Chapter 13
The Importance of Organic Nitrogen Transport Processes for Plant Productivity and Nitrogen Use Efficiency

Mechthild Tegeder and Molly Perchlik

Abbreviations

AAP	Amino Acid Permease
AMT	Ammonium Transporter
CAT	Cationic Amino acid Transporter
DUR	Degradation of urea (urea transporter)
LHT	Lysine-Histidine-type Transporter
NRT	Nitrate Transporter
PTR	Peptide Transporter
NPF	NRT1/PTR Family
ProT	Proline Transporter
UmamiT	Usually Multiple Acids Move In and out Transporter
UPS	Ureide Permease

Summary

Plants need large amounts of nitrogen for growth, development, and reproduction. Generally, inorganic nitrogen is acquired from the soil or atmosphere and reduced in nodules, roots, or photosynthetically active source leaves to amino acids or ureides. These organic compounds present the main nitrogen forms transported from source to sink, and their regulated partitioning is critical for plant metabolism, growth, and efficient nitrogen use. Nitrogen uptake and long-distance transport of organic nitrogen from root to leaf to seed requires the function of plasma membrane transporters. Amino acid and ureide transporters are localized to critical positions along the path where they control nitrogen acquisition, export from nodules,

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M. Tegeder (✉) · M. Perchlik
School of Biological Sciences, Washington State University, Pullman, WA 99164, USA
e-mail: tegeder@wsu.edu

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xylem-to-phloem transfer, phloem loading, and seed import. These transporters present important targets for manipulation of nitrogen partitioning to improve seed yield and quality, and nitrogen use efficiency.

Introduction

Nitrogen (N) is an essential macronutrient that is needed in large amounts for plant growth and development. It is an important component of many biological molecules, including amino acids, which are the building blocks of proteins and enzymes. Amino acids are also used as N precursors or donors for the synthesis of nucleic acids, ureides, chlorophyll, and many other N metabolites, such as alkaloids, which are involved in plant defense (Lam et al. 1996; Epstein and Bloom 2005; Zrenner et al. 2006; Miret and Munné-Bosch 2014; Züst and Agrawal 2016). In addition, amino acids are the main long-distance N transport forms in most plant species.

Plants generally acquire inorganic N from the soil through uptake of nitrate and ammonium via transport proteins (Fig. 13.1; Loqué and von Wirén 2004; Krapp et al. 2014). Uptake of organic N compounds also occurs (e.g., amino acids and peptides), especially in ecosystems with low soil N mineralization and in cropping systems that use organic fertilizers such as manure or compost (Farley and Fitter 1999; Rentsch et al. 2007; Näsholm et al. 2009; Tegeder and Rentsch 2010). In addition, legumes can acquire atmospheric dinitrogen through a symbiotic relationship with bacteria that reside in root nodules. This fixed N is reduced to amino acids and ureides in nodules of temperate and tropical legumes, respectively (Bergersen 1971; Schubert 1986; Tegeder 2014). Inorganic N that is taken up by the root is assimilated into amino acids either in roots or in leaves, depending on the plant species, N availability, and the diurnal cycle (Fig. 13.1; Andrews et al. 1992; Lam et al. 1996; Stöhr and Mäck 2001; Ferrario-Méry et al. 2002; Miller et al. 2007; Xu et al. 2012; Krapp 2015). Amino acids that are taken up or synthesized in roots are either metabolized within root cells or transported to the shoot for use (Miflin and Lea 1977; Schobert and Komor 1990).

In plants that mainly reduce N in roots, the newly produced amino acids are translocated in the xylem transpiration stream to source leaves (Fig. 13.1; Miflin and Lea 1977; Schobert and Komor 1990). Along this path, amino acids can be transferred from the xylem to the phloem to directly supply developing sinks, such as young leaves, flowers, and seeds with N (Pate et al. 1975; van Bel 1984; Zhang et al. 2010). However, many plant species preferentially transport nitrate to source leaves where photosynthesis provides the reductants and carbon skeletons for amino acid synthesis (Andrews 1986; Lam et al. 1996; Lewis et al. 2000; Nunes-Nesi et al. 2010; Tegeder and Masclaux-Daubresse 2017).

