Transporters involved in source to sink partitioning of amino acids and ureides: opportunities for crop improvement

Mechthild Tegeder*

School of Biological Sciences, Washington State University, Pullman, WA 99164-4236, USA

* To whom correspondence should be addressed. E-mail: tegeder@wsu.edu

Received 8 November 2013; Revised 12 December 2013; Accepted 17 December 2013

Abstract

In most plant species, amino acids are the predominant chemical forms in which nitrogen is transported. However, in nodulated tropical or subtropical legumes, ureides are the main nitrogen transport compounds. This review describes the partitioning of amino acids and ureides within the plant, and follows their movement from the location of synthesis (source) to the sites of usage (sink). Xylem and phloem connect source and sink organs and serve as routes for long-distance transport of the organic nitrogen. Loading and unloading of these transport pathways might require movement of amino acids and ureides across cell membranes, a task that is mediated by membrane proteins (i.e. transporters) functioning as export or import systems. The current knowledge on amino acid and ureide transporters involved in long-distance transport of nitrogen is provided and their importance for source and sink physiology discussed. The review concludes by exploring possibilities for genetic manipulation of organic nitrogen transporter activities to confer increases in crop productivity.

Key words: Allantoin, allantoic acid, amino acid and ureide transporters, biomass production, genetic manipulation, metabolism, nitrogen fixation and assimilation, nitrogen use efficiency, nutritional quality, photosynthesis, plant defence, root uptake, seed loading, source and sink transport.

Introduction

Plants require relatively high amounts of nitrogen (N) for their growth and development. N is used to synthesize amino acids. These compounds are the building blocks of proteins and act as precursors or amino group donors for synthesis of an array of N metabolites essential for cell structure and function, and plant defence. Most plants take up inorganic N compounds from the soil solution. However, uptake of amino acids plays a role in some environments (Rentsch et al., 2007; Näsholm et al., 2009). Following their uptake, nitrate and ammonium are reduced to amino acids that are the major transport forms of N in most plant species.

Synthesis of amino acids mainly occurs in roots or mature leaves (sources) that export N to supply sinks such as root tips, flowers, fruits, and seeds with the essential nutrient for growth or product synthesis (i.e. starch, proteins, and fatty acids/oils; Rentsch et al., 2007). In nodulated tropical and subtropical legumes (e.g. soybean, common bean, chickpea, and cowpea), ureides represent the major form for long-distance transport of N (Atkins and Smith, 2007). Following fixation of atmospheric di-nitrogen (N₂), allantoin and allantoic acid are produced and transported to the shoot, where they are catabolized, releasing ammonia (NH₃) for re-assimilation into amino acids (Smith and Atkins, 2002; Todd et al., 2006). While root to shoot transport of amino acids and ureides occurs in the xylem, transport of organic N from source leaves to sinks occurs in the phloem.

A fundamental process is the selective partitioning of amino acids and ureides among different organelles, cells, tissues, and organs mediated by various transport mechanisms. These mechanisms must be coordinated and regulated to achieve normal physiological cell functions (Stitt et al., 2002; Lalonde et al., 2003). Membrane transport proteins
contribute to the selective partitioning of amino acids and ureides (Tegeder and Weber, 2006; Tegeder and Rentsch, 2010, Ladwig et al., 2012).

This review follows the route of amino acids and ureides from their location of synthesis to sites of usage. It describes what is known about transporter function in moving organic N from source to sink cells, and addresses the potential of altering amino acid and ureide transport processes for crop improvement.

**Nitrogen uptake, nitrogen assimilation, and xylem loading of amino acids and ureides**

Plants utilize a range of N forms such as the inorganic N molecules nitrate (NO$_3^-$) and ammonium (NH$_4^+$), and organic N compounds including amino acids, peptides, and proteins (Rentsch et al., 2007; Nacry et al., 2013). If inorganic N is taken up, it might be reduced in the root to amino acids that are then transported in the xylem to the shoot (Fig. 1). Alternatively, NO$_3^-$ is loaded into the xylem for reduction to amino acids in the leaves. Root versus shoot N assimilation strongly depends on the plant species but is also influenced by environmental conditions and diurnal rhythm (Pate, 1983, Lam et al., 1995).

Nodulated legumes can use atmospheric N through N$_2$-fixing rhizobia that form a symbiotic relationship with plant roots (Fig. 2). In nodules of tropical and subtropical legumes, the ammonia from N$_2$ fixation is primarily utilized for the synthesis of ureides (Smith and Atkins, 2002). In temperate legumes, such as pea and fava bean, amides are produced (Atkins and Bevers, 1990; Tajima et al., 2004). Following their synthesis, amino acids or ureides are exported from nodules through the nodule vascular system, that is connected to the root vasculature, to ultimately reach the shoot in the xylem transpiration stream.

**Nitrogen uptake by the root and xylem loading**

While some uptake might occur via the root tip, in general, radial N transport in roots follows symplasmic or apoplasmic routes (Fig. 1). In the symplasmic pathway, N is imported into root hairs or epidermal cells and moves from cell to cell via plasmodesmata to the vascular cylinder (Rentsch et al., 2007). Alternatively, N travels in the cell wall space and is imported by the cortical or endodermal cells. Transporter-mediated import into the root symplasm has to take place at or before the endodermis since the endodermal Casparian strip blocks any further inward apoplasmic nutrient flow to

