kit Titan™ One Tube RT-PCR System (Boehringer Mannheim). Two primers derived from the sequence conserved in *Arabidopsis thaliana* were used. The nucleotide sequence of AMT1 mRNA was obtained through GenBank as accession number X75879. The primer sequences were as follow: the 5' primer was 5'-TGG TTT GGA TGG TAC GGA TTT AAC-3' and the 3' primer was 5'-GAA GAG AGC CGT GAA TAT TAG TCC-3'. RT-PCR products were separated electroforetically on 1.8 % agarose gels and stained in ethidium-bromide.

Results

Carbohydrates were found to be an important signal for the modulation of ammonium uptake. When intact rutabaga plants were grown for 11 h in the nutrient solutions containing 0.1 mM NH_4^+ as the nitrogen source, in the presence of externally added 1 mM glucose or sucrose, ammonium uptake increased continuously from 1 to 11 h (Fig. 1). Ammonium uptake by field pea seedlings deprived cotyledons and treated with 1 mM glucose and 0.1 mM NH_4^+ concentration was also enhanced during the same period. After 7 h the field pea plants with cotyledons took up more ammonium than the seedlings deprived cotyledons (Fig. 2). The effect of both glucose and sucrose treatments, as

Table. Changes in the accumulation of free amino acids, glucose, and total reducing sugars in roots of field pea seedlings growing in nutrient medium containing 0.5 mM NH_4^+. The parts of plants were exposed to 1 mM sucrose or 1 mM glucose.
(+C) - plants with cotyledons; (-C) - plants without cotyledons.
Values are the mean of two independent samples.

Compound	Amino acid and sugar accumulation (μmol·g^{-1} FW roots)C					
	(+C)	(-C)	(-C+ 1 mM sucrose)	(-C +1 mM glucose)	(+C)	(-C)
	5 h				24 h	
Alanine	1.8	2.3	2.2	2.0	4.6	1.2
Asparagine	9.3	44.0	41.8	42.5	56.3	44.3
Aspartate	1.3	1.8	1.7	1.6	1.2	1.3
Glutamate	1.3	1.4	1.4	1.6	2.1	1.5
Glutamine	5.3	3.6	3.1	2.7	4.3	2.2
Histidine	1.1	1.5	1.3	1.4	1.4	0.6
Isoleucine	0.5	0.8	0.4	0.6	0.5	0.5
Lysine	0.5	0.5	0.3	0.3	1.1	0.6
Proline	1.1	1.1	0.8	1.0	1.1	0.6
Serine	9.8	14.6	13.1	14.1	8.7	8.3
Valine	1.7	2.2	1.6	1.8	1.3	1.3
Glucose	29.4	30.9	-	-	18.5	11.4
Total reducing sugars	46.0	46.3	-	-	48.6	35.0

well as the cotyledons absence was also visible, although less significant, when NH_4^+ concentration in ammonium nutrient solution rose to 0.5 mM (Fig. 3).

Plant cotyledons are known to contain a high concentration of proteins and carbohydrates as storage reserves. To lower the carbohydrate concentration in roots of young field pea, the cotyledons were removed from plants. No significant changes in the contents of glucose and total reducing carbohydrates in roots were observed after 5 h (Table). However, over a period of 24 h the concentration of sugars in roots decreased markedly. The effect of cotyledon excision on the accumulation of free amino acids in field pea roots is also shown in Table. Most of the amino acids, which were measured, showed an increase in concentration 5 h after cotyledons cutting, followed by a slight decline upon sugar treatment. Moreover, there were several amino acids such as glutamate, glutamine, lysine and proline, which did not increase in their concentration. The major contribution to the increase in amino acid levels was due to asparagine. However, after prolonged time (24 h) the concentration of amino acid in roots of field pea plants without cotyledons showed significantly decline, except aspartate, isoleucine, serine and valine.