In leaves, the organic N compounds are either used for metabolism, stored as amino acids, ureides or proteins, or exported into the phloem to supply developing sink tissues with N (Ellis 1979; Millard 1988; Liu et al. 2005; Lee et al. 2014;

Table 13.1 Overview on genetic approaches used to increase nitrogen transporter expression in crop plants and their impact on seed yield, protein levels, and nitrogen use efficiency when plants were grown with varying nitrogen (N) supply

Species	Genetic approach	N supply	N regime	Seed yield	NUE	NUpE	NUtE	Protein	Additional phenotype	References
Rice	*PTR9/NPF8.20* OE	0 N	none	↑↑	↑↑			↕ seed	↑ biomass	Fang et al. (2013)
		0.25 mM $(NH_4)_2SO_4$	low	↑	↑			↑ seed	↑ biomass	
		5 mM $(NH_4)_2SO_4$	high	↑	↑			↑ seed	↑ biomass	
		2 mM $NaNO_3$	medium	↑	↑			↑ seed	↑ biomass	
		1 mM NH_4NO_3	medium	↑↑	↑↑			↕ seed	↑ biomass	
Rice	*NRT2.1* OE	180 kg urea/ha	low	↑	↑				↑ biomass	Chen et al. (2016)
									↑ biomass	
		300 kg urea/ha	normal	↑	↑				↑ shoot N	
Rice	*AMT1-3* OE	0, 0.02 g NH_4NO_3	low	↓	↓			↕ leaf	↓↓ biomass	Bao et al. (2015)
		0.2, 1 g NH_4NO_3	high	↓	↓			↑ leaf	↓ biomass	
Rice	*AMT1.1* OE	0.03 mM $(NH_4)_2SO_4$	low	↑					↑ biomass	Ranathunge et al. (2014)
		0.3 mM $(NH_4)_2SO_4$	optimum	↑					↕ biomass	
		3 mM $(NH_4)_2SO_4$	high	↕					↓ biomass	
Rice	*AMT1.1* OE	10 µM NH_4^+	low						↑ NH_4 uptake	Kumar et al. (2006)
									↓ biomass	
		2 mM NH_4^+	high						↑ NH_4 uptake	
									↕ biomass	

(continued)

Table 13.1 (continued)

Species	Genetic approach	N supply	N regime	Seed yield	NUE	NUpE	NUtE	Protein	Additional phenotype	References
Pea	*ScMMP1*	Peter's 20-20-20*	high	↑				↑ seed	↑ biomass	Tan et al. (2010)
Pea	*AAP1* OE	5 mM NH_4NO_3	low	↑	↑	↕	↑	↓ seed	↑ biomass	Zhang et al. (2015), Perchlik and Tegeder (2017)
		10 mM H_4NO_3	medium	↑	↑	↑	↑	↕ seed	↑ biomass	
		2x 10 mM NH_4NO_3	high	↑	↑	↑	↕	↑ seed	↑ biomass	
Soybean	*PvUPS1* OE	atmospheric N_2		↑					↑ N fixation	Carter and Tegeder (2016)

Arrows refer to an increase (↑), decrease (↓), or no change (↕). AAP, amino acid permease; AMT, ammonium transporter; N_2, atmospheric dinitrogen NH_4^+, ammonium; NO_3^-, nitrate; NPF; NRT1/PTR family; NRT, nitrate transporter; NUE, nitrogen use efficiency; NUpE, nitrogen uptake efficiency; NUtE, nitrogen utilization efficiency; OE, overexpression; PTR, peptide transporter; *Pv*UPS1, *Phaseolus vulgaris* ureide transporter 1, *Sc*MMP1, *Saccharomyces cerevisiae* S-methyl-methionine transporter

*Peter's 20-20-20 contains 20% N consisting of 50% urea-N, 30% nitrate-N and 20% ammonium-N

