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Capacities and constraints of amino acid utilization in *Arabidopsis*

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Summary

• Various amino acids, including both L- and D-enantiomers, may be present in soils, and recent studies have indicated that plants may access such nitrogen (N) forms. Here, the capacity of *Arabidopsis* to utilize different L- and D-amino acids is investigated and the constraints on this process are explored.

• Mutants defective in the lysine histidine transporter 1 (LHT1) and transgenic plants overexpressing LHT1 as well as plants expressing D-amino acid-metabolizing enzymes, were used in studies of uptake and growth on various N forms.

• *Arabidopsis* absorbed all tested N-forms, but D-enantiomers at lower rates than L-forms. Several L- but no D-forms were effective as N sources. Plants deficient in LHT1 displayed strong growth reductions and plants overexpressing LHT1 showed strong growth enhancement when N was supplied as amino acids, in particular when these were supplied at low concentrations. Several D- amino acids inhibited growth of wild-type plants, while transgenic *Arabidopsis*-expressing genes encoding D-amino acid-metabolizing enzymes could efficiently utilize such compounds for growth.

• These results suggest that several amino acids, and in particular L-Gln and L-Asn, promote growth of *Arabidopsis*, and increased expression of specific amino acid transporters enhances growth on amino acids. The efficiency by which transgenic plants exploit D-amino acids illustrates how plants can be engineered to utilize specific N sources otherwise inaccessible to them.

Key words: D-amino acid, D-amino acid oxidase, D-serine dehydratase, growth, lysine histidine transporter 1, metabolism, nitrogen uptake.

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Introduction

The soil solutions of both managed and unmanaged terrestrial ecosystems contain wide ranges of nitrogen (N) compounds, including various amino acids (Schulten & Schnitzer, 1998; Senwo & Tabatabai, 1998; Nordin *et al.*, 2001, 2004; Henry & Jefferies, 2002; Yu *et al.*, 2002; Kielland *et al.*, 2006, 2007; Kranabetter *et al.*, 2007). Soil amino acids are to a large extent derived from the exoenzymatic decomposition of proteins and peptides originating from decaying organisms (Kielland *et al.*, 2007), and thus proteinoic L-amino acids usually dominate soil amino acid pools. However, some D-amino acids may also be abundant, including D-Glu and D-Ala from peptidoglycans of eubacterial cell walls and D-Ser from lombricine of earthworms (Ennor & Rosenberg, 1962). Thus, soils may contain significant fractions of both L- and D-amino acids (Amelung & Zhang, 2001).

Research dating back several decades has shown that plant roots are capable of absorbing both inorganic and organic forms of N (Virtanen & Linkola, 1946; Wright, 1962; Valle & Virtanen, 1965). More recent studies have also demonstrated

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that a number of plant species, including representatives of several major mycorrhizal (M) types (including arbuscular-, ericoid- and ecto-M) and nonM plants can absorb organic N in the form of amino acids (Persson & Näsholm, 2001). Furthermore, the affinities of roots for uptake of amino acids are in the same range as those of various soil microorganisms (Shobert & Komor, 1987; Lipson & Näsholm, 2001; Jämtgård *et al.*, 2008). Thus, results from a range of studies suggest that amino acids are potential N sources for plants. However, several investigations have also indicated that nonM plants may be unable to utilize amino acids for growth. For instance, Stribley & Read (1980) and Turnbull *et al.* (1995) studied the capacity of obligate mycorrhizal plants (*Vaccinium macrocarpon* and *Eucalyptus grandis/E. maculata*, respectively) to use various organic N compounds and concluded that these plants have a severely restricted capacity to utilize Gly (Stribley & Read, 1980), L-His, L-Arg, Gly and the protein BSA (Turnbull *et al.*, 1995) in the absence of their mycobionts. Nevertheless, the latter study also showed that plants lacking mycobionts grew relatively well when supplied with L-Gln as the sole source of N. Bonner *et al.* (1992) studied the responses of *Nicotiana sylvestris* protoplasts exposed to 1–10 mM of amino acids and found all amino acids, with the notable exception of L-Gln, to be toxic. In addition, Bonner & Jensen (1996, 1997) suggested that L-Gln might counteract the toxicity of several amino acids. Bollard (1966) assessed the capabilities of a fungus (*Neurospora crassa*), an alga (*Chlorella vulgaris*) and a plant (*Spirodela oligorrhiza*) to grow on various N compounds, and found that the plant was able to grow on substantially fewer of the tested substances (just a few amino acids, such as L-Gln, L-Glu and L-Asn) than the other two test organisms. In the same study, it was also found that a number of amino acids (e.g. L-Arg, L-Ile and L-Val) inhibited growth of the plant. Furthermore, its growth was inhibited by all tested D-enantiomers of the amino acids (D-Ala, D-Ser, D-Asp, D-Asn, D-Glu and D-Gln). Strong growth-inhibiting effects have also been shown for a number of D-enantiomers on both pea and barley (Valle & Virtanen, 1965), as well as on rice, soybean, tobacco, corn and ryegrass (Aldag & Young, 1970; Manabe & Ohira, 1981), suggesting that D-enantiomers generally may not be utilizable as sources of N for plants and that they can interfere with plant metabolism.

Clearly, data on plant utilization of organic N sources are conflicting. While studies on short-term uptake suggest that plants have the capacity to absorb a wide range of compounds, studies of plant performance on organic N substances suggest that a limited number of such substances actually promote growth, and that several amino acids may inhibit plant growth. The prospects for gaining new knowledge regarding plant organic N nutrition depend, naturally, on advances in our understanding of the basic mechanisms whereby plants take up and metabolize such compounds. Towards that end, recent results presented by Hirner *et al.* (2006) and Svennerstam *et al.* (2007) suggest that root uptake of a number of amino acids in *Arabidopsis thaliana* (*Arabidopsis*) is mediated, at least partly, by the lysine histidine transporter 1 (LHT1). This suggests that mutants with altered expression of LHT1 can be used to study the extent to which root uptake capacities govern plants' ability to use such compounds as N sources. Furthermore, Erikson *et al.* (2004, 2005) found that the apparent toxicity of D-amino acids, such as D-Ala and D-Ser, was abolished in transgenic plants expressing genes encoding D-amino acid-metabolizing enzymes such as the *Rhodutorula gracilis* D-amino acid oxidase (DAAO) or the *Escherichia coli* D-serine deaminase (DSDA). These findings suggest that such transgenic plants may be used to study the effects of changes in plants' capacities to metabolize absorbed amino compounds on their ability to utilize such compounds for growth.

Our aim in the current study was to investigate the capacity of the natively nonM plant *Arabidopsis* to utilize various L- and D-amino acids and unravel the constraints exerted by root uptake and internal metabolism for this process. For this purpose, we used both plants with altered capacities for root amino acid uptake and transgenic plants expressing genes encoding D-amino acid-metabolizing enzymes.

Materials and Methods

Plant material and growth conditions

In the experiments described below, *Arabidopsis thaliana* (L.) Heynh. (*Arabidopsis*) wild-type plants (ecotype Col-0), *Arabidopsis* plants either deficient in LHT1 (*lht1-4* or *lht1-5*; Svennerstam *et al.*, 2007), or overexpressing it (*35SLHT1-1*, *35SLHT1-2*; see below), and plants expressing the *Escherichia coli* gene D-serine deaminase (35S*dsdA*; Erikson *et al.*, 2005) or the *Rhodotorula gracilis* gene encoding D-amino acid oxidase (*35Sdao1*; Erikson *et al.*, 2004) were used.

Unless stated otherwise, the following information applies to all the experiments performed on plants growing in plant agar (Duchefa Biochemie BV, Haarlem, the Netherlands). Seeds were surface-sterilized in ethanol (1 min in 70% ethanol + 0.05% Tween, followed by quick wash in 95% ethanol) and sown on half-strength N-free MS (Murashige & Skoog, 1962), 0.5% sucrose, 0.5% agar medium, buffered to pH 5.8 using MES at 3.6 mM. After sowing, the agar plates were incubated in a cold room at 5°C for 48 h, to optimize for an even germination of *Arabidopsis* seeds, and then transferred to a climate chamber with a 16 : 8 h light : dark (200 μmol photon m^{-2} s^{-1}) and 23 : 18°C (day : night) regime. After 20 d, plants were harvested, agar was removed from their roots and the plant material was dried overnight at 60°C and then weighed. Any plate with visible contamination was discarded.