---

**Fig. 1.** Root nitrogen uptake, assimilation and transport to the vasculature. Inorganic and forms of organic nitrogen are taken up by roots, and finally transported in the xylem to the shoot, or in the phloem to the growing root tip. This scheme describes uptake of nitrate (NO$_3^-$) and amino acids (AA). NO$_3^-$ is reduced in root cortical cells via nitrite (NO$_2^-$) and ammonium (NH$_4^+$) to amino acids. In roots of tropical legumes, ureides (Ur) are also synthesized. Following uptake into root hairs or root epidermal cells, or synthesis in the cortex, nitrogen movement towards the vasculature might follow the symplasmic pathway from cell to cell through plasmodesmata. Alternatively, nitrogen moves apoplasmically into the root, and is taken up into the cortical or endodermal cells by importers (cylinder with arrow). Uptake into the symplasm occurs by cells located on the outer side of the Casparian strip (grey line) that blocks inward radial movement through the apoplasm to the vascular system. Once the Casparian strip is circumvented, amino acids, ureides, or nitrate are released into the vascular apoplasm by export/efflux systems (rectangle with arrow) for xylem loading, or they are loaded via the apoplasmic route into the sieve element–companion cell (SE–CC) complex of the phloem. At any point along the symplasmic transport pathway, nitrogen may leak into the root apoplasm and be retrieved into the root symplasm. Nitrogen in the xylem sap might be transferred from xylem to phloem (blue arrow; for details, see Fig. 4). The different colours used for the exporters and importers refer to the transport substrate: amino acids, green; ureides, white; nitrate, black.
the root vasculature. Once N passes this barrier, it might move symplastically through the pericycle and vascular parenchyma cells. For xylem loading, N is released by exporters into the apoplasm from the endodermis, pericycle, or xylem parenchyma cells. N loading into the sieve element–companion cell (SE–CC) complexes of the phloem is likely to be via an apoplasmic pathway (Lalonde et al., 2003).

In crop species, plant roots mainly import NO$_3^-$ and NH$_4^+$, and a relatively large number of AMT (ammonium) and NRT (nitrate) transporters are involved in this step (see recent reviews by Wang et al., 2012; Xu et al., 2012; Nacry et al., 2013). Following uptake, the inorganic N might be reduced to amino acids (Fig. 1). Most of the 20 protein amino acids are produced in plastids and, following their synthesis, are released by unknown transporters into the cytosol, for transport to the shoot. Soils of some ecosystems or in organic agriculture contain relatively high amounts of amino acids that are taken up by roots (Näsholm et al., 2009). Using uptake studies and Arabidopsis mutants, four transporters have been shown to play a role in root acquisition of amino acids. These are amino acid permeases AtAAP1 (Lee et al., 2007) and AtAAP5 (Svennerstam et al., 2008, 2011), lysine/histidine-type transporter AtLHT1 (Hirner et al., 2006; Svennerstam et al., 2007), and the compatible solute/proline transporter AtProT2 (Lehmann et al., 2011). These transporters vary with respect to their substrate affinity and specificity (Rentsch et al., 2007; Tegeder, 2012).

For root to shoot transport, amino acids or nitrate are loaded into root xylem elements, thus requiring export systems (Fig. 1). This task is performed for nitrate by NRT1.5, a low-affinity nitrate transporter located in the plasma membrane of pericycle cells (Lin et al., 2008). Similarly, an Arabidopsis amino acid exchanger (SIAR1) was suggested to function in exporting amino acids, including glutamine, from the pericycle for xylem loading (Ladwig et al., 2012). While its cellular localization in roots remains to be resolved, the bidirectional amino acid transporter, BAT1, might also release organic N into the xylem apoplast for root to shoot transport (Dündar, 2009; Dündar and Bush, 2009). Alternatively, it may play a role in phloem unloading. The carrier-mediated amino acid export seems to be regulated by GDU1 (glutamine dumper) and LOG2 (LOSS OF GDU) potentially by controlling transporter protein turnover (Pilot et al., 2004; Pratelli and Pilot, 2006; Pratelli et al., 2010; Guerra et al., 2013).

Some of the apoplasmic amino acids and nitrate of the vascular cylinder seem to be loaded into the phloem for transport to the root tip, as indicated by expression of Arabidopsis nitrate transporter NRT1.9 and amino acid transporter AAP3 in the companion cells (Okumoto et al., 2004; Wang and Tsay, 2011; Fig. 1). However, unlike nrt1.9 mutants that show reduced downward transport of NO$_3^-$ through the phloem, no phenotype has been detected in aap3 mutants.
In non-nodulated tropical and subtropical legumes, N assimilation in roots mostly leads to synthesis and long-distance transport of amino acids (Fig. 1). However, ureide synthesis also occurs, and allantoin and allantoic acid can make up significant amounts of organic N in the xylem sap, dependent on the developmental stage of the plant (Matsumoto et al., 1977; McNeil and Larue, 1984). While root export systems for xylem loading are yet to be found, ureide importers (i.e. UPS1 proteins) have recently been discovered in common bean (PvUPS1; Pélissier et al., 2004) and soybean (GmUPS1-1 and GmUPS1-2; Collier and Tegeder, 2012). PvUPS1 is localized to the root phloem and presumably functions in N supply to the growing root tip (Pélissier and Tegeder, 2007).

Atmospheric nitrogen fixation and nodule to shoot transfer of amides and ureides

Following reduction of N₂ to NH₄⁺ in bacteroids, NH₄⁺ or amino acids (Day et al., 2001; Lodwig et al., 2003) are released into the cytosol of infected nodule cells, where they are used for glutamine synthesis (Fig. 2). In temperate legumes, asparagine is then synthesized, while in tropical and subtropical legumes the N goes through the de novo purine synthesis pathway followed by purine degradation via xanthine and uric acid (Todd et al., 2006; Werner and Witte, 2011). The ureide allantoin is finally produced in the peroxisomes of non-infected nodule cells, and allantoinic acid is probably synthesized in the smooth endoplasmic reticulum after import of allantoin into this compartment (Werner et al., 2008).