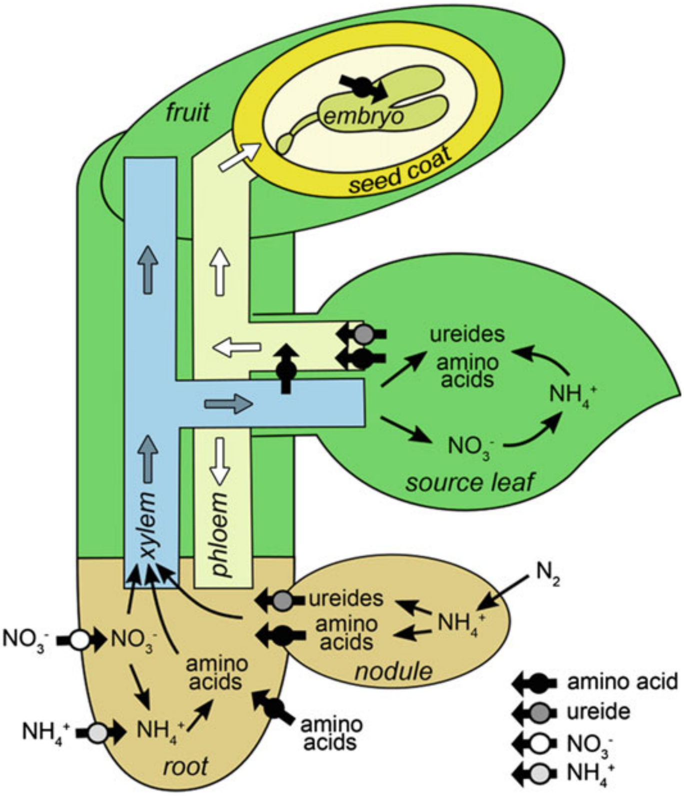

Fig. 13.1 Model of nitrogen (N) fixation, uptake, assimilation, and partitioning in plants. Legumes fix atmospheric N_2 through a symbiotic relationship with bacteria in root nodules. In tropical and temperate legumes, the fixed N is reduced to ureides or amino acids, which are transported from the nodules via the xylem to the shoot. Alternatively, plants take up amino acid and inorganic N (ammonium, NH_4^+; nitrate, NO_3^-), the later being from assimilated into amino acids in the roots or shoot. Root amino acids (and NO_3^-) are moved in the xylem to the shoot. Some of the xylem amino acids are transferred along the transport path to the phloem for direct N supply of sinks. However, the majority of amino acids are delivered with the transpiration stream to source leaves. Root-derived amino acids and ureides, and leaf-synthesized amino acids are loaded into the phloem for N supply of developing sinks such as fruits and seeds. Ureides are converted within the seed coat to amino acids. Amino acids are released from the seed coat into the seed apoplast followed by import into embryo (for details, see recent reviews Tegeder 2014; Tegeder and Masclaux-Daubresse 2017). Soil-to-root and root-to-shoot-to-seed N transport require a series of membrane transport steps. Some transporters (arrows with a circle) that are localized in key positions where they control N uptake and partitioning to sinks are indicated (see text and also Table 13.1)

Tegeder and Masclaux-Daubresse 2017). The N compounds enter the phloem primarily in leaf minor veins and are then moved toward sinks using an osmotic pressure gradient (Fig. 13.1; Pate 1980; Knoblauch et al. 2016). Once in the sink, N compounds are symplasmically unloaded from the phloem and move toward the sink cells. Within the seed coat of seed sinks, organic N catabolism, transamination, and re-assimilation processes may occur (Atkins et al. 1975; Rainbird et al. 1984; Weber et al. 1995; Gallardo et al. 2007), followed by release of amino acids into the seed apoplast and import into the embryo for development and storage compound accumulation (Patrick 1997; Offler et al. 2003).

Efficient plant uptake, allocation, and use of N in source and sink are essential for plant biomass production and reproductive success (Fig. 13.2). In modern cropping systems, large amounts of industrially produced N fertilizers are supplied in order to guarantee N availability for maximum seed yields. However, many crop plants inefficiently acquire and use N, and an increase in N fertilization is often not proportional to increases in yield production (Ju et al. 2004; Delin and Stenberg 2014; Lassaletta et al. 2014; Mueller et al. 2014; Zhu et al. 2016). Depending on the crop species, soil conditions, and N supply, plants may take up less than half of the N fertilizer (Raun and Johnson 1999; Kumar and Goh 2002; Yang et al. 2015; Zhu et al. 2016). While a number of factors may impact N uptake and usage, including inorganic N import into the roots, N assimilation, and its regulation may (Kumar et al. 2006; Tsay et al. 2011; Ruffel et al. 2011; Nacry et al. 2013; Bao et al. 2015; Stahl et al. 2016), recent studies suggest that amino acid and/or ureide partitioning processes within the plant also play essential roles in plant productivity and N use efficiency (Fig. 13.2; Rolletschek et al. 2005; Schmidt et al. 2007; Weigelt et al. 2008; Tan et al. 2010; Zhang et al. 2010, 2015; Carter and Tegeder 2016; Santiago and Tegeder 2016).

Long-distance partitioning of amino acids and ureides requires plasma membrane-bound transport proteins that facilitate movement of the organic N from nodules or roots to leaves and finally to sinks (Fig. 13.1; Delrot et al. 2001; Rentsch et al. 2007; Tegeder 2014). This review discusses key transporters that control N root uptake, and root-to-shoot and leaf-to-seed partitioning of organic N, and examines their importance for plant growth, development, and seed production. Further, we evaluate the importance of amino acid transporters in source and sink for the efficiency of N uptake and use.

Importance of Nitrogen Root Uptake Systems for Plant Performance

Root N uptake from the soil is mediated by plasma membrane transporters, influenced by the availability of soil N, and controlled by plant N assimilation processes and the N demand of the plant (Fig. 13.1; Ruffel et al. 2011; Nacry et al. 2013; Stahl et al. 2016). A range of inorganic and organic N transporters with varying substrate specificities and affinities are present in roots (Wang et al. 1998; Sonoda et al. 2003; Yan et al. 2011; Haynes 2012; Fan et al. 2017). This diversity enables the root to regulate uptake in response to varying soil environments, including different N forms and concentrations.

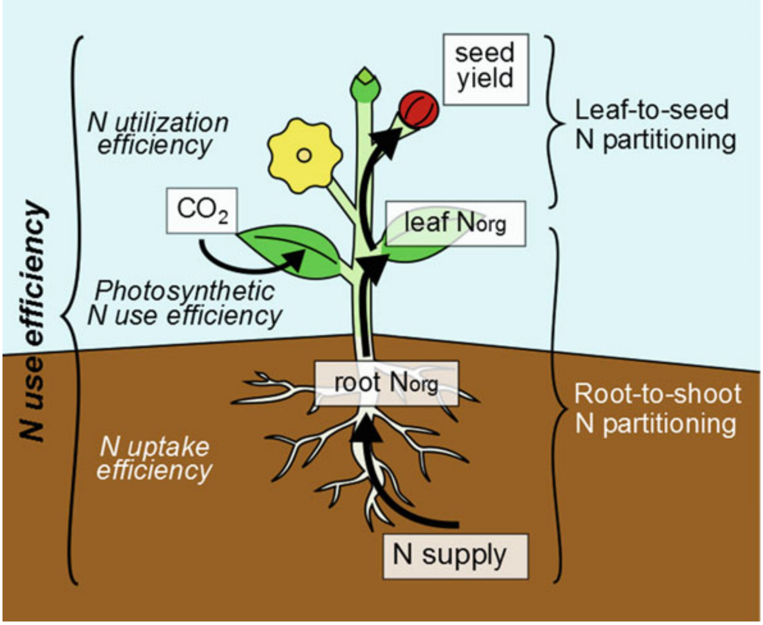

Fig. 13.2 Model on nitrogen (N) uptake, partitioning, and N use efficiency. Uptake of inorganic and organic N (N_{org}) and root-to-sink partitioning of N_{org} are indicated. N use efficiency (NUE) is defined as the amount of seed yield relative to N supply (Moll et al. 1982). NUE is comprised of two components: N uptake (NUpE) and utilization efficiency (NUtE). NUpE is the proportion of N in the shoot relative to the N supply, and it is influenced by the amount of N uptake and root-to-shoot N partitioning. NUtE describes the amount of shoot N used for seed production, which is influenced by leaf-to-seed N partitioning. Additionally, NUE is affected by photosynthetic N use efficiency, or the rate of CO_2 fixation per area leaf N (Commichau et al. 2006; Makino 2011)

Inorganic Nitrogen Uptake

Nitrate uptake is achieved by transporters of the NRT1 family (Nitrate Transporter1), also named NPF transporters (Nitrate Transporter1/Peptide Transporter Family), and the NRT2 family (Fan et al. 2017). NRT1 transporters are mostly low-affinity systems (Huang et al. 1999; Liu et al. 1999; Guo et al. 2002; Léran et al. 2014), while NRT2 transporters primarily function in high-affinity nitrate uptake (Wang et al. 1998; Okamoto et al. 2003; Li et al. 2007; Yan et al. 2011). Studies on natural variation and using manipulation of *NRT1* or *NRT2* expression have shown that NRT function affects N acquisition, plant productivity, and N use for seed production (Table 13.1). For example, overexpression of *ZmNRT1.1* and *ZmNRT1.3* in maize roots led to improved nitrate uptake, seed yield, and N use efficiency (Allen et al. 2016). Likewise, variation in *NRT1.1B/OsNPF6.5* expression in root and shoot tissue of the *Oryza sativa* (rice) subspecies *indica* and *japonica* seems to correlate with efficient use of N for seed development (Hu et al. 2015). Further, *OsNRT2.1* overexpression in rice roots and leaves resulted in increased biomass, seed yield, and N use efficiency (Chen et al. 2016; 2017). Similar successes have also been reported when *NRT1* or *NRT2* transporters were constitutively overexpressed in rice (Table 13.1; Fang et al. 2013; Fan et al. 2016a, b; Feng et al. 2017).

Uptake of ammonium is regulated by saturable, high-affinity ammonium transporters (AMTs) and non-saturable, low-affinity systems (*i.e.*, aquaporins or cation channels) (Glass et al. 2002; Sonoda et al. 2003; Jahn et al. 2004; Loqué et al. 2005; Lea and Azevedo 2006; Guo et al. 2007; Bárzana et al. 2014). In *Arabidopsis thaliana*, six AMT genes are present, while 10 AMTs have been identified in rice. Up to date, genetic manipulation of ammonium transporters had relatively little success. For example, overexpression of *OsAMT1;1* and *OsAMT1;3* in rice under control of the CaMV-35S promoter resulted in increased ammonium uptake, but biomass and seed yield were either not changed or decreased (Table 13.1; Kumar et al. 2006; Bao et al. 2015). Nevertheless, the use of a ubiquitin promoter-*OsAMT1;1* construct led to more biomass and seed productivity (Table 13.1; Ranathunge et al. 2014). Overall, manipulation of ammonium transport processes to improve plant N uptake and use may be challenging, since alterations in cellular ammonium pools or excess ammonium can be toxic for the plant cell (Britto and Kronzucker 2002; Bittsánszky et al. 2015).

Uptake of Organic Nitrogen

High amounts of organic N may be found in cropping systems that rely on manure or compost for N nutrition (Khan 1971; Gregorich et al. 1994; Senwo and Tabatabai 1998). Although peptides, proteins, and other N compounds can be acquired by the plant, research on root uptake of organic N has mainly focused on amino acids (Rentsch et al. 2007; Komarova et al. 2008; Paungfoo-Lonhienne et al. 2008; Tegeder and Masclaux-Daubresse 2017). In Arabidopsis, five transporters have been shown to affect amino acid uptake by roots, and these include Amino Acid Permeases AAP1 and AAP5, Proline Transporter ProT2, and Lysine-Histidine-type Transporters LHT1 and LHT6 (Grallath et al. 2005; Hirner et al. 2006; Lee et al. 2007; Svennerstam et al. 2007, 2008, 2011; Lehmann et al. 2011; Perchlik et al. 2014; Ganeteg et al. 2017). However, all of these amino acid transporters were characterized using Arabidopsis mutants and it still remains to be examined if and how their increased expression in roots affects N acquisition and usage, and plant growth.

Soils often contain considerable amounts of urea (Kojima et al. 2006). Following microbial hydrolysis, a majority of the urea-N is accessible to the plant as ammonium, but direct urea uptake also occurs (Mérigout et al. 2008). As shown in Arabidopsis and rice, urea is actively taken up and transported within root tissues via the high-affinity urea transporter DUR3 (Kojima et al. 2007; Wang et al. 2012;

Bohner et al. 2015). Constitutive expression of rice *DUR3* in Arabidopsis *dur3-1* mutants resulted in increased urea uptake and shoot growth, suggesting manipulation of root urea import as a potential approach to improve plant performance (Wang et al. 2012).

Function of Nodule Ureide Transporters in Atmospheric Nitrogen Fixation and Plant Growth

Legumes can access the large atmospheric N pool through a symbiotic interaction with rhizobia that are housed in root nodules (Fig. 13.1). The final organic products available to the plant are amides in nodules of temperate legumes (e.g., pea, *Pisum sativum*) and the ureides allantoin and allantoic acid in case of tropical legumes such as soybean (*Glycine max*) and common bean (*Phaseolus vulgaris*) (Streeter 1979; Scharff et al. 2003; Tajima et al. 2004; Todd et al. 2006; Atkins and Smith 2007). While the molecular mechanisms for nodule amide transport processes remain to be identified, transport of ureides out of the nodules requires the function of UPS1 (Ureide Permease 1) proteins (Pélissier et al. 2004; Collier and Tegeder 2012; Carter and Tegeder 2016). When overexpressing the common bean *UPS1* transporter (Pélissier et al. 2004; Pélissier and Tegeder 2007) in soybean nodules, ureide transport from nodules to shoot and finally to seeds were increased resulting in a significantly improved seed yield (Table 13.1; Carter and Tegeder 2016). In addition, atmospheric N fixation was enhanced in the transgenic versus control nodules by around 100%. Together, these results support that organic N export out of the nodules is a key regulatory step in N fixation, shoot N supply, and seed development in legumes. The data further suggest significant improvements in plant N acquisition and use efficiency.

Role of Amino Acid Transporters in Root-to-Shoot Nitrogen Supply and Photosynthetic Use Efficiency

Amino acids that are taken up from the soil or synthesized in roots or nodules are translocated in the xylem mainly to photosynthetically active, transpiring leaves (Figs. 13.1 and 13.2; Miflin and Lea 1977; Schobert and Komor 1990). The rate of N transport from the root to leaves is influenced by the rate of N flux from the root cells to the xylem, transpiration rate and its associated hydrostatic pressure gradient between root and leaf, and import of N into the mesophyll cells (Engels et al. 1992; Gouia et al. 1994; Windt et al. 2006). Amino acid xylem loading requires the export of amino acids into the apoplast from either nodule or root endodermal cells, the pericycle or vascular parenchyma cells (Tegeder 2014). Root-localized Usually Multiple Acids Move In and out Transporters (UmamiTs) are predicted to function in this efflux step (Ladwig et al. 2012; Müller et al. 2015; Besnard et al. 2016).

Import of the organic N into the leaf cells is, at least in part, mediated by LHT1, since a mutation in the transporter results in decreased uptake of amino acids by mesophyll cells and their accumulation in the apoplast, overall negatively affecting growth (Hirner et al. 2006; Liu et al. 2010; Svennerstam et al. 2011). Future studies will need to address if an increase in amino acid import into the source leaf cells affects N flux rates from the roots to the shoot and, subsequently, root N uptake and assimilation.

Not all amino acids that are transported out of the root are directed to leaves. In particular during vegetative phase, up to 21% (van Bel 1984) of the organic N may be retrieved from the transpiration stream along the pathway for metabolism (Bailey and Leegood 2016), establishment of N storage pools (Streeter 1979; Millard 1988), or xylem-to-phloem transfer to directly supply growing sinks with N (Fig. 13.1; Dickson et al. 1985; Pate et al. 1975; van Bel 1984; 1990). Arabidopsis AAP6 is localized to the vascular parenchyma and is thought to be involved in amino acid removal from the xylem (Hunt et al. 2010), while AAP2 has been shown to function in amino acid loading into the transport phloem (Zhang et al. 2010). Mutants of *aap6* and *aap2* both demonstrated reduced phloem amino acid levels, and in *aap2* plants less N was transported to developing sinks, resulting in decreased seed protein levels (Hunt et al. 2010; Zhang et al. 2010). However, no negative effects were observed with respect to *aap2* seed yield or seed germination rates (Zhang et al. 2010). On the contrary, in *aap2* mutants, xylem allocation of amino acids to leaves was elevated, leading to increased carbon fixation (Zhang et al. 2010). Overall, leaf carbon metabolism and partitioning to *aap2* siliques and seeds were enhanced, which resulted in higher fatty acid levels per seed, seed number, and seed oil yields. This suggests that at least for oil (or starch) crop plants, optimizing N allocation to photosynthetically active source leaves presents a promising approach to increase seed carbon/oil/starch yields, and potentially photosynthetic N use efficiency (Makino and Osmond 1991; Escudero and Mediavilla 2003; Dordas and Sioulas 2008).

Influence of Phloem Loading of Amino Acids and Source-to-Sink Transport on Seed Development

Xylem-derived and leaf-synthesized amino acids are used for leaf metabolism, transiently stored in amino acid or protein pools, or loaded into the phloem for translocation to sinks (Fig. 13.1 and 13.2; Tegeder and Masclaux-Daubresse 2017). The amount of N that is allocated to sinks and used for seed development is affected by several physiological factors including N uptake, metabolism, and source-to-sink allocation (Habash et al. 2001; Tsay et al. 2011; Girondé et al. 2015). In particular during senescence, leaves are considered strong sources for amino acids, and effective N mobilization during leaf senescence and redistribution to sinks can significantly impact the efficiency of N utilization for seed development (Moll et al. 1982; Muurinen et al. 2007; Masclaux-Daubresse and Chardon 2011). The phloem is primarily comprised of sieve elements and companion cells (SEs/CCs), which accommodate the long-distance transport of amino acids to sink (Kempers et al. 1998; Oparka and Turgeon 1999). Depending on the plant species and frequency of functional plasmodesmata, phloem loading occurs either symplasmically via plasmodesmata, or apoplastically involving cellular export and import processes (van Bel 1993; Rennie and Turgeon 2009). At least with respect to sucrose, many crop plants are considered to be apoplastic phloem loaders (Geiger et al. 1973; Winter et al. 1992; Aoki et al. 2004; Slewinski et al. 2009; Chen et al. 2012), and a similar phloem-loading mechanism is assumed for amino acids and other N-containing compounds (Servaites et al. 1979; Lohaus et al. 1995; Fischer et al. 1998). In apoplastic loading, amino acids are passively exported from parenchyma or bundle sheath cells into the cell wall space. In Arabidopsis, UmamiT18/SIAR1 is involved in this efflux step (Ladwig et al. 2012) and potentially BAT1 (Dündar and Bush 2009). The amino acids move within the apoplastic space to the SE-CC complex of the phloem where they are actively taken up (Dündar and Bush 2009; Ladwig et al. 2012; Santiago and Tegeder 2016). Based on localization studies in Arabidopsis, pea, and common bean, several members of the AAP transporter family have been identified as potential phloem loaders (Tegeder et al. 2007; Tan et al. 2008; Tegeder and Rentsch 2010; Tegeder and Ward 2012). However, up to date a function in amino acid import into the SEs/CCs has only been demonstrated for AAP8 (Santiago and Tegeder 2016). Analysis of Arabidopsis *aap8* mutants showed decreased amino acid import into the phloem resulting in reduced seed yield. The study suggests that amino acid transporter function in phloem loading regulates seed number and size, and is most probably important for efficient N utilization for seed development (Santiago and Tegeder 2016).

The importance of amino acid phloem loading for sink development has further been demonstrated by overexpressing a yeast sulfur (S)-methyl-methionine transporter (*i.e.*, *MMP1*) in the leaf phloem (and embryo) of pea plants (Tegeder et al. 2007; Tan et al. 2010). Long-distance transport of S-containing amino acids, including S-methyl-methionine, was increased in the transgenic plants, positively co-regulating amino acid metabolism and source-to-sink allocation, and seed N import (Tan et al. 2010). Together, this led to increased biomass production, seed yield, and seed protein levels. However, S import into the embryo was unchanged. S-methyl-methionine is converted in seed coats to methionine, and results suggest that the ‘pulling force’ for methionine uptake by the embryo was limited in the transgenic plants and that seed loading of methionine or other S compounds may present a bottleneck in increasing S-rich, high-quality seed storage proteins (Tan et al. 2010).

Amino Acid Transporter Function in Seed Sinks and Their Importance for Nitrogen Storage Pools

Developing fruits and seeds are major sinks for N during reproductive phase. Phloem unloading in seeds is generally assumed to occur via the symplasmic pathway through plasmodesmata (Patrick 1997). However, post-phloem transport of amino acids into seeds involves both apoplastic and symplasmic transport routes dependent on the developmental stage and seed tissue (Peoples et al. 1985; Patrick 1997; Offler et al. 2003; Stadler et al. 2005; Müller et al. 2015). Symplasmic isolations occur between the outer and inner integuments of the seed coat (Schneitz et al. 1995; Stadler et al. 2005). Further, the maternal seed coat encircles the endosperm and the developing embryo, which are all symplasmically disconnected (Stadler et al. 2005). Overall, the lack of plasmodesmata necessitates a sequence of export and import steps to finally release the amino acids into the seed apoplast for uptake by the embryo. Arabidopsis transporters that are involved in amino acid movement toward the embryo for development and/or storage protein synthesis include UmamiTs, the Cationic Amino acid Transporter CAT6 and AAPs (Fig. 13.1; Hammes et al. 2006; Schmidt et al. 2007 Sanders et al. 2009; Ladwig et al. 2012, Müller et al. 2015).

The majority of N uptake by the embryo happens via the outer cotyledon epidermal cells exposed to the seed apoplast, although some of the N may move apoplastically and is taken up into the storage cells of the cotyledon parenchyma (Offler et al. 2003). Up to date, only AAP1 has been shown to function in import of amino acids into the embryo (Sanders et al. 2009). Studies with Arabidopsis *aap1* mutants resolved that decreased amino acid uptake by embryo epidermis cells (and potentially parenchyma cells) led to reduced seed protein levels. In addition, source leaf N metabolism and source-to-sink N allocation seemed negatively affected, ultimately resulting in decreased silique development (Sanders et al. 2009). These results suggest that transporter function in seed sinks controls seed N storage pool, and may negatively feedback regulate sink development. However, when overexpressing *AAP1* in the storage parenchyma cells of pea and *Vicia narbonensis* cotyledons, N uptake into the embryo was increased, but seed yields were not altered in the transgenic legumes (Rolletschek et al. 2005, Weigelt et al. 2008). Together with the function of phloem transporters (see above), this indicates that both amino acid loading into the phloem and import into the embryo may present bottlenecks for efficient use of N for seed development and establishment of seed N pools.

Effects of Concurrent Increases in Amino Acid Phloem and Seed Loading on Seed Yield and Plant Nitrogen Use Efficiency

Plants generally display a trade-off between seed number and seed protein accumulation (Martre et al. 2003; Seiffert et al. 2004; Gambín and Borrás 2010; Drechsler et al. 2015; Santiago and Tegeder 2016). The amount of amino acids loaded into the phloem and allocated to sinks could therefore impact fruit and seed number, and/or seed N level (Santiago and Tegeder 2016; Tan et al. 2010). In addition, seed import processes influence seed protein pools (Lemaître et al. 2008; Sanders et al. 2009; Tan et al. 2010, Drechsler et al. 2015; Santiago and Tegeder 2016; Rolletschek et al. 2005, Weigelt et al. 2008; see above). To increase both, the amount of N that is ‘pushed’ into the phloem and the amount that is ‘pulled’ into the seed, recently an *AAP1* amino acid transporter was simultaneously overexpressed in the phloem and embryo of pea plants (Zhang et al. 2015). In these plants, phloem loading and seed import of amino acids were increased leading to higher seed numbers and significantly enhanced seed yields and seed storage protein levels when grown in very high N environments. In addition, N uptake and metabolism were upregulated, probably via feedback control.

The *AAP1* pea plants were further examined with respect to plant nitrogen use efficiency (NUE) and by evaluating the seed yield relative to high, moderate and low N applications (Table 13.1; Perchlik and Tegeder 2017). Regardless of the N supply, the *AAP1* plants performed better than controls and exhibited improved NUE. In addition, the transgenic plants achieved the same seed yield as controls with half the amount of N fertilizer. When analyzing the different components of NUE, specifically N utilization efficiency (NUtE) and N uptake efficiency (NUpE), some variations were observed (c.f. Fig. 13.2). Under high N, only NUpE efficiency was improved, while under low N, NUtE was enhanced. However, both NUpE and NUtE were significantly increased when N supply was moderate (Perchlik and Tegeder 2017). Overall, the data suggest that engineering amino acid partitioning from leaf to seed provides a promising approach for plant breeding not only to facilitate improved seed yield and quality, but also to support efficient plant N use.

Conclusions

Organic N allocation is an essential component for establishing seed yield and seed N pools. Nodule ureide transporters and amino acid transporters involved in N root-to-shoot movement, xylem-to-phloem transfer, phloem loading, and seed import are critical in regulating N partitioning to sinks. The function of these transporters also has significant impact on N soil uptake, N utilization in source and sink, and overall plant N use. Repression or targeted overexpression of key ureide and amino acid transport proteins can positively affect both N and carbon metabolism and partitioning in plants leading to increases in biomass production, seed development, and N use efficiency. However, current data mostly derive from studies with plants grown in controlled environments, and it will now be crucial test their performance under field conditions. Further, organic N transport processes from root to seed involve a series of export and import steps for a range of amino acids (and ureides), and many of the responsible transporters have not yet been characterized, although they may present promising targets to alter N allocation to specific organs or tissues and to optimize plant N use efficiency.

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