REVIEWS IN ADVANCE

Plant Nitrogen Assimilation and Use Efficiency

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Annu. Rev. Plant Biol. 2012. 63:5.1–5.30

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

This article's doi: 10.1146/annurev-arplant-042811-105532

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1543-5008/12/0602-0001$20.00

Keywords

nitrate, ammonium, nitrogen uptake, nitrogen remobilization, carbohydrate metabolism, phytohormone

Abstract

Crop productivity relies heavily on nitrogen (N) fertilization. Production and application of N fertilizers consume huge amounts of energy, and excess is detrimental to the environment; therefore, increasing plant N use efficiency (NUE) is essential for the development of sustainable agriculture. Plant NUE is inherently complex, as each step—including N uptake, translocation, assimilation, and remobilization—is governed by multiple interacting genetic and environmental factors. The limiting factors in plant metabolism for maximizing NUE are different at high and low N supplies, indicating great potential for improving the NUE of current cultivars, which were bred in well-fertilized soil. Decreasing environmental losses and increasing the productivity of crop-acquired N requires the coordination of carbohydrate and N metabolism to give high yields. Increasing both the grain and N harvest index to drive N acquisition and utilization are important approaches for breeding future high-NUE cultivars.

Contents

INTRODUCTION

Nitrogen (N) is a primary constituent of the nucleotides and proteins that are essential for life. Because most nonlegume plants require 20–50 g of N taken up by their roots to produce 1 kg of dry biomass, the natural supply of soil N usually limits plant yields in most agricultural cropping systems (132). Together with crop breeding, the production and application of chemical N

fertilizers during the past five decades has resulted in greatly increased global food production and decreased world hunger (46, 67). The Declaration of the World Summit on Food Security (35) calls for an average annual increase in food production of 44 million metric tons to feed approximately 9 billion people by 2050 (157). Accordingly, N fertilizer application is expected to increase by approximately threefold in the next 40 years (46) unless N use efficiency (NUE) is significantly increased.

The biological conversion of N_2 in the air to plant-available ammonium by symbiotic bacteria is another major source of N input in agriculture besides chemical N fertilizers. The global annual N inputs through biological N_2 fixation in various agricultural systems total approximately 50–70 Tg (53). Several recent reviews have described the limiting factors for increasing N_2 fixation in plants (27, 53, 134) and the prospects for genetically engineering N_2-fixing cereals (11), so this review will not cover this topic for crops.

The benefits of N added to cropping systems come with well-documented energy and environmental costs. In a collaborative report, the International Fertilizer Industry Association (**http://www.fertilizer.org**) and United Nations Environment Programme estimated that production of 1 metric ton of fertilizer N synthesized through the Haber-Bosch process consumes 873 m^3 of natural gas (19a, table 3.3). For many crops, N fertilization has become the highest input cost, and this cost will only increase as resources become scarcer. Excess N compounds released from agricultural systems threaten the quality of air, water, and soil. Increased soil leaching into drainage water and the release of atmospheric nitrous oxide and reactive N gases (NO_x, NH_3) into the troposphere accelerate the eutrophication of waterways and acidify soils (48, 132). Because the intricate effects of reactive N cascade through its many chemical forms, N pollution poses an even greater challenge than carbon (C); excess N in the environment is also currently costing the European Union between €70 billion and €320 billion per year (150). Improving NUE is therefore crucial, and represents a significant challenge.

As a function of multiple interacting genetic and environmental factors, NUE is inherently complex. The definition of NUE itself is also complex, and the term can mean different things in different contexts, including N use efficiency (NUE), N uptake efficiency (NUpE), N utilization (assimilation) efficiency (NUtE), apparent N recovery rate (ANR), agronomy efficiency of fertilizer N (AE), N physiological use efficiency (NpUE), N transport efficiency (NTE), and N remobilization efficiency (NRE) (see the definitions presented in the margin of this review). A number of reviews have summarized broader aspects of NUE (31, 40, 44, 46, 54, 105, 132). In general, two plant physiological components—NUpE and NUtE—contribute to plant NUE. Owing to the effects that adding external N has on the complex N form interconversions governed by soil microbial activity, the different mobilities of soil N forms, and the loss of gaseous N from the soil/plant canopy, it is difficult to quantify the "real" amount of fertilizer N available or actually acquired by plants.