From the place of synthesis, the amides or ureides are transported to the nodule vasculature for long-distance transport to the shoot via the xylem (McClure and Israel, 1979; Streeter, 1979; Atkins et al., 1982; Fig. 2). Similar to the root transport pathways, movement of organic N out of the nodule might occur through a symplastic or apoplastic pathway (see above). Like the root vasculature, the nodule vascular system is surrounded by an endodermis with a Casparian band blocking apoplastic transport. To pass this barrier, apoplastic ureides or amides are re-loaded into the symplasm, an import step that may take place in the cortex or endodermal cells. Up to now, membrane proteins involved in amide transport out of nodules and in amino acid xylem loading have not been found. However, two of a total of three recently identified ureide transporters in soybean, specifically GmUPS1-1 and GmUPS1-2, as well as the common bean PvUPS1, were localized to plasma membranes of the nodule cortical and endodermal cells (Pélissier et al., 2004; Collier and Tegeder, 2012). Repression of the UPS1 transporters in soybean nodules led to decreased export of allantoin and allantoic acid from the nodules, and to reduced N supply of the shoot (Collier and Tegeder, 2012).

While the mechanism(s) of ureide export out of vascular parenchyma cells for xylem loading remains to be resolved, UPS1 transporters were expressed in the nodule phloem (Fig. 2). This localization supports their role in ureide loading into SE–CC complexes to supply root tips with N (Pélissier et al., 2004; Collier and Tegeder, 2012).

Xylem to phloem transfer of amino acids and ureides

The organic N taken up directly from the soil, or synthesized in nodules and roots is transported in the xylem to the shoot. Along the long-distance transport pathway, transfer of amino acids or ureides from the xylem to the phloem (i.e. transport phloem) can occur for direct N delivery to fast growing sinks (Pate et al., 1975; van Bel, 1990; Atkins, 2000; Fig. 3). In ureide-transporting legumes, the N retrieved from the stem xylem also serves as a transient storage pool (Herridge et al., 1978; Rainbird et al., 1984; Diaz-Leal et al., 2012). N storage and transfer might involve the import of amino acids and ureides, which escape by diffusion from the tracheary elements, into the xylem parenchyma with subsequent symplastic movement to the phloem parenchyma cells. Ultimately, amino acids and ureides are released into the apoplasm followed by uptake into the phloem. Thus, transient storage and exchange of N between the xylem and phloem requires three transport events across the plasma membrane. In some plant species, xylem and phloem transfer cells could underpin high assimilate exchange rates (Offer et al., 2003).

The importance of these transfer processes for N distribution within the plant has been demonstrated by physiological studies (Pate et al., 1975; Atkins et al., 1982; Pate, 1983; van Bel, 1984; Atkins, 2000). Furthermore, localization studies of amino acid and ureide transporters to the transport phloem in Arabidopsis (AtAAP2; Hirner et al., 1998; Zhang et al., 2010), pea (PsAAP1; Tegeder et al., 2007), and common bean (PvAAP1, Tan et al., 2008; PvUPS1, Pélissier and Tegeder, 2007) revealed that active import systems are required (Fig. 3). Studies of AAP2 function in Arabidopsis proved that phloem loading of amino acids along the transport highway is essential for N supply to seed sinks (Zhang et al., 2010). In addition, the high-affinity transporter AAP6, for acidic and neutral amino acids (Okamoto et al., 2002), has been localized to the xylem parenchyma cells in Arabidopsis, and reduced phloem amino acid levels in aap6 mutants support its function in exchange of N between xylem and phloem (Hunt et al., 2010). Based on its localization to xylem parenchyma cells, a similar function might be assigned to the common bean UPS1 ureide transporter (Pélissier et al., 2007).

Phloem loading of amino acids and ureides

Due to high transpiration rates, the majority of nodule/root synthesized amino acids or ureides may arrive in mature source leaves through the xylem, where xylem-phloem exchange might occur in the major veins (e.g. AAP2; Zhang et al., 2010; see above), or the reduced N is imported into leaf mesophyll cells (Fig. 4). For instance, Arabidopsis LHT1 is localized to the plasma membrane of mesophyll cells and it has been demonstrated to be responsible for uptake of acidic and neutral amino acids from the leaf apoplasm (Hirner et al., 2006).

For source to sink translocation, newly synthesized amino acids or those resulting from metabolic processes such as photorespiration and protein degradation in leaves (Buchanan-Wollaston, 1997; Rachmilevitch et al., 2004) are
loaded into SE–CC complexes of minor veins (i.e. collection phloem) (Tegeder and Rentsch, 2010). Generally, all protein amino acids are found in the phloem, with glutamate, glutamine, asparagine, aspartate, alanine, and serine often dominating (Riens et al., 1991; Lam et al., 1995; Lohaus and Möllers, 2000; Hunt et al., 2006; Tan et al., 2010; Zhang et al., 2010). Loading of N into the collection phloem might follow an apoplasmic or symplasmic route, dependent on the plant species, the plasmodesmal connectivity between phloem parenchyma and companion cells, and the frequency of plasmodesmata (Turgeon and Wolf, 2009; Patrick, 2013; Fig. 4). In the symplasmic pathway, amino acids diffuse down their respective concentration gradients towards the phloem. Apoplasmic phloem loading is generally found in crop plants and involves the release of amino acids into the cell wall space (e.g. from bundle sheath cells). This transport step is mediated Arabidopsis by SIARS1 (Siliques Are Red; Ludvig et al., 2012) and potentially by BAT1 (Dündar and Bush, 2009), or other as yet unidentified export systems (Okumoto and Pilot, 2011; Tegeder, 2012). In addition, GDU and LOG proteins might regulate amino acid efflux into the leaf apoplasm for phloem loading (Pilot et al., 2004; Pratelli and Pilot, 2006; Pratelli et al., 2010; Guerra et al., 2013).

To date, the function of Arabidopsis transporters in amino acid loading of minor vein phloem has not been demonstrated, although AAPs involved in transport of glutamate and neutral amino acids are predicted to play a major role (Koch et al., 2003; Tegeder and Ward, 2012). For legumes, AAP transporters have been localized to the collection phloem (Tegeder et al., 2007; Tan et al., 2008).

Based on expression and localization studies (Toufighi et al., 2005; Brady et al., 2007), amino acid transporters such as Arabidopsis CAT6 and CAT9 (cationic amino acid transporter; Su et al., 2004; Hammes et al., 2006) and proline transporter ProT1 (Rentsch et al., 1996; Grallath et al., 2005) may also function in phloem loading. On the other hand, ANT1, an aromatic–neutral amino acid transporter in Arabidopsis, might remove organic N from the phloem, as the sieve tube amino acid content was increased in ant1 mutants (Hunt et al., 2006). Transport of amino acids from the companion cells to the sieve element is through plasmodesmata, and NHL26-like proteins might be needed to facilitate this step (Vilaine et al., 2013).

Ureides, unloaded from the transpiration stream, are imported into mesophyll cells probably by UPS transporters (Fig. 4). For example, the common bean UPS1 transporters...
are expressed throughout the leaf, supporting their function in ureide uptake from the leaf apoplast (Pélissier and Tegeder, 2007). Once imported, ureides might be catabolized, and the released NH$_3$ used for re-assimilation into amino acids for leaf metabolic processes or, in soybean, for transient storage within the paraveinal mesophyll cells in the form of vegetative storage proteins (Atkins et al., 1982; Costigan et al., 1987; Lansing and Franceschi, 2000; Todd et al., 2006). Alternatively, ureides are moved into mesophyll cell vacuoles to serve as temporary reserves, or immediately loaded into the phloem for transport to sinks. While an efflux carrier for allantoin and allantoic acid release into the leaf apoplast still needs to be identified, UPS transporters are localized to SE–CC complexes of minor veins, supporting their function in phloem loading (Pélissier and Tegeder, 2007).

**Phloem unloading and transport to seeds**

Unloading mechanisms from the phloem of roots and stems vary along the meristematic, elongation, and maturation zones, and involve symplasmic and apoplastic movement of assimilates (reviewed by Patrick, 2013). In terminal sinks such as root apices, sink leaves, flowers, fruits, and seeds, initial transport of amino acids and ureides from the release phloem to the adjacent parenchyma cells may be symplasmic (Fig. 5).

![Symplasmic transport](image.png)

However, dependent on the sink and its developmental stage, symplasmic or apoplastic transport steps follow (recently reviewed by Tegeder and Rentsch, 2010; Tegeder et al., 2013). In ureide-transporting legumes, much of the allantoin and allantoic acid is symplasmically unloaded into cells comprising the pod wall where ureide catabolism occurs and the released NH$_3$ is re-assimilated into amino acids (Atkins et al., 1982; Rainbird et al., 1984; Atkins and Beevers, 1990; Todd et al., 2006). Transporters are probably needed for recovery of ureides leaked into the apoplasm, although such proteins have not yet been identified. Some of the pod ureides are redirected through the phloem to developing seeds to combine with those being directly delivered from source leaves; and UPS1 seems to function in loading of ureides into SE–CC complexes located in pod walls (Pélissier and Tegeder, 2007).

Phloem unloading into the seed coat occurs symplasmically (Fig. 5). However, since the filial parts of the seed (endosperm and embryo) are largely symplasmically isolated, organic N needs to be released from the maternal seed coat into the seed apoplasmic space, followed by uptake into filial cells (Offler et al., 2003; Tegeder and Weber, 2006; Patrick, 2013). In endospermic seeds (e.g. *Arabidopsis*), besides the export of amino acids from the seed coat and import into the embryo, two additional transport steps might be needed. These are the import of amino acids into the endosperm and subsequent release into the seed apoplasm.

Ureides are catabolized in the seed coat of legumes, and most of the N transported to the cotyledons is in the form of amino acids (Rainbird et al., 1984; Todd et al., 2006; Fig. 5). Expression of UPS1 transporters in seed coats supports ureides, leaked to the seed apoplasm, being retrieved for degradation and final synthesis to amino acids (Pélissier and Tegeder, 2007). Only small amounts, if any, of ureides are taken up by legume cotyledons (Atkins et al., 1982; Rainbird et al., 1984; Streeter, 2005) requiring some activity of an importer (Pélissier and Tegeder, 2007).

In *Arabidopsis* amino acids delivered to, or synthesized in, seed coats are exported into the seed apoplasm by SIAR1, and potentially by SIAR1-related transporters or BAT transporters (Dündar and Bush, 2009; Ladwig et al., 2012). However, their physiological function in N delivery to the embryo axis
or cotyledons still needs to be demonstrated. Transport of amino acids to the growing embryo is developmentally regulated. *Arabidopsis* AAP8 plays an essential role in importing amino acids into the endosperm as about half the fertilized ovules abort in *aap8* siliques (Schmidt et al., 2007). Based on expression and localization studies, *Arabidopsis* transporters AAP1 and CAT6 (Hirner et al., 1998; Hammes et al., 2006), and pea and common bean AAP1 transporters (Tegeder et al., 2000, 2007; Tan et al., 2008) were suggested to import amino acids into the embryo epidermal cells that face the seed apoplasm. In some species, such as broad bean and pea, these epidermal cells are modified to transfer cells (Offler et al., 2003). Evidence for the importance of transporters in amino acid uptake by the embryo has recently been provided for the *Arabidopsis* AAP1 (Sanders et al., 2009). In embryos of *aap1* mutants, amino acid levels were reduced and protein levels decreased.

Following uptake into the embryo through the epidermal cell layer, amino acids move symplasmically to the parenchyma cells (Fig. 5). Here, the organic N is used to support growth or, at later stages of seed development, storage compound synthesis (i.e. oils and starch) and/or accumulation (i.e. storage proteins). In addition, some amino acids might move apoplasmically from the seed apoplasm to embryonic axis/cotyledon parenchyma cells. Supported by expression and localization studies, uptake of the amino acids into these parenchyma cells is most probably mediated, in *Arabidopsis*, pea, and broad bean, by AAP1 transporters (Tegeder et al., 2000; Miranda et al., 2001, 2003; Tan et al., 2008; Sanders et al., 2009).

**Alteration of source to sink transport for crop improvement**

Manipulation of amino acid and ureide transport processes with the goal of improved plant productivity (biomass and seed yield), nutritional quality, and/or adaptation to environmental challenges requires detailed knowledge of the localization (i.e. cell type and membrane), substrate specificity, affinity, and regulation of the transporters, and their integration in source and sink physiology. Key positions for altering N distribution lie with the nature of the long-distance transport pathway and its symplasmic discontinuity (see Figs 1–5). Apoplastic transport steps provide excellent opportunities to regulate assimilate partitioning from source to sink, and generally the export from, or import into, plant cells, or both, might be manipulated.

**Phloem loading supporting vegetative and reproductive growth**

While source-sink relationships are highly complex, sink (fruits and seeds) development and sink N levels depend on
the amounts of N [and carbon (C)] that are transported in the phloem sap. It can generally be assumed that xylem to phloem transfer along the long-distance transport pathway (see Fig. 3) and phloem loading (see Fig. 4) of amino acids and ureides in source leaves are determining factors for seed growth and storage compound accumulation. For example, in *Arabidopsis siar*1 mutants, export of amino acids into the leaf apoplasm and subsequent phloem loading and delivery of amino acids to fruits are decreased, resulting in N-stressed, red siliques (Ladwig *et al.*, 2012). Consequently, increased export from leaf bundle sheath or vascular parenchyma cells mediated by SIAR or other export proteins, such as BAT transporters (Dündar and Bush, 2009), might positively affect amino acid partitioning to sinks. Equally important might be the regulation of export function by, for example, GDU proteins and the ubiquitin ligase LOG2 (Pilot *et al.*, 2004; Pratelli and Pilot, 2006; Pratelli *et al.*, 2010). Overexpression (OE) of GDU proteins led to an increase in amino acid levels of the leaf apoplasm and phloem. In addition, a mutation in *LOG2* suppressed this *GDU1-OE* phenotype, supporting both GDU and LOG2 function in regulating amino acid export from plant cells. However, overexpression of *GDUs* seems not to be a suitable approach for crop improvement since growth of *GDU-OE* plants was retarded, at least when their endogenous, non-cell-specific promoters were used to drive their overexpression (Pratelli *et al.*, 2010).

Recent studies have shown that import of amino acids into the collection phloem is a critical step in regulating long-distance transport (Tan *et al.*, 2010; see Fig. 4). Expression of a yeast S-methyl-methionine transporter in the pea phloem led to increased N as well as sulphate (S) transport to sinks. In addition, positive effects were observed for leaf biomass production, seed yield (i.e. increased pod and seed number, and weight), and seed storage protein accumulation. Of further importance, alteration in phloem loading of the S-containing amino acid not only positively affected sink development and physiology, it also enhanced, probably by feedback regulatory mechanisms, N and S uptake and assimilation, and overall amino acid transport (Tan *et al.*, 2010). Current, yet unpublished work in the Tegeder laboratory overexpressing a broad-spectrum AAP transporter, as well as a UPS ureide transporter, in the phloem of pea and soybean confirms the critical role of these transporters in N import into the phloem as well as their regulatory control over uptake, primary metabolism, and transport processes upstream and downstream of their location. Consequently, alteration of amino acid and ureide import into the collection phloem has a high potential for successful application for plant improvement, leading to higher biomass production (i.e. vegetative growth) and increased fruit or seed development (i.e. reproductive growth).

**Xylem loading supporting nitrogen allocation to leaves and photosynthesis**

Many studies document that biomass formation and plant productivity depend on N supply and its efficient use for photosynthesis (Walch-Liu *et al.*, 2005; Zhu *et al.*, 2007) as well as on proper allocation and recycling of N within the plant (Hirel *et al.*, 2007; Lea and Azevedo, 2007). It is generally agreed that C to N balance is central to feedback control of photosynthesis (Paul and Pellny, 2003), and increased N supply positively regulates photosynthesis and therefore improves utilization of carbohydrates in sink tissues for vegetative and reproductive growth (Sage, 1994; Nakano *et al.*, 1997). Interestingly, in *Arabidopsis aap2* mutants, decreased xylem–phloem transfer and thus increased delivery of amino acids to, and import into, source leaves (see Figs 3 and 4), led to increased leaf protein/RuBisCo levels and strongly enhanced photosynthesis (Zhang *et al.*, 2010). In addition, leaf area was increased and leaf senescence delayed in the mutants, demonstrating that not only C assimilation per leaf area can be enhanced when N allocation to leaves is improved, but also the total photosynthetic leaf area and duration of photosynthesis. The changes in C metabolism in *aap2* plants led to increased source to sink partitioning of sucrose with positive effects on seed fatty acid levels (Zhang *et al.*, 2010) probably due to changes in phloem C level or altered C:N ratios (Corbesier *et al.*, 2002; Lawlor, 2002). Flower and silique development were also improved in *aap2* plants, resulting in an overall seed yield and oil yield increase of 20% or higher dependent on the mutant. It will now be important to apply this approach to crop plants such as canola and soybean. However, effects of alteration of xylem–phloem transfer on leaf physiology and sink development might vary, strongly dependent on the plant species and whether amino acids (or ureides) are mainly synthesized in leaves versus roots during the photoperiod.