To examine the mechanism that could explain root NH_4^+ uptake capacity in response to carbohydrate supply, the transcript level of gene encoding ammonium transport system (AMT1) in rutabaga roots was studied. It should be noted that the AMT1 gene from rutabaga we reported here is probably not identical in sequence to any members of AtAMT1 gene family of *Arabidopsis thaliana*. When rutabaga seedlings were incubated for 11 h in solutions containing 1 mM glucose or sucrose, an induction of AMT1 mRNA level was observed (Fig. 4). Therefore, the enhancement in the ammonium uptake, induced by glucose and sucrose was accompanied by parallel increase in mRNA for the high affinity ammonium transporter.

Discussion

It is well established that nitrogen incorporation into organic components requires carbon skeletons and energy (Huppe and Turpin 1994). Therefore it may be sensitive to changes in the carbohydrate contents. During early stages of higher plant development the allocation of carbohydrates from shoots to the roots is insufficient, and degradation of carbohydrates in cotyledons or endosperm supplied carbon substrates for nitrogen metabolism in roots. Field pea and rutabaga show a different pattern dur-

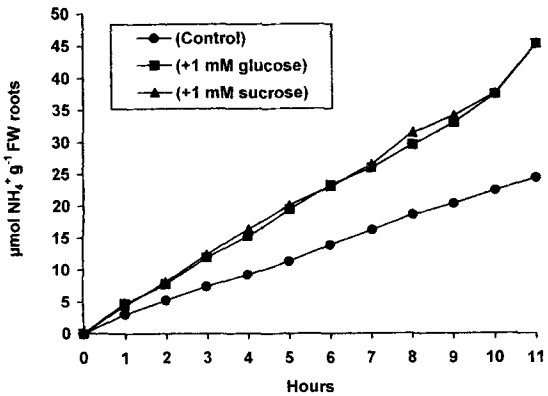

Fig. 1. Ammonium uptake by rutabaga seedlings treated with 1 mM glucose or 1 mM sucrose in ammonium nutrient solutions containing 0.1 mM NH_4^+.
Groups of 5 seedlings were placed in beakers with 18 ml of ammonium nutrient solutions. Glucose and sucrose were added to solutions at the start of uptake experiment.

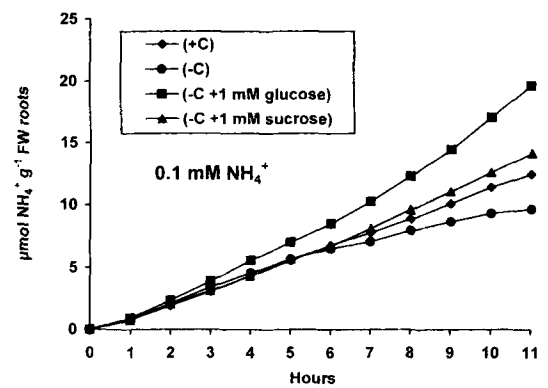

Fig. 2. Ammonium uptake by field pea seedlings treated with 1 mM glucose or 1 mM sucrose in ammonium nutrient solutions containing 0.1 mM NH_4^+.
(+C) - plants with cotyledons; (-C) - plants without cotyledons.
Groups of 5 seedlings were placed in beakers with 45 ml of ammonium nutrient solution. Glucose and sucrose were added to solutions at the start of uptake experiment.

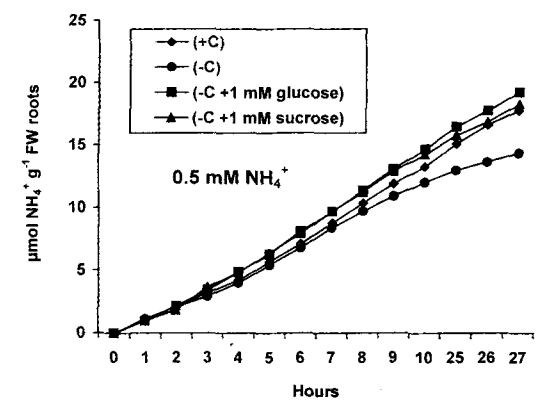

Fig. 3. Ammonium uptake by field pea seedlings treated with 1 mM glucose or 1 mM sucrose in ammonium nutrient solutions containing 0.5 mM NH_4^+.
(+C) - plants with cotyledons; (-C) - plants without cotyledons.
Groups of 17 seedlings were placed in beakers with 100 ml of ammonium nutrient solution. Glucose and sucrose were added to solutions at the start of uptake experiment.