Construction of *35S-LHT1 Arabidopsis* plants

The open reading frame (ORF) of *LHT1* was cloned into the binary vector pB7WG2D (Karimi *et al.*, 2002) using

Gateway technology (Invitrogen, Carlsbad, CA, USA). Sequence-specific primers with Gateway linkers were used to amplify *LHT1* (F, 5′-attB1-TCACCATGGTAGCTCAAGCTCCT; R, 5′-attB2-GCGTTTATGAGTAAAACTTGTA) by PCR. The resulting construct was used to transform *Arabidopsis* (sets of three to four plants in each of 10 pots) via *Agrobacterium tumefasciens*-mediated gene transfer by the floral dip method (Clough & Bent, 1998). Seeds from the plants were collected and screened separately for BASTA resistance (the selectable marker included in the construct) to ensure that lines originating from individual transformation events were isolated. Seeds from two individual lines (T2 generation; *35SLHT1-1*, *35SLHT1-2*) were isolated showing a 3 : 1 resistant : sensitive to BASTA segregation ratio, suggesting that they each originated from a single insertion event, and T3 seed batches were obtained from each of them. No BASTA-sensitive plants were detected amongst those that germinated from sets of 300 T3 seeds screened from each line, indicating that they were homozygous for the *35S:LHT1* insert.

Overexpression of *LHT1* was confirmed by RT-PCR, as follows. Total RNA was prepared from leaves of three T3 individuals from each line, cDNA was synthesized using a first-strand synthesis kit (Amersham Biosciences, Piscataway, NJ, USA) as recommended by the vendor, then *LHT1* was amplified using the sequence-specific primers 5′-AGTCATCG-TTGCTTACATCGTCGT (F) and 5′-TGGCGATAGGACC-ATCAAGAAAAGA (R) with three technical replicates for each cDNA reaction. To confirm that approximately equal amounts of RNA were present in each reaction mixture, the constitutively expressed *Arabidopsis* actin gene *ACT2* was amplified using the primers 5′-CCAATCGTGTGTGACAA-TGGTACCG (F) and 5′-GGTTGTACGACCACTGGCGT-ACAAG (R) (An *et al.*, 1996).

Amino acid uptake

Wild-type plants were grown as described earlier, but on vertical plates with increased agar content (0.8%) to avoid roots penetrating the agar surface, enabling the plants to be easily harvested with minimum damage of roots. After 18 d (19 d for the CCCP-treated plants), plants were removed from the vertical agar plates and their roots were rinsed in 0.5 mM $CaCl_2$ and blotted on a tissue. Thereafter, the root parts of the intact plants were placed in 2 ml of uptake solution and the total setup incubated in a climate chamber (200 μmol photon m^{-2} s^{-1}; 23°C) on a shaking table at 70 rpm. Five replicate samples, each comprising three plants, were incubated for each type of uptake solution (i.e. $n = 5$). Two different uptake solutions were used: one for studies of uptake of NH_4^+ and L-enantiomers of amino acids, and the other for studies of uptake of D-enantiomeric amino acids. The uptake solution for L-amino acids contained the following compounds: L-Gln, L-Asn, L-Glu, L-Asp, Gly, L-Ala, L-Ser, L-Arg, L-Val, L-Ile as well as NH_4^+, each at a concentration of 10 μM. The uptake solution for D-amino acids contained the following compounds: D-Ala, D-Ser, D-Arg, D-Val and D-Ile, each at a concentration of 10 μM. Both solutions also contained 0.5 mM $CaCl_2$. Solution samples were taken after 1, 2 and 4 h. After the last sampling, the roots were dried and weighed. Passive amino acid uptake was measured in a separate experiment by submerging roots in 100 μM CCCP (carbonyl cyanide 3-chlorophenylhydrazone) for 1 h before the uptake experiment, then treating them identically in all other respects to those used in the uptake experiment described earlier. CCCP inhibits the formation of ATP subsequently, resulting in depolarization of membrane potential. Root uptake rates (μmol g^{-1} DW root h^{-1}) for each N compound supplied were calculated from the decline in their concentrations in the solutions, by regressing measurements of samples taken after 0, 1, 2 and 4 h against time. These regressions displayed r^2 values between 0.89 and 0.99 for all compounds except for L-Glu and L-Asp, which displayed lower values (0.59 and 0.78, respectively). For L-Arg, the linear phase was restricted to the three first measurements (i.e. 0, 1 and 2 h) and hence only these time points were used in the L-Arg regression. Furthermore, for NH_4^+, rates of depletion in the incubation solution were very high, so its uptake rate was based on the measurements taken solely after 1 h. For CCCP pretreated plants, none of the regressions of measurements of samples taken at the different time points were significant.

Growth of *Arabidopsis* on various N sources

Wild-type plants were grown on agar media amended with each of the following N sources, both alone and in combination with 3.0 mM NO_3^-, at concentrations equivalent to 3 mM N in each case: NH_4^+, L-Gln, L-Asn, L-Glu, L-Asp, Gly, L-Ala, L-Ser, L-Arg, L-Val, L-Ile, D-Ala, D-Ser, D-Arg, D-Val and D-Ile. Nitrogen-free half-strength MS medium was used as a reference ('no N') for plants supplied with amino acids as the sole N source and the same medium, amended with 3.0 mM NO_3^-, was used as a reference for plants grown on combinations of amino acids and NO_3^-. Each treatment was replicated four times (i.e. $n = 4$).

Plant acquisition of ^{15}N-labeled amino acids

Seeds from wild-type plants and *lht1-4* mutants were sown on 0.65% agar plates amended with 3.0 mM NO_3^- and L-^{15}N-Gln or L-^{15}N-Ala (> 98% ^{15}N in both cases). Labeled amino acids were supplied at two concentrations: 30 μM and 3.0 mM. Eight replicate plates, each with three seeds, were prepared for each genotype and treatment. After 14 d for the 30 μM treatment and 19 d for the 3.0 mM treatment, plants from five randomly selected plates per treatment and genotype were harvested ($n = 5$). Roots were rinsed and cleaned thoroughly three times in 0.5 mM $CaCl_2$ to remove ^{15}N from their surfaces. The harvested plants were then dried at 60°C overnight, weighed and homogenized.

Studies of plants with altered expression of LHT1

Two experiments were conducted to study the effects of changes in the plants' capacities for absorbing amino acids on their growth on amino acids. In the first experiment, wild-type and *lht1-5* seeds were sown on 0.65% agar media amended with 0.5, 1.0 and 1.5 mM L-Gln. Plants from at least five plates were harvested after 19–21 d ($n \geq 5$). In the second experiment, wild-type, *35SLHT1-1* and *35SLHT1-2* seeds were sown on 0.65% agar media containing 0.5, 1.5 or 5.0 mM of L-Gln, L-Glu, or L-Asn. Seeds sown on 3 mM NO_3^- provided references. All three lines were sown onto the same plates, with three seeds per genotype, each plate contained 100 ml of media. Seven plates were harvested per treatment ($n = 7$).

Growth of *Arabidopsis* expressing D-amino acid-metabolizing enzymes

One of our aims was to study the effects of altering *Arabidopsis* plants' capacity to metabolize D-enantiomeric amino acids on their growth performance when such N forms are present both as sole N sources and together with other N sources. For this purpose, we used two sets of plants, one expressing the *E. coli* gene D-serine dehydratase (*dsdA*; Erikson *et al.*, 2005) and another expressing the *R. gracilis* gene D-amino acid oxidase (*dao1*; Erikson *et al.*, 2004). In a first experiment, wild-type, *35S:dsdA* and *35S:dao1* plants were grown on agar media amended with 0, 0.3, 3.0 or 30 mM D-Ser (*35S:dsdA*) or D-Ala (*35S:dao1*) as the sole N sources. In a second experiment, sets of wild-type plants and *35S:dsdA* plants were grown on media with 20 mM NO_3^-, 20 mM NO_3^- + 10 mM NH_4^+, 20 mM NO_3^- + 10 mM D-Ser, 10 mM NH_4^+ and 10 mM D-Ser. A further set was also grown on medium with no added N. In these experiments, 10 plants of each genotype were grown per plate and four plates were prepared per treatment (i.e. $n = 4$). Plants were grown for 21 d following germination.

Analyses

Concentrations of amino acids in uptake solutions were measured using the UPLC-AccQTag method (UPLC Amino Acid Analysis System Solution, application note 720001683EN; www.waters.com). Since the uptake of L- and D-enantiomers of amino acids was assessed separately, enantiomeric separation of compounds was not required. Plant ^{15}N and total N (i.e. $^{14}N + ^{15}N$) contents were analyzed using a Europa Scientific Isotope Ratio Mass Spectrometer according to Ohlson & Wallmark (1999).

Treatment of data

ANOVA followed by the Dunnet's post-hoc test was used to test the significance of differences in plant biomass between reference and amino acid treatments and ANOVA followed by Tukey's post-hoc test was used to test the significance of difference between LHT1 mutants or overexpressors and wild-type plants, and to test the significance of difference between plants expressing D-amino acid metabolizing enzymes and wild-type plants. Bars marked with asterisks in the figures indicate treatments that resulted in significant differences from the respective references at the $P \leq 0.05$ level.

Results

Root uptake of amino acids

Uptake rates, estimated from the rates of depletion of amino acids in the buffer during the incubation period, were highest for NH_4^+ and L-Arg (5.7 and 3.6 μmol g^{-1} DW h^{-1}, respectively), intermediate for L-Asn, L-Gln L-Ala, Gly, L-Ser, L-Val, L-Ile, D-Ala and D-Ser (1–2 μmol g^{-1} DW h^{-1}) and low (0.5–1 μmol g^{-1} DW h^{-1}) for L-Glu, L-Asp, D-Arg, D-Val and D-Ile (Fig. 1a,b). Uptake rates of D-enatiomers were comparable to those of the corresponding L-enantiomers for Ser and Ala, but significantly lower for Arg, Ile and Val.

Pretreatment with the protonophore CCCP reduced amino acid uptake of plants so that rates were not significantly different from zero for any of the tested amino acids (data not shown).

Growth of *Arabidopsis* on amino acids

All tested N sources promoted growth to a lesser extent than nitrate (Fig. 2a). Compared with the 'no N' reference, six of the tested organic N sources (L-Gln, L-Asn, L-Asp, Gly, L-Ala and L-Arg) resulted in significantly higher biomass, while seven resulted in significantly less biomass and two of the N-forms had no significant effect on growth (L-Glu and D-Ile). The amides L-Gln and L-Asn, together with L-Ala, were most effective as N sources for biomass production, while L-Val, D-Ala and D-Ser were most effective as inhibitors of growth. When amino acids were supplied with 3.0 mM nitrate, only L-Gln, L-Asn and L-Asp significantly stimulated growth more strongly than the nitrate reference, while nine of the N forms reduced growth compared with the reference treatment, and three had no effect (L-Glu, Gly and D-Ile; Fig. 2b). When tested in combination with nitrate, D-Ala and D-Ser were again the most powerful inhibitors of growth, but L-Val and L-Ile also reduced growth significantly, and to a much greater extent than the corresponding D-enantiomers. Thus, the amides (L-Gln, L-Asn) and L-Asp were effective as N sources both when supplied as single N sources and when supplied in combination with nitrate. By contrast, L-Ala and L-Arg had positive effects on growth when supplied as the sole N source, but a negative growth effect when supplied in combination with nitrate. Ammonium stimulated growth, both when supplied as the single N source and when supplied together with nitrate, but to a lesser extent than either of the amides. None of the tested D-enantiomers of amino acids

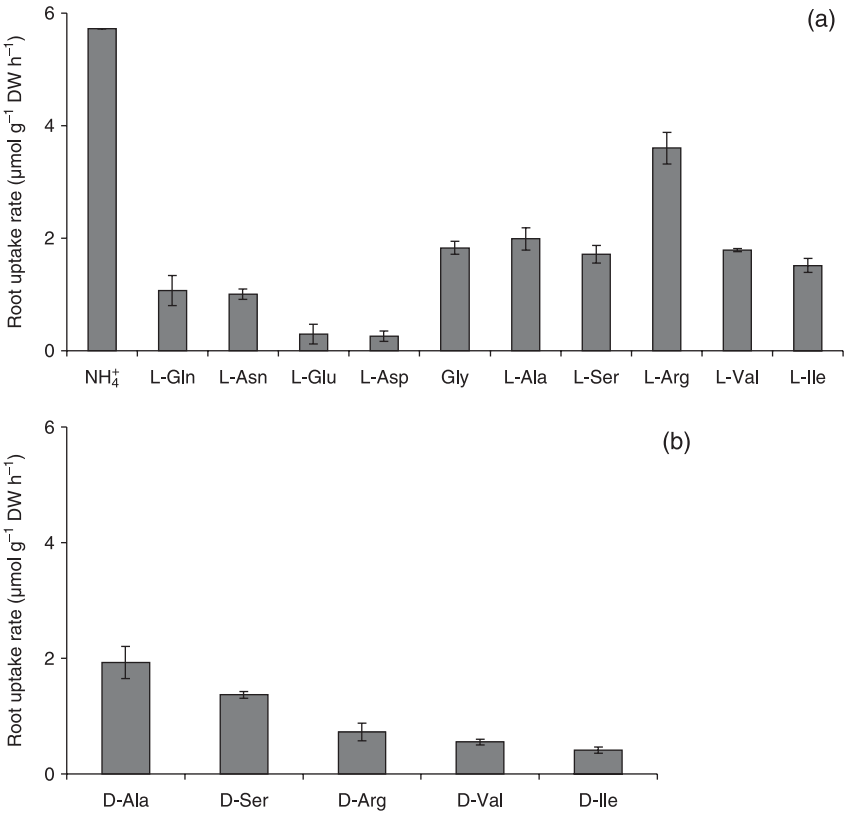

Fig. 1 Uptake of ammonium and various amino acids by roots of *Arabidopsis*, assessed by immersing roots of intact, sterile-grown seedlings in a mixture of 10 μM of each of 10 different L-amino acids and ammonium (a) or a mixture of 10 μM of each of five different D-amino acids (b), and monitoring the decrease in concentration of the respective nitrogen (N) source in the test solution over a 4 h incubation period. Bars represent means ± SE, $n = 5$.

promoted growth more strongly than the control, and all D-forms except D-Ile caused decreased growth compared with the control. Among D-amino acids, D-Ala and D-Ser were absorbed at the highest rates and were also the most efficient growth inhibitors, while D-Val and D-Ile were absorbed at lower rates and were also less toxic.

Rates of growth on the various N forms did not always correspond well to the respective uptake rates of these forms. For instance, L-Arg, which was the most rapidly absorbed amino acid, was a very poor N source for growth of *Arabidopsis*. Conversely, L-Gln, and L-Asn, which promoted growth of plants most efficiently, were only absorbed at intermediate rates.

Growth and N acquisition of LHT1 mutants on amino acids

To assess the extent (if any) to which plants' capacity to take up amino acids through their roots affects their growth performance on them, we used *Arabidopsis* mutants with deficiencies in the amino acid transporter LHT1 (*lht1-4* and *lht1-5*; Svennerstam *et al.*, 2007). To study the growth of *LHT1* mutants, plants were exposed to various concentrations of L-Gln (Fig. 3). Differences between mutants and wild-type plants were greater at low than at high concentrations; at 0.5, 1 and 1.5 mM L-Gln, mutant growth rates were 53, 66 and 91%, respectively, of wild-type rates.

These findings suggested to us that changes in the expression of LHT1 would more strongly affect plant acquisition of amino acids at low than at high external concentrations of amino acids. To test this hypothesis, plants were grown on agar amended with 3 mM NO_3^- and ^{15}N-labeled L-Gln or L-Ala, supplied at concentrations of either 0.03 or 3 mM, and the proportions of plant N derived from amino acid uptake in the presence of each of these permutations were measured (Fig. 4). At the lower concentration of L-Gln and L-Ala, *c.* 2.7 and 1.9%, respectively, of the N in wild-type plants was derived from the amino acids, while the corresponding figures for *LHT1* mutants were 1.2 and 0.8%, corresponding to 56 and 57% reductions in L-Gln and L-Ala acquisition, respectively (Fig. 4a). At the higher amino acid concentration, the proportions of N in wild-type plants derived from the respective amino acids were 36.6 and 45.7% (Fig. 4b), while the corresponding figures for *LHT1* mutants were 29.4 and 46.2%, respectively. Thus, the *LHT1* mutation had no significant effect on L-Ala acquisition and reduced L-Gln acquisition by only 20% at the high concentration of the amino acids. These results confirm that the lack of LHT1 expression had a much stronger effect on amino acid acquisition at the 0.03 mM than at the 3 mM concentration.

Growth of LHT1 overexpressors on amino acids

We compared the growth of wild-type plants and plants expressing the *Arabidopsis LHT1* gene under the control of the CaMV 35S promoter on the amino acids L-Glu, L-Gln and

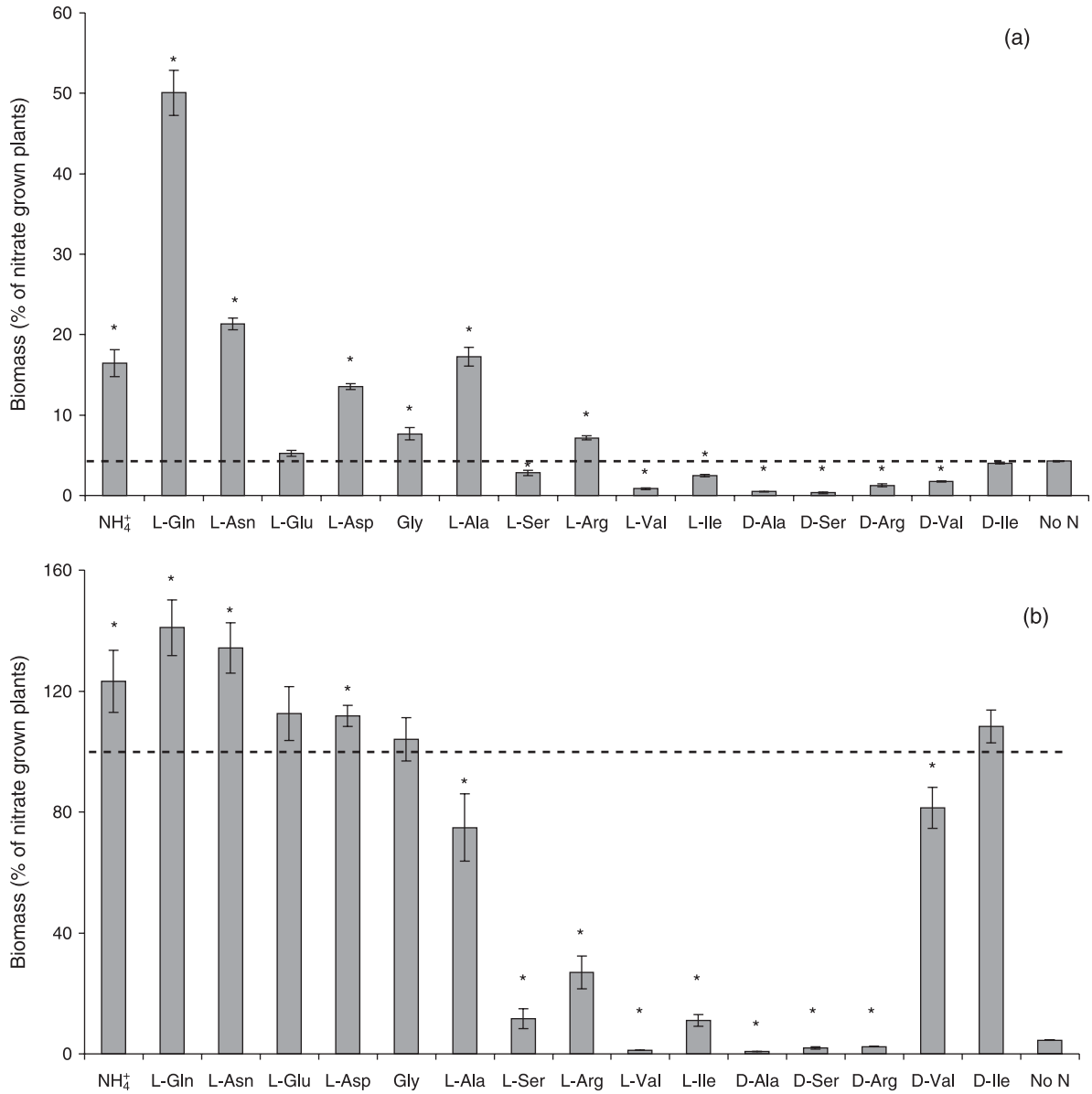

Fig. 2 Growth of *Arabidopsis* on various nitrogen (N) sources on axenic agar plates. Plants were grown for 20 d on half-strength N-free MS medium (a) or half-strength N-free MS medium amended with 3 mM nitrate (b), containing one of the N sources shown in the figure legend at a concentration corresponding to 3 mM N. Thus, (a) represents growth on amino acids only, whereas (b) represents growth on amino acids plus nitrate. Bars show mean biomass as a percentage of the biomass of plants grown on 3 mM nitrate ± SE, $n = 4$. The 3 mM nitrate treatment corresponds to a biomass of *c*. 5.7 mg DW per plant. The horizontal dotted lines indicate the biomass of plants grown without added N (a; no N) and a 3 mM nitrate reference (b). *, statistically significant deviations ($P \leq 0.05$) from the respective references (no N or 3mM nitrate).

L-Asn. Two lines were obtained (*35SLHT1-1*, and *35SLHT1-2*; see the Materials and Methods section), both displaying clearly elevated *LHT1* mRNA concentrations (Fig. 5). Overexpressors showed increased growth compared with wild-type plants for all concentrations of L-Gln tested, but the relative increase over wild-type plants was higher at low than at high concentrations (Fig. 6). *35SLHT1* plants produced, on average, three times more biomass than wild-type plants at the 0.5 mM concentration, while there was only a 25% difference between these plant types at 5 mM. On L-Glu, growth was poorer than on L-Gln, and the highest concentration of L-Glu tested resulted in slower growth than the two lower concentrations. Both *35SLHT1* lines displayed increased growth compared with wild-type at all tested concentrations of L-Glu but, again, the relative effect was higher at low concentrations. Plant growth on L-Asn was intermediate between growth on L-Gln and L-Glu, and growth of wild-type plants increased with increases in L-Asn concentration. The two *35SLHT1* lines displayed higher growth than wild-type plants at 0.5 mM, slightly increased growth at 1.5 mM and lower growth than wild-type at 5 mM concentration of L-Asn (Fig. 6).

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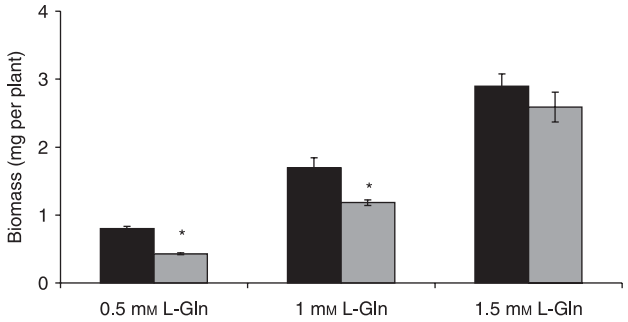

Fig. 3 Growth (mg DW per plant) of *Arabidopsis* on various concentrations of L-Gln. Wild-type plants and *LHT1* mutants were grown for 20 d on half-strength N-free MS media amended with 0.5, 1 or 1.5 mM L-Gln, corresponding to N concentrations of 1, 2 and 3 mM N, respectively. Bars represent means ± SE, $n \geq 5$. *, statistically significant differences ($P \leq 0.05$) between plant types.

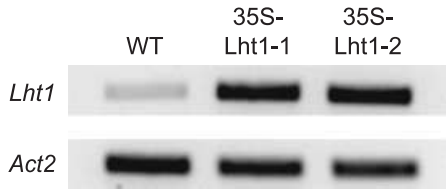

Fig. 5 Characterization of two LHT1 overexpressing *Arabidopsis* lines. RT-PCR analysis was performed on total RNA from wild-type (WT) and three T3 individuals each of *35SLHT1-1* and *35SLHT1-2* using *LHT1* gene-specific primers. For clarity, the results of only one reaction per line are shown. Primers amplifying the *Arabidopsis* gene Actin2 (*ACT2*) were used as controls.

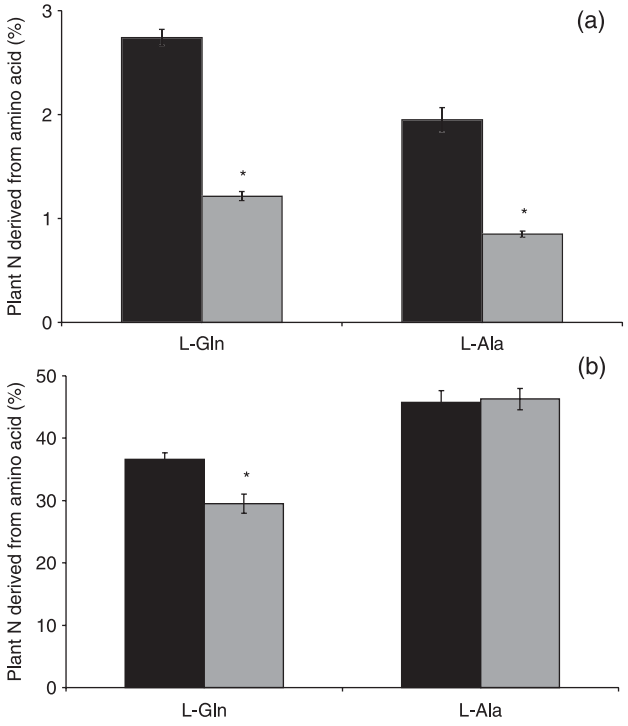

Fig. 4 Fractions of nitrogen (N) derived from the uptake of ^{15}N-labeled amino acids in wild-type *Arabidopsis* plants (black bars) and *LHT1* mutants (gray bars). Plants were grown on N-free half-MS media with N administered as a mixture of 3mM nitrate and 0.03 mM (a) or 3 mM (b) of the ^{15}N-labeled amino acids indicated on the *x*-axis. Bars represent means ± SE, $n = 5$. *, statistically significant differences ($P \leq 0.05$) between plant types.