Plant growth is generally projected to increase with rising CO₂ levels through increased rates of photosynthesis (Long *et al.*, 2006). However, the increased capacity to use the additional C is often limited by N (Sims *et al.*, 1988; Farage *et al.*, 1998; Stitt and Krapp, 1999). Deficiency in N may cause an increased negative feedback on photosynthesis due to a limited capacity to utilize carbohydrates in sink tissue (Geigenberger *et al.*, 1999). The response of photosynthesis to long-term exposure to elevated CO₂ depends on species and growth conditions, and is ultimately determined by sink utilization (Stitt, 1991; Sage, 1994). Current knowledge on N partitioning suggests that biomass (sink) production in plants can be improved by elevating amino acid allocation to photosynthesis and thereby increasing N use for CO₂ fixation. Since N availability and sink capacity can promote acclimation of plants to elevated CO₂, altered N distribution within the plant and N transport to seeds might prove to be important to maintain or enhance plant growth and productivity under global change conditions.

**Embryo loading supporting seed development and accumulation of storage compounds**

Delivery of N to sinks generally influences development of pods/siliques and seed number, and seed storage compound accumulation (Crawford, 1995; Schmidt *et al.*, 2007; Sanders *et al.*, 2009). Besides manipulating the amount of N loaded into the phloem (see above), increasing import of amino acids...
into the endosperm, embryonic axis, or cotyledons might be equally important for seed quality and yield improvement (see Fig. 5). Principally, this can be achieved by altering unloading of amino acids from seed coats into the apoplasm and increasing assimilate flow through the endosperm via altering the expression and activity of exporters such as SIAR1 (Ladwig et al., 2012) and BAT1 (Dündar and Bush, 2009), and endosperm importers such as AAP8 (Schmidt et al., 2007). Uptake of amino acids into the cotyledon via their epidermal cells, abutting the seed apoplastic space, will be the final crucial step in sink N supply. To date it has not been resolved whether targeted expression of amino acid transporters to this cell layer will enrich seed N and storage protein levels. However, the up-regulation of AAP1 transporters in the cotyledon parenchyma cells of *Vicia narbonensis* and pea led to an increased uptake of amino acids that move apoplastically to these storage cells (Rolleschek et al., 2005; Weigelt et al., 2008).

While alteration of import systems in the embryo/cotyledon generally affects seed/sink protein accumulation (Rolleschek et al., 2005; Weigelt et al., 2008; Sanders et al., 2009), it needs to be tested if the sink number can be improved when increasing amino acid uptake into embryo epidermal cells. Studies with *Arabidopsis aap1* mutants show that decreased N import into the embryo and increased amino acid levels in seed endosperm/apoplast affect source leaf activity, probably by a sink feedback mechanism, finally resulting in decreased sink/silique development (Sanders et al., 2009). In contrast, increased import of amino acids into the legume storage parenchyma cells had no effect on seed yield (Rolleschek et al., 2005; Weigelt et al., 2008). Therefore, to achieve both improved seed yield and storage protein accumulation, simultaneous up-regulation of source–sink translocation of organic N (i.e. phloem loading) and seed loading may be the most promising approach (see Figs 4 and 5).

A balanced intake of amino acids is important for human and animal nutrition. Suboptimal content of essential amino acids, such as methionine and cysteine, occurs in seeds of many crop species including grain legumes and cereals such as maize. In the past, much research has focused on manipulating amino acid synthesis pathways in seeds, with moderate success (Frizzi et al., 2008; Ufaz and Galili, 2008; Jander and Joshi, 2010), but enhanced source to sink translocation of desired amino acids might present a promising alternative. While, to date, no transporters for specific essential amino acids have been found in plants, in a recent approach the yeast *S*-methyl-methionine (*MMPI*) transporter was expressed in the phloem of pea plants to enhance *S*-methionine delivery to seeds and ultimately methionine-rich proteins (i.e. legumin) (Tan et al., 2010). *S*-Methyl-methionine is generally transported in the phloem and converted in the seed coat to methionine (Bourgis et al., 1999; Gallardo et al., 2007; Lee et al., 2008) that is then taken up by the embryo. While *MMPI* expression was successful in improving organic *S* (and *N*) partitioning to developing pea sinks, legumin levels per seed were not improved. This was most probably due to a strongly increased seed number (up to 27%) resulting in a distribution of *S*-methyl-methionine between a large number of sinks. In addition, import of methionine into cotyledons might have caused a bottleneck, and improved expression of a methionine transporter in the epidermal cells of pea cotyledons might solve this problem. Most importantly, this strategy demonstrates that source to sink partitioning of specific, low abundant amino acids can be altered, and future genetic approaches would need to consider both phloem and seed loading of the respective amino acid. It is also noteworthy that cellular export processes of amino acids in *MMPI* plants seem not to be a limiting factor in source–sink translocation of amino acids (Tan et al., 2010).

**Primary nitrogen acquisition and shoot–root balance**

To improve plant productivity, N allocation to photosynthesis and developing sinks might be enhanced by increasing N uptake from the soil or the atmosphere, and its subsequent delivery to the photosynthetic active leaves (see Figs 1 and 2). This might potentially be achieved by overexpressing amino acid, ureide, or inorganic N transporters in the root or nodule cells or indirectly by improved positive shoot feedback regulation.

Root-localized inorganic and organic N transporters control the access to soil N (see Fig. 1). To improve N availability for plant growth, efforts have mainly focused on inorganic N transporters and N assimilation (Masclaux-Daubresse et al., 2010; McAllister et al., 2012; Wang et al., 2012; Xu et al., 2012). However, these approaches have proven to be complicated, as both inorganic N uptake and reduction is strongly regulated by plant N (Lam et al., 1995; Miller and Cramer, 2005; Lea and Azevedo, 2006, 2007; Nacry et al., 2013). For example, high concentrations of amino acids down-regulate expression of nitrate transporters and reductase (Dzuibany et al., 1998; Krapp et al., 1998; Zhuo et al., 1999). While external supply of amino acids negatively regulates N uptake and assimilation, N deficiency or low N tissue concentrations seem to increase expression of inorganic N transporters (Gazzarrini et al., 1999; Sohlenkamp et al., 2000; Loque and von Wirén, 2004), suggesting that they react to plant nutrient status rather than external concentrations (Miller et al., 2008; Amtmann and Blatt, 2009). Similarly, acquisition of amino acids from the soil might be regulated to adjust N uptake to its metabolic and nutritional needs (Tegeder, 2012). Therefore, overexpression of root N transporters might be a successful approach for increasing N uptake and assimilation, if tissue N pools are kept low; for example, by increasing export of nitrate or amino acids from the root or by their accelerated delivery to the growing root tips. This might be achieved in roots by enhanced xylem loading through overexpressing nitrate (NRT; Lin et al., 2008) and amino acid exporters (e.g. SIARs or BAT; Dündar and Bush, 2009; Ladwig et al., 2012) or by accelerating phloem loading via NRT or AAP transporters (Okumoto et al., 2004; Wang and Tsay, 2011). A similar approach might be needed in legume nodules (see Fig. 2), where high internal or soil N levels negatively affect nodule development and N fixation (Herridge et al., 1984; Imsande, 1986). For nodule to shoot delivery of ureides, UPS1 transporters are essential, and their repression led to accumulation
of ureides in nodules, with negative consequences for nodule development and N\textsubscript{2} fixation (Collier and Tegeder, 2012). Increasing ureide or amide export from nodules could potentially be achieved by overexpressing, for example, UPS\textsubscript{1} and amino acid transporters, respectively, in the nodule cortex and endodermis.

N uptake or fixation, and subsequent root to shoot transfer of ureides and amino acids, might, on the other hand, be regulated by shoot N pools and transporters, and associated shoot–root signalling processes (Tegeder, 2012), rather than by xylem loading. Evidence is provided by studies with the acidic and neutral amino acid transporter, LHT\textsubscript{1}, that is strongly expressed in the plasma membrane of leaf mesophyll cells and less so in root tips (Hirner et al., 2006). Lht\textsubscript{1} mutants accumulate high amounts of amino acids in the leaf apoplasm, and concurrently show severely reduced root uptake of amino acids (Svennerstam et al., 2007, 2011), supporting source leaf regulation of N uptake. Further, in aap\textsubscript{2} plants with altered xylem–phloem transfer and increased N import into leaf cells, N acquisition is positively adjusted to the increased requirements of aap\textsubscript{2} shoots (Zhang et al., 2010). In line with this, in legumes, positive feedback, resulting from increased phloem loading of amino acids and ureides, regulates N and S uptake and their subsequent assimilation in roots (Tan et al., 2010; unpublished results). Therefore, and based on our current knowledge, a successful strategy for increasing plant N availability might be increasing the activity of root/nodule transporters to improve N uptake in combination with altering shoot N partitioning processes, to keep the N tissue levels low to avoid negative feedback of N uptake (see Figs. 1, 2, and 4).

**Producing nitrogen-efficient crops**

In cropping systems, a sufficient supply of N is achieved through applying industrially produced N fertilizer. To cover future energy and food needs, N fertilization will need to intensify (Good et al., 2004). This in turn will have detrimental impacts on the biosphere through eutrophication of freshwater and marine ecosystems as well as gaseous emissions, exaggerating environmental changes (Hirel et al., 2007). Strategies will need to be developed to meet growing needs for alternative sources of energy and food security as well as the challenge to protect the planet from harmful pollutants. Crops with improved N use efficiency might require less energy-expensive N fertilization and thereby also reduce N run-offs into the environment. The different aspects of N use efficiency are well addressed in some recent reviews (e.g. Masclaux-Daubresse et al., 2010; Chardon et al., 2012; McAllister et al., 2012; Xu et al., 2012).

Organic N transporters are involved directly or indirectly in plant processes that are crucial for improving N use efficiency. These include N assimilation and partitioning of the N assimilates within the cell, translocation of N over short and long distances, as well as uptake and usage of N in sinks (Tegeder and Weber, 2006; McAllister et al., 2012; Xu et al., 2012; Tegeder et al., 2013). In addition, transporters are required for movement of amino acids from NH\textsubscript{3} recycling through photorespiration or ureide catabolism, and for remobilization of transiently stored amino acids, ureides, and proteins (e.g. vegetative storage proteins) as well as for N remobilization from older and senescing leaves (Delrot et al., 2001; Rentsch et al., 2007). While amino acid and ureide transporters have been identified that accommodate some of the above transport events, many key players are still unknown (Okumo and Pilot, 2012; Tegeder, 2012; this review).

Nevertheless, studies in *Arabidopsis* and pea demonstrated that biomass formation, sink number, seed yield, and storage compounds (i.e. protein or oil) are enhanced when amino acid transport processes are manipulated, most probably by affecting photosynthesis and C/N metabolism (Tan et al., 2010; Zhang et al., 2010). Increased biomass formation and sink capacity in these studies further indicate that N acquisition is adjusted in the transporter mutants/overexpressors. Therefore, plants in which altered amino acid, and potentially ureide, transport processes lead to improved growth and productivity provide promising material to discover clues on how N partitioning is coordinated with other metabolic processes and how it influences photosynthetic and general N use efficiency. To expose organic N transporter mutants/OE plants to sufficient versus low N environments would help to resolve whether amino acid partitioning through these transporters plays an essential role in maintaining or improving N supply for C fixation and source/sink metabolism even under N limitation.

**Role of amino acid transporters in plant defence**

Many studies are available describing the link between primary metabolism and plant-pathogen interaction, and recent reviews provide an excellent overview on the current state of knowledge (e.g. Bolton, 2009; Schultz et al., 2013; Seifi et al., 2013; Zeier, 2013). In general, the N status of the host influences infection and plant resistance to the pathogen, respectively. Host plants often provide organic N in the form of amino acids, and, dependent on the pathogen (e.g. fungi, bacteria, or virus), high or low amino acid levels may present a favourable environment for the invader (Zeier, 2013). Altering amino acid pools via manipulating transporters might therefore ultimately result in two opposite scenarios: support of a plant defence strategy finally to form an effective resistance response or promoting and facilitating infection.

Little is known about the role of amino acid transporters in balancing N pools for plant defence. However, in a recent study analysing fungal infection in the *lht1* mutant, in which amino acid transport into the leaf cells was reduced (Hirner et al., 2006; cf. Fig. 3), it was found that the *lht1* plants displayed increased resistance against a broad spectrum of fungi (Liu et al., 2010). The resistance was caused by enhanced host defence responses promoting hypersensitive cell death in a salicylic acid-dependent manner. Changes in cellular redox status and glutamine levels seem to be responsible for the improved disease resistance. Currently available amino acid transporter
mutants and overexpressors of *Arabidopsis* and crop plants provide an important resource to test a broad spectrum of pathogens and their potential for plant infection in relation to amino acid tissue levels, and to draw conclusions on the importance N transport processes for plant disease resistance.

Amino acids further represent the precursors for lignin and polyamine synthesis as well as for a large spectrum of other secondary metabolites involved in plant defence and attraction of pollinators (Alburquerque et al., 2006; Maeda and Dudareva, 2012). Examples of natural products derived from amino acids include the alkaloids, flavonoids, cyanogenic glycosides, and non-protein amino acids (Maeda and Dudareva, 2012). While these many N metabolites might be synthesized in different cellular compartments, chloroplasts are the major intracellular site of amino acid biosynthesis in plants; and there is a gap in knowledge on chloroplast transporters exporting amino acids from the chloroplast (Weber and Linka, 2011; Tegeder, 2012; see Fig. 4). These plastidic transporters might be fundamental in feeding amino acids into highly diverse and competing pathways of primary and secondary metabolism, including the biosynthesis of many protective plant natural products that finally affect the plant immune system.

Work on plant–parasite interaction demonstrated that the *Arabidopsis* amino acid transporter CAT6 plays a role in supplying N to nematode-induced feeding structures (Hammes et al., 2006). Further, nematode growth and success of progeny were significantly reduced in mutants of root-localized AAPs (Elashry et al., 2013; Marella et al., 2013). Crop losses though nematode infestation are huge worldwide (Sasser et al., 1987), and repression of *AAP* and *CAT* transporters in roots of crop plants might be successful in improving nematode resistance and thereby crop safety, as long as root metabolism and N distribution in the modified plants are not negatively affected.

**Concluding remarks**

Membrane-localized transport proteins are involved in partitioning of the numerous amino acids and ureides within and between cells, and in intermediate and long-distance N transport between tissues and organs. While still only few amino acid and ureide transporters have been functionally analysed in plants, current work demonstrates that they are essential for vegetative and reproductive growth, and that they regulate metabolic and transport processes up- and downstream of their location. Consequently, they are excellent candidates for genetic engineering of crop plants to alter plant biomass, seed yield, and quality, as well as plant performance under environmental challenges and limited N nutrition. However, any genetic approach has to consider the physiology of the plant and differences in N metabolism, N assimilates, and required transporters, as seen for example between plant species. This includes variations in the location of N assimilation (roots versus shoot; nodules versus roots) or in the composition and levels of amino acids and ureides in specific cells, tissues, and organs. In this context, and dependent on the goal of a genetic manipulation, highly specific versus broadly specific transporters, high-affinity versus low-affinity systems, passive versus active transporters, and exporter versus importer might be chosen. Using different amino acid/ureide transporters for crop improvement most certainly will produce different outcomes. Finally, although plants generally display phenotypic plasticity, which would enable them to adapt to changes in N pools when N transport is altered, competition between metabolic pathways, feedback regulation, or changed susceptibility to environmental stresses might need to be carefully evaluated in the transgenic plants. Overall, for successful crop improvement, and for predicting effects of N availability and climate change on future food and energy safety, it will be crucial to understand: (i) the mechanisms controlling N partitioning in plants; (ii) the importance of N transport processes for C assimilation (photosynthesis), N assimilation, and plant N use efficiency; (iii) the role of N transport in natural product synthesis and plant defence; and (iv) how N limitation and elevated CO₂ affect these processes.

**Acknowledgements**

Our work was funded by US National Science Foundation grant 1021286 and Agricultural and Food Research Initiative Competitive grant 2010-65115-20382 from the US Department of Agriculture, National Institute of Food and Agriculture.

**References**