ing germination. In rutabaga seedlings the green cotyledons become a source of photoassimilates and cannot be removed from the young plants. The non-green cotyledons of field pea provide reserves during transition from embryo to seedling. After excision of cotyledons from field pea seedlings the uptake of ammonium was strongly reduced. The results agree with those of Sasakawa and Yamamoto (1978), Macduff and Jackson (1992), Rideout *et al.* (1994) who found that root or shoot excision, defoliation or endosperm removing diminished the uptake of ammonium. In roots of field pea deprived cotyledons the contents both of glucose and total reducing sugars were markedly lower than in control plants. These results indicate that decrease of NH_4^+ uptake correlate with carbohydrate limitation in roots. Exogenously supplied sucrose or glucose increased ammonium uptake activity in field pea plants deprived of cotyledons. Sucrose enhanced ammonium uptake by rice seedlings (Sasakawa and Yamamoto 1978) but sucrose or glucose did not enhance the rate of NH_4^+ absorption in excised barley roots (Bloom and Caldwell 1988). Depression of NH_4^+ uptake at low carbohydrate level in roots could occur as a consequence of carbon skeleton deficiency for amino acid biosynthesis and the decrease in necessary amount of ATP. However, the NH_4^+ uptake capacity of field pea roots increased in response to supplied sugars without a similar change in amino acid concentrations. Although the content of glutamine decreased significantly in 5 h after cotyledon excision, its concentration in tissues was not enhanced by sugars. It was established that ammonium uptake depends on glutamine level in roots (Lee *et al.* 1992, Kubik-Dobosz and Buczek 1999). Recently, it was shown that high concentration of glutamine in *Arabidopsis* roots reduced AMT1 gene expression (Rawat *et al.* 1999). Glutamine concentration in roots of field pea was not restored by exogenous sugars which suggested that feedback control by glutamine concentration rather is not the mechanism for enhanced ammonium up-

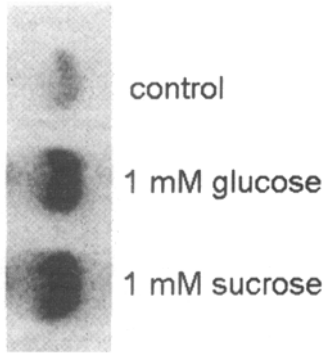

Fig. 4. Expression of AMT1 gene in roots of rutabaga seedlings treated for 11 h with
1 mM glucose or 1 mM sucrose in mineral solution containing 0.1 mM NH_4^+ as the sole nitrogen source.
Glucose and sucrose were added to ammonium solutions at the start of experiment. To avoid the possibility of a serious bacterial or fungal infection the solutions were changed every hour.

take in sugar treated plants. Moreover, the activity of glutamine synthetase in roots of field pea plants deprived cotyledons and in sugar-treated plants did not change markedly within 7 h (data not shown). Glutamate dehydrogenase was slightly stimulated in roots of field pea plants with excised cotyledons, probably as a result of increase in its catabolic activity. The supply of exogenous sugars to field pea plants rapidly decreased the activity of this enzyme (data not shown). At 0.5 mM NH_4^+ the effect of both sugars on ammonium uptake was less significant, what coincides with the minor role of HATS system at higher ammonium concentrations (Wang *et al.* 1993).

Recently, it has been established that AtAMT1;3 transcription was strongly induced after several hours of light period (Gazzarrini *et al.* 1999). Sugars play an important role in regulation the expression of genes encoding some proteins involved in photosynthesis, respiration or nitrogen metabolism (Koch 1996, Jang *et al.* 1997, Felitti and Gonzalez 1998). Glucose and sucrose strongly stimulated the ammonium uptake and AMT1 gene expression in rutabaga plants. The findings suggest that signals derived from sugar levels in rutabaga roots may be involved in changes in AMT1 gene expression, resulting in a reprogramming of ammonium uptake rate. In this way the uptake of low ammonium concentration appears to be controlled by sugars on the molecular level, although we do not know whether signals involved in changes in AMT1 gene expression derive from sugar itself or from metabolites formed during carbohydrate assimilation.

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