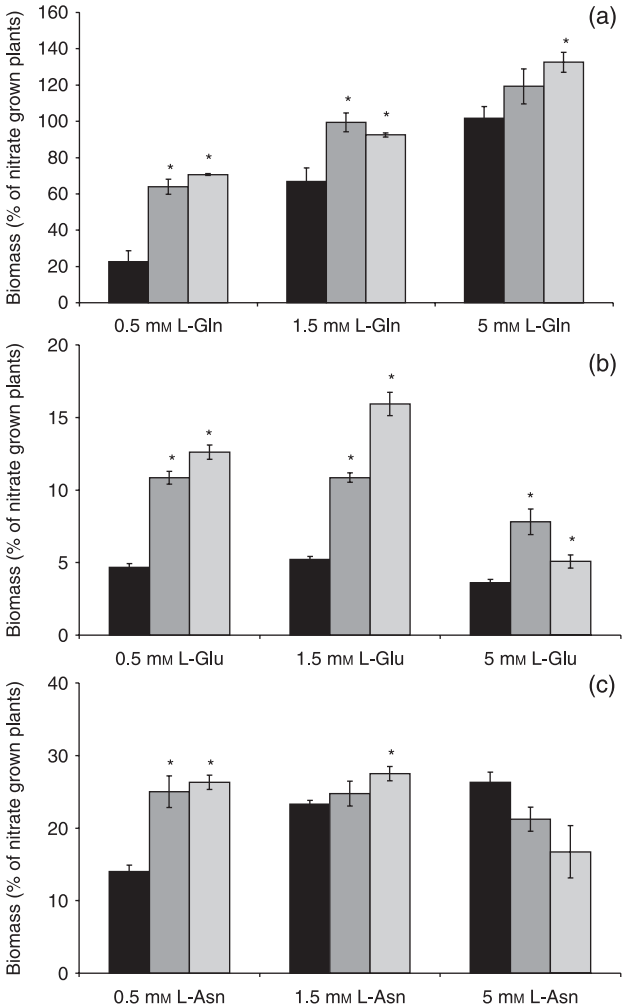

Fig. 6 Growth of *Arabidopsis* wild-type (black bars) and *35SLHT1-1* and *35SLHT1-2* (dark gray and light gray bars, respectively) plants on N-free half-MS media amended with 0.5, 1.5 or 5 mM L-Gln (a), L-Glu (b) or L-Asn (c). Bars represent means ± SE, $n = 7$. *, statistically significant differences ($P \leq 0.05$) between plant types within each concentration.

Growth of plants expressing the *dsdA* or *dao1* genes on D-amino acids

As shown earlier, *Arabidopsis* has the capacity to absorb D-amino acids, but cannot utilize them for growth (Figs 1, 2). To examine the extent to which the lack of metabolic capabilities contributes to its inability to utilize D-amino acids, we performed growth

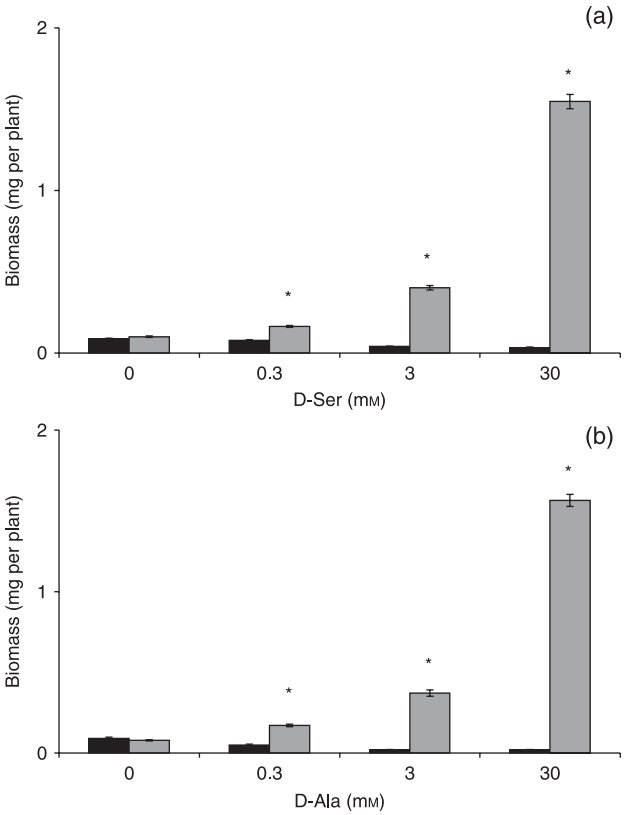

Fig. 7 Growth (mg DW per plant) of *Arabidopsis* wild-type and D-serine dehydratase (*dsdA*)-expressing plants (a) or wild-type and D-amino acid oxidase (*dao1*)-expressing plants (b) on half-strength N-free MS amended with different concentrations of D-serine (a) or D-alanine (b). Black bars represent wild-type plants and gray bars transgenic plants (*dsdA* and *dao1*). Bars represent means ± SE, $n = 4$. *, statistically significant differences ($P \leq 0.05$) between plant types.

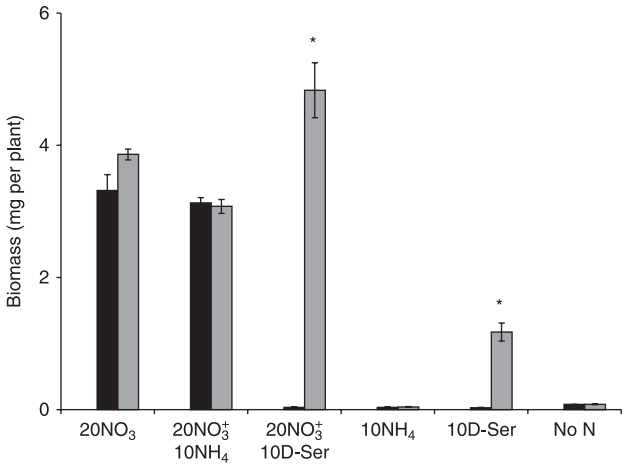

Fig. 8 Growth (mg DW per plant) of *Arabidopsis* wild-type and D-serine dehydratase (*dsdA*)-expressing plants on half-strength N-free MS with N supplied as 20 mM nitrate alone, 20 mM nitrate + 10 mM of either ammonium or D-serine, and either ammonium or D-serine as sole N sources. Black bars, wild-type plants; gray bars, transgenic plants expressing the *E. coli* gene *dsdA*. Bars represent mean ± SE, $n = 4$. *, statistically significant differences ($P \leq 0.05$) between plant types.

experiments using two types of transgenic *Arabidopsis* plants: one expressing the *E. coli* gene *dsdA* encoding D-serine deaminase, and one expressing the *dao1* gene from *R. gracilis* encoding D-amino acid oxidase (cf. Erikson *et al.*, 2004, 2005). D-serine deaminase catalyses the breakdown of D-Ser, D-Thr and D-alloThr into the respective keto acids, NH_4^+ and water, while D-amino acid oxidase catalyses the oxidation of a wide range of amino acids, including D-Ser, D-Ala, D-Val and D-Ile, into the respective keto acids, NH_4^+ and hydrogen peroxide. *dsdA*-expressing plants exposed to a range of D-Ser concentrations produced increasing amounts of biomass with increasing concentrations of D-Ser (Fig. 7a). By contrast, the growth of wild-type plants was inhibited even at the lowest D-Ser concentration, 0.3 mM (Fig. 7a). Similarly, plants expressing the *dao1* gene displayed increased biomass with increasing concentrations of D-Ala, while wild-type plants were inhibited even at the lowest-tested D-Ala concentration of 0.3 mM (Fig. 7b). The increases in growth of *dao1*-expressing plants with increasing D-Ala concentrations were similar to those of *dsdA*-expressing plants on D-Ser (Fig. 7a,b).

In a separate experiment, *dsdA*-expressing plants and wild-type plants were exposed to NO_3^- and NH_4^+, both alone and in combination with D-Ser. On NO_3^- alone, NH_4^+ alone and the combination of 20 mM NO_3^- plus 10 mM NH_4^+, no significant differences in growth rates between plant types were found (Fig. 8). In addition, growth rates of plants on the 2 : 1 mixture of NO_3^- and NH_4^+ were similar to those on NO_3^- alone, suggesting that NH_4^+ did not promote growth at this concentration. This conclusion was corroborated by the results of supplying NH_4^+ alone, for which growth was even lower than with the 'no N' reference. Including D-Ser in the NO_3^- medium resulted in complete inhibition of the growth of wild-type plants but stimulated the growth of *dsdA*-expressing plants by *c.* 20%. D-Ser alone inhibited the growth of wild-type plants, relative to the 'no N' controls, but stimulated the growth of transgenic plants expressing the *dsdA* gene by similar degrees to the growth added from D-Ser in combinations of NO_3^- and D-Ser (Fig. 8).

Discussion

Numerous studies in recent decades have investigated whether various N forms can function as sources of N for plant growth (Hutchinson & Miller, 1911; Valle & Virtanen, 1965; Bollard, 1966; Turnbull *et al.*, 1995). A general conclusion from these studies is that a limited number of L-amino acids are the only forms of organic N that can support the growth of higher plants. By contrast, studies of root uptake capacities indicate that plants are capable of absorbing a wide range of amino acids (Soldal & Nissen, 1978; Shobert & Komor, 1987; Persson & Näsholm, 2001), suggesting that limitations in the plants'

capacity to metabolize absorbed amino compounds are the primary constraints on their utilization. Several studies have indicated or shown that various plant species are capable of absorbing amino compounds from the soil (Kielland, 1994; Schimel & Chapin, 1996; Raab *et al.*, 1996, 1999; Näsholm *et al.*, 1998, 2000, 2001; Schmidt & Stewart, 1999; Nordin *et al.*, 2001, 2004; Henry & Jefferies, 2002; McFarland *et al.*, 2002; Persson *et al.*, 2003; Kielland *et al.*, 2006; Harrison *et al.*, 2007). However, the extent to which such compounds actually contribute to plant growth is still unclear, and in this context the above-mentioned discrepancy between results from uptake studies and growth studies using amino acids needs to be resolved.

Our data regarding the capacity of *Arabidopsis* to utilize various amino acids for growth correspond well to findings presented by Valle & Virtanen (1965), Bollard (1966), Ferguson (1970) and Turnbull *et al.* (1995). The amino acid that promoted plant growth most efficiently was L-Gln, while L-Asn, L-Asp, Gly, L-Arg and L-Ala also resulted in a higher biomass than the N-free reference (Fig. 2a). However, exposure to L-Val, L-Ile, L-Ser and all tested D-amino acids except D-Ile resulted in lower biomass than the reference. L-Ala and L-Arg have been found to inhibit growth when supplied in combination with nitrate, but to promote growth when supplied as the sole N sources. Joy (1969) found L-Arg to stimulate and L-Ala to inhibit growth of *Lemna minor* when supplied in combination with nitrate and suggested L-Arg to differ from all other amino acids in this respect. Bonner *et al.* (1992) suggested that all amino acids except L-Gln cause growth inhibition and named this phenomenon 'general amino acid inhibition'. These authors also showed that such inhibition could be neutralized, at least partly, by the addition of L-Gln (Bonner & Jensen, 1996, 1997). Our findings show that *Arabidopsis* can utilize several L-amino acids for growth, and hence do not lend support to the hypothesis that amino acids generally inhibit growth. Notably, the range of compounds that promote growth is smaller when nitrate is supplied together with the amino acid, suggesting that compounds that are utilized for growth may also be growth-inhibiting when other sources of N are available.

Root uptake of amino acids and growth

It has been suggested that the amino acid transporter LHT1 may play an important role in root amino acid uptake (Hirner *et al.*, 2006; Svennerstam *et al.*, 2007) and recent evidence implies that AAP1 may also be involved in amino acid absorption by roots (Lee *et al.*, 2007). In our experiment, growth of *LHT1* mutants plants were substantially more strongly reduced than wild-type controls when the concentration of L-Gln was decreased (Fig. 3).

We showed earlier that acquisition of amino acids during growth in sterile culture can be assessed by including ^{15}N-labeled amino acids in the growth media (Svennerstam *et al.*, 2007). In the current study, we expanded these studies to include two concentrations of amino acids (Fig. 4) to study how *Arabidopsis* acquire amino acids at low and at high external concentrations and the effect that loss of LHT1 may have on these processes. In wild-type plants, the amount of N derived from uptake of amino acids was higher than the corresponding fraction of amino acid N in the growth substrate for both L-Gln and L-Ala when tested at the low (corresponding to 30 μM amino acid-N) amino acid concentration but lower than that fraction when tested at a high concentration (corresponding to 3 mM amino acid-N). This suggests capture of amino acids during growth is especially effective at low concentrations. Moreover, differences in ^{15}N contents between wild-type plants and mutants were much larger following the 30 μM than after the 3 mM treatment (Fig. 4), corroborating results presented in Fig. 3 in which effects of the mutation were more clearly displayed at lower concentrations of amino acids. Interestingly, L-Ala-derived N accounted for nearly half of plant N of wild-type plants in the 3 mM amino acid treatment but in spite of this efficient acquisition of L-Ala-N, plant growth was negatively affected (Fig. 2b) suggesting internal metabolism to be the major bottleneck for L-Ala utilization at this concentration.

Hirner *et al.* (2006) found that *LHT1* mutants displayed retarded growth relative to wild-type plants when N was supplied as L-Asp, L-Glu or L-Gln at a concentration of 5 mM, while mutants did not differ from wild-type plants on GABA and actually grew better than wild-type plants when N was supplied as L-Asn, an effect attributed to the fact that L-Asn at this concentration has a growth-retarding effect on wild-type plants and that this effect is alleviated by the impaired L-Asn uptake in the mutant. Similarly, Svennerstam *et al.* (2007) found that *LHT1* mutants display retarded growth on L-Gln as well as on a mixture of four amino acids. From the analysis presented here, we suggest that any consequence of the *LHT1* mutation is likely to have greater effects on growth and on amino acid acquisition during growth at low concentrations of amino acids. In accordance with this notion, we found that plants overexpressing the *LHT1* gene displayed increased growth on all tested amino acids (L-Gln, L-Glu and L-Asn) at 0.5 mM, but the positive effect was less pronounced at 1.5 mM, and at 5 mM, *35SLHT1* and wild-type plants were similar in size (for L-Glu) or smaller (for L-Asn) than wild-type plants. Similarly, Hirner *et al.* (2006) found that growth of *35SLHT1* were retarded on 5 mM L-Asn compared with wild-type plants. Clearly, excess L-Asn uptake had negative consequences for plants. LHT1 is expressed in several tissues (Chen & Bush, 1997; Hirner *et al.*, 2006) and is probably involved in several processes in addition to the process of root uptake. Hirner *et al.* (2006) showed that LHT1 is active in mesophyll cells, retrieving apoplastic amino acids from intracellular spaces as well as functioning in root amino acid uptake. Hence, alterations in expression of LHT1 would probably cause a number of effects, in addition to that on root uptake, and such effects will, of course, limit our ability to infer that variation in root uptake of amino acids causes changes in plant

growth. In a previous study (Svennerstam *et al.*, 2007), we found that growth of *LHT1* mutants was not significantly different from that of wild-type plants during the first 3 wk of growth. In the current study, all measurements on *LHT1* mutants were conducted on plants less than 3 wk old and growth of these mutants was not affected when N was supplied as nitrate (data not shown).

The increased growth of *35SLHT1* plants on low amino acid concentrations suggests that constraints on the uptake of amino acids may impose a *de facto* limit on growth, especially when they are present at low concentrations. This raises the possibility that transgenic plants overexpressing root amino acid transporters may produce more biomass than wild-type counterparts in environments where soils have high organic contents and amino acids are prevalent N forms. LHT1, as with most of the amino acid transporters that have been characterized, seems to be active in transport of a wide range of amino acids (Fischer *et al.*, 1998; Hirner *et al.*, 2006). Thus, any alteration in LHT1 expression would probably affect uptake of a wide range of amino acids. Soil solutions contain a wide range of protenoic and nonprotenoic amino acids in relatively low concentrations and it is therefore possible that growth of plants with altered expression of LHT1 would be even more affected, at least on a relative scale, when grown in natural, high-organic soils compared with controlled environments such as those used in the current study. Increased growth of LHT1-overexpressing plants on amino acids may at first seem an obvious and anticipated effect. For nitrate uptake, however, overexpression of nitrate transporter did not result in increased nitrate accumulation (Fraisier *et al.*, 2000). Thus, for inorganic N, net uptake is strongly affected by efflux and any alteration in one or several of the ammonium and nitrate carriers may be compensated for by an altered rate of efflux of these ions (cf. Britto & Kronzucker, 2004). In this context, the clear growth response recorded on plants overexpressing LHT1 on different amino acids is surprising and may suggest that efflux of amino acids does not compensate for increased influx in plants overexpressing the LHT1 transporter.

D-amino acids

D-amino acids are not major components of the soil solution, but the proportions of Ala, Glu, Asp and Ser present as D-enantiomeric forms may exceed 10% and, thus, such D-amino acids may be available for root absorption in various soils (Amelung & Zhang, 2001). From the study of root uptake capacities (Fig. 1), we can conclude that all tested D-forms are absorbed by *Arabidopsis* roots but that except for D-Ala and D-Ser, rates of uptake are significantly lower compared with the respective L-form. None of the D-amino acids could be used as N sources by wild-type *Arabidopsis* plants, and several compounds, in particular D-Ala and D-Ser, strongly inhibited their growth (Fig. 2a,b). Growth inhibitory effects of various D-amino acids have been described by several researchers (Valle & Virtanen, 1965; Bollard, 1966; Aldag & Young, 1970; Manabe & Ohira, 1981; Erikson *et al.*, 2005), but the underlying mechanism responsible for this inhibition has remained obscure. D-amino acids may compete for tRNA binding with L-amino acids and hence affect protein synthesis through depletion of endogenous tRNA pools (Soutourina *et al.*, 1996). Alternatively, D-amino acid toxicity may depend on competitive inhibition of amino transferases. Thus, D-Ala and D-Ser could inhibit serine-glyoxalate aminotransferase (EC 2.6.1.45) and/or serine hydroxymethyltransferase (EC 2.1.2.1) (Schirch *et al.*, 1985).

Earlier studies have suggested that higher plants differ from algae, mosses, fungi and bacteria in the way absorbed D-amino acids are metabolized (Pokorny *et al.*, 1970), since plant metabolism of absorbed D-amino acids mainly occurs via conjugation with malonylic- or acetic acids, while other organisms oxidize them, thus forming keto acids and NH_4^+ as the major products (Pokorny *et al.*, 1970). This may, at least partly, explain the occurrence of D-amino acids in various tissues of different plant species (Brückner & Westhauser, 2002).

In the present study, a new route for D-amino acid metabolism was introduced in the transgenic plants. This enabled for plants to utilize the two D-amio acids, D-Ser and D-Ala, compounds that had strong growth-inhibiting effects on wild-type plants. To our knowledge, this is the first demonstration of how plants can be engineered to utilize N forms that are inaccessible to wild-type plants. The clear positive growth effects observed of increasing concentrations of D-Ser on *dsdA*-expressing plants and D-Ala on *dao1*-expressing plants (Fig. 7a,b) suggest that the primary decomposition of D-amino acids is the main bottleneck for plant utilization of D-amino acids. D-serine deaminase metabolism of D-Ser results in the formation of ammonium, pyruvate and water. Similarly, DAAO metabolism of D-Ala results in the formation of ammonium, pyruvate and hydrogen peroxide. Hence, growth of transgenic plants on either D-Ser or D-Ala could be compared with that of wild-type plants on ammonium. We therefore studied the growth of wild-type plants and *dsdA*-expressing plants on combinations of NO_3^-, NH_4^+ and D-Ser (Fig. 8). In this comparative experiment, the growth of transgenic plants on D-Ser, both alone and in combination with NO_3^-, was significantly greater than their growth on NO_3^- alone. By contrast, NH_4^+ had no positive effect on plant growth when supplied in combination with NO_3^-, and when supplied as the sole N source it inhibited the growth of both plant types. Clearly, plant growth is severely hampered by high concentrations of NH_4^+ while D-Ser does not cause such growth inhibition. We conclude that plants expressing D-amino acid-metabolizing enzymes can escape the toxicity mechanism acting on wild-type plants (see earlier) and efficiently utilize D-amino acids for growth and that these compounds stimulated the growth of the transgenic plants even in the presence of nitrate. The growth inhibition caused by ammonium could be due to excessive uptake of this N form (Britto & Kronzucker, 2004), and the different responses

towards D-Ser and ammonium could be related to the much higher uptake rate of the latter (Fig. 1). The growth utilization of D-amino acids (D-Ser and D-Ala) by transgenic plants was thus as unproblematic as the L-Gln utilization by wild-type plants. Utilization of single amino acids for growth may be constrained by a general control mechanism of amino acid biosynthesis (Noctor *et al.*, 2002; Voll *et al.*, 2004). This mechanism clearly does not apply, however, to transgenic plants exposed to D-amino acids.

Conclusions

We wanted to study the capacity of the model plant *Arabidopsis* to utilize amino acids as N sources and used both transgenic plants and knock-out mutants to identify the potential bottlenecks in this process. Of the 15 amino acids tested, only six promoted growth when supplied as the sole N source. The results (presented in Fig. 1) indicate that the inability of *Arabidopsis* to use the other N forms is probably not a result of a lack of uptake capacity but rather a result of metabolic constraints. This is especially likely for some of the D-enantiomeric amino acids tested because plants expressing genes encoding D-amino acid-metabolizing enzymes were able to utilize both D-Ser and D-Ala efficiently for growth over a wide range of concentrations, while wild-type plants were severely inhibited by these substances. The amino acids that promoted the growth of wild-type plants were still poor N substrates compared with nitrate. However, plants overexpressing the LHT1 transporter showed increased growth on L-Gln, L-Glu and L-Asn, particularly at low concentrations, suggesting that the growth of wild-type plants may be limited by rates of uptake under such conditions. Concentrations of amino acids in soil solutions are generally in the low micromolar range, hence uptake limitation may be a common restriction for plant amino acid utilization, suggesting that for plants such as *Arabidopsis*, or various crop plants, it may be possible to increase their rates of N uptake from organic soils by increasing their expression of root amino acid transporters.

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References

Aldag RW, Young JL. 1970. D-amino acids in soils. I. Uptake and metabolism by seedling maize and Ryegrass. *Agronomy Journal* **62**: 184–189.

Amelung W, Zhang X. 2001. Determination of amino acid enantiomers in soils. *Soil Biology & Biochemistry* **22**: 553–562.

An Y-Q, McDowell JM, Huang SR, McKinney EC, Chambliss S, Meagher RB. 1996. Strong, constitutive expression of the *Arabidopsis* ACT2/ACT8 actin subclass in vegetative tissues. *Plant Journal* **10**: 107–121.

Bollard EG. 1966. A comparative study of the ability of organic nitrogenous compounds to serve as sole sources of nitrogen for the growth of plants. *Plant & Soil* XXV: 153–166.

Bonner CA, Jensen RA. 1996. Antagonism by L-glutamine of toxicity and growth inhibition caused by other amino acids in suspension cultures of *Nicotiana silvestris. Plant Science* **113**: 43–58.

Bonner CA, Jensen RA. 1997. Recognition of specific patterns of amino acid inhibition of growth in higher plants, uncomplicated by glutamine-reversible 'general amino acid inhibition'. *Plant Science* **130**: 133–143.

Bonner CA, Rodrigues AM, Miller JA, Jensen RA. 1992. Amino acids are general growth inhibitors of *Nicotiana silvestris* in tissue culture. *Physiologia Plantarum* **84**: 319–328.

Britto DT, Kronzucker HJ. 2004. Bioengineering nitrogen acquisition in rice: can novel initiatives in rice genomics and physiology contribute to global food security? *BioEssays* **26**: 683–692.

Brückner H, Westhauser T. 2002. Chromatographic determination of L- and D-amino acids in plants. *Amino acids* **24**: 43–55.

Chen L, Bush DR. 1997. LHT1, a lysine- and histidine-specific amino acid transporter in Arabidopsis. *Plant Physiology* **115**: 1127–1134.

Clough SJ, Bent AF. 1998. Floral dip: a simplified method for *Agrobacterium* mediated transformation of *Arabidopsis thaliana*. *Plant Journal* **16**: 735–743.

Ennor AH, Rosenberg H. 1962. The isolation of N-Phosphoryl-lombricine from earthworms. *Biochemical Journal* **83**: 14–17.

Erikson O, Hertzberg M, Näsholm T. 2004. A conditional marker gene allowing both positive and negative selection in plants. *Nature Biotechnology* **22**: 455–458.

Erikson O, Hertzberg M, Näsholm T. 2005. The *dsdA* gene from *Escherichia coli* provides a novel selectable marker for plant transformation. *Plant Molecular Biology* **57**: 425–433.

Ferguson AR. 1970. Nitrogen metabolism of *Spirodela oligorrhiza*. *Planta* **90**: 365–369.

Fischer W-N, Andre B, Rentsch D, Krolkiewicz S, Tegeder M, Breitkruz K, Frommer WB. 1998. Amino acid transport in plants. *Trends in Plant Science* **3**: 188–195.

Fraisier V, Gojon A, Tillard P, Daniel-Vedele F. 2000. Constitutive expression of a putative high-affinity nitrate transporter in *Nicotiana plumbaginifolia*: evidence for post-transcriptional regulation by a reduced nitrogen source. *Plant Journal* **23**: 489–496.

Harrison KA, Bol R, Bardgett RD. 2007. Preferences for different nitrogen forms by coexisting plant species and soil microbes. *Ecology* **88**: 989–999.

Henry HAL, Jefferies RL. 2002. Free amino acid, ammonium and nitrate concentrations in soil solutions of a grazed coastal marsh in relation to plant growth. *Plant, Cell & Environonment* **25**: 665–675.

Hirner A, Ladwig F, Stransky H, Okumoto S, Keinath M, Harms A, Frommer WB, Koch W. 2006. *Arabidopsis* LHT1 is a high-affinity transporter for cellular amino acid uptake in both root epidermis and leaf mesophyll. *Plant Cell* **18**: 1931–1946.

Hutchinson HB, Miller NHJ. 1911. The direct assimilation of inorganic and organic forms of nitrogen by higher plants. *Centbl Bakt II* **30**: 513–547.

Jämtgård S, Näsholm T, Huss-Danell K. 2008. Uptake of organic nitrogen by Barley. *Plant & Soil* **302**: 221–231.

Joy KW. 1969. Nitrogen metabolism of Lemna minor. I. Growth, nitrogen sources and amino acid inhibition. *Plant Physiology* **44**: 845–848.

Karimi M, Inze D, Depicker A. 2002. GATEWAY vectors for Agrobacterium-mediated plant transformation. *Trends in Plant Sciences* **7**: 193–195.

Kielland K. 1994. Amino-acid-absorption by arctic plants – implications for plant nutrition and nitrogen cycling. *Ecology* **75**: 2373–2383.

Kielland K, McFarland J, Olson K. 2006. Amino acid uptake in deciduous and coniferous taiga ecosystems. *Plant & Soil* **288**: 297–307.

Kielland K, McFarland JW, Ruess RW, Olson K. 2007. Rapid cycling of organic nitrogen in taiga forest ecosystems. *Ecosystems* **10**: 360–368.

Kranabetter JM, Dawson CR, Dunn DE. 2007. Indices of dissolved organic nitrogen ammonium and nitrate across productivity gradients of boreal forests. *Soil Biology & Biochemistry* **39**: 3147–3158.

Lee YH, Foster J, Chen J, Voll LM, Weber APM, Tegeder M. 2007. AAP1 transports uncharged amino acids into roots of *Arabidopsis*. *Plant Journal* **50**: 305–319.

Lipson D, Näsholm T. 2001. The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. *Oecologia* **128**: 305–316.

Manabe H, Ohira K. 1981. Effects of D- and L-Ala on the growth of suspension-cultured rice, soybean and tobacco cells. *Soil Science & Plant Nutrition* **27**: 383–386.

McFarland JW, Ruess RW, Kielland K, Doyle AP. 2002. Cycling dynamics of NH_4^+ and amino acid nitrogen in soils of a deciduous boreal forest ecosystem. Ecosystems 5: 775–788.

Murashige T, Skoog FA. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiologia Plantarum* **15**: 310–313.

Näsholm T, Ekblad A, Nordin A, Giesler R, Högberg M, Högberg P. 1998. Boreal forest plants take up organic nitrogen. *Nature* **392**: 914–916.

Näsholm T, Huss-Danell K, Högberg P. 2000. Uptake of organic nitrogen in the field by four agriculturally important plant species. *Ecology* **81**: 1155–1161.

Näsholm T, Huss-Danell K, Högberg P. 2001. Uptake of glycine by field grown wheat. *New Phytologist* **150**: 59–63.

Noctor G, Novitskaya L, Lea PJ, Foyer CH 2002. Co-ordination of leaf minor amino acid contents in crop species: significance and interpretation. *Journal of Experimental Botany* **53**: 939–945

Nordin A, Högberg P, Näsholm T. 2001. Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. *Oecologia* **129**:125–132.

Nordin A, Schmidt IK, Shaver GR. 2004. Nitrogen uptake by arctic soil microbes and plants in relation to soil nitrogen supply. *Ecology* **85**: 955–962.

Ohlson KEA, Wallmark PH. 1999. Novel calibration with correction for drift and nonlinear response for continuous flow isotope ratio mass spectrometry applied to the determination of ^{15}N, total nitrogen, ^{13}C and total carbon in biological material. *Analyst* **124**: 571–577.

Persson J, Högberg P, Ekblad A, Högberg M, Nordgren A, Näsholm T. 2003. Nitrogen acquisition from inorganic and organic sources by boreal forest plants in the field. *Oecologia* **137**: 252–257.

Persson J, Näsholm T. 2001. Amino acid uptake: a widespread ability among boreal forest plants. *Ecology Letters* **4**: 434–438.

Pokorny M, Marcenko E, Keglevic D. 1970. Comparative studies of L- and D-Methionine metabolism in lower and higher plants. *Phytochemistry* **9**: 2175–2188.

Raab TK, Lipson DA, Monson RK. 1996. Nonmycorrhizal uptake of amino acids by roots of the alpine sedge *Kobresia myosuroides*: implications for the alpine nitrogen cycle. *Oecologia* **108**: 488–494.

Raab TK, Lipson DA, Monson RK. 1999. Soil amino acid utilization among species of the Cyperaceae: plant soil processes. *Ecology* **80**: 2408–2419.

Schimel JP, Chapin FS. 1996. Tundra plant uptake of amino acid and NH_4^+ nitrogen in situ: plants compete well for amino acid N. *Ecology* **77**: 2142–2147.

Schirch V, Hopkins S, Villar E, Angelaccio S. 1985. Serine hydroxymethyltransferase from Escherichia coli: purification and properties. *Journal of Bacteriology* **163**: 1–7.

Schmidt SK, Stewart GR. 1999. Glycine metabolism by plant roots and its occurrence in Australian plant communities. *Australian Journal of Plant Physiology* **26**: 253–264.

Schulten H-R, Schnitzer M. 1998. The chemistry of soil organic nitrogen: a review. *Biology & Fertility of Soils* **25**: 1–15.

Senwo ZN, Tabatabai MA. 1998. Amino acid composition of soil organic matter. *Biology & Fertility of Soils* **26**: 235–242.

Shobert C, Komor E. 1987. Amino-acid-uptake by *Ricinus communis* roots – characterization and physiological significance. *Plant, Cell & Environment* **10**: 493–500.

Soldal T, Nissen P. 1978. Multiphasic uptake of amino acids by barley roots. *Physiologia Plantarum* **43**: 181–188.

Soutourina J, Plateaus P, Delort F, Peirots EA, Blanquet S. 1996. Functional charactersization of the D-Tyr-tRNA^{Tyr} deacylase from *Escherichia coli*. *Journal of Biological Chemistry* **274**: 19109–19114.

Stribley DP, Read DJ. 1980. The biology of mycorrhiza in the Ericaceae. VII. The relationship between mycorrhizal infection and the capacity to utilise simple and complex organic nitrogen sources. *New Phytologist* **86**: 365–371.

Svennerstam H, Ganeteg U, Bellini C, Näsholm T. 2007. Comprehensive screening of *Arabidopsis* mutants suggests the Lysine Histidine Transporter 1 to be involved in plant uptake of amino acids. *Plant Physiology* **143**: 1853–1860.

Turnbull MH, Goodall R, Stewart GR. 1995. The impact of mycorrhizal colonisation upon nitrogen source utilization and metabolism in seedlings of *Eucalyptus grandis* Hill ex Maiden and *Eucalyptis maculata* Hook. *Plant, Cell & Environment* **18**: 1386–1394.

Valle E, Virtanen AI. 1965. On the injurious and growth promoting effects in peas and barley of various amino acids given together with nitrate. *Acta Agralica Fennica* **107**: 308–319.

Virtanen AI, Linkola H. 1946. Organic nitrogen compounds as nitrogen nutrition for higher plants. *Nature* **158**: 515.

Voll LM, Allaire, EE, Fiene G, Weber APM. 2004. The *Arabidopsis* phenylalanine insensitive growth mutant exhibits a deregulated amino acid metabolism. *Plant Physiology* **136**: 3058–3069.

Wright DE. 1962. Amino acid uptake by plant roots. *Archives of Biochemsiry & Biophysics* **97**: 174–180.

Yu Z, Zhang Q, Kraus TEC, Dahlgren RA, Anastasio C, Zasoski RJ 2002. Contribution of amino compounds to dissolved organic nitrogen in forest soils. *Biogeochemistry* **61**: 173–198