Here we comment on the N-regulated biological components of NUE and the genes identified as being important for NUE, as well as the effect of a plant's environment on the expression of those genes. Based on current knowledge, we propose some possible approaches to improve NUE by breeding and molecular manipulation in the future.

PATHWAY OF NITROGEN FROM RHIZOSPHERE TO SEEDS

Root-Induced Changes in Nitrogen Forms and Concentrations in the Rhizosphere

In aerobic soils, the major form of inorganic N is nitrate; in flooded wetland or acidic soils, the major form is ammonium. In the rhizosphere, the root can release oxygen and exudates that greatly influence local redox potential and the density and activity of microbial populations,

Nitrogen use efficiency (NUE): the total biomass or grain yield produced per unit of applied fertilizer N; it is an integration of NUpE and NUtE

Nitrogen uptake efficiency (NUpE): the capacity of plant roots to acquire N from the soil (commonly referred to as the percentage of fertilizer N acquired by plant)

Nitrogen utilization (assimilation) efficiency (NUtE): the fraction of plant-acquired N to be converted to total plant biomass or grain yield

Apparent nitrogen recovery rate (ANR): The ratio of net increased total N uptake by the plant with and without N fertilization to total amount of fertilizer N

Agronomy efficiency of fertilizer nitrogen (AE): The ratio of net increased grain weight of the plant with and without N fertilization to total amount of fertilizer N

Nitrogen physiological use efficiency (NpUE): the ratio of net increased grain weight to net increased N uptake with and without application of fertilizer N

Nitrogen transport efficiency (NTE): The ratio of total N transported into the above ground parts to total N in the whole plant

Nitrogen remobilization efficiency (NRE): the ratio of N remobilization from source or senescent leaves to that of sink leaves or developing grains (seeds)

Rhizosphere: a narrow region of the soil surrounding the roots that is directly influenced by root secretions and associated soil microorganisms

GS: glutamine synthetase

GOGAT: glutamine-2-oxoglutarate aminotransferase

Asparagine synthetase (AS): enzyme that catalyzes the formation of asparagine and glutamate from glutamine and aspartate

GDH: glutamate dehydrogenase

Photorespiration: a process by which a C_3 plant consumes oxygen and releases carbon dioxide during leaf photosynthesis

which in turn can interconvert soil N forms, including those derived from fertilizer. For example, rice roots in paddy soils release oxygen via their aerenchyma and generate rapid nitrification on their surface, and thus take up N as nitrate at a rate comparable with that of ammonium uptake (72, 91). Direct molecular evidence for nitrate uptake in rice has been presented (173). Ammonium or nitrate N uptake by roots commonly results in acidification or alkalization of the rhizosphere, which in turn changes the soil N availability for plants (102).

Nitrogen Acquisition

To cope with the heterogeneity and dynamic variations of nitrate and ammonium concentrations, which range from lower than 100 μM to higher than 10 mM in soil solutions (109), plant roots have uptake systems for both nitrate and ammonium with different affinities. Each high- and low-affinity nitrate transport system is composed of constitutive and nitrate-inducible components (109). Numerous membrane proteins function in nitrate uptake, compartmentation, translocation, and remobilization (24). Both the root architecture and the activities of ammonium and nitrate transporters regulated by N form and concentration, diurnal fluctuations, and temperature fluctuations affect N acquisition by roots (40, 43, 44).

Nitrogen Assimilation

For many plants, some nitrate taken up by the roots is assimilated into the roots, but the larger part is transported to the shoot, where it is first reduced to nitrite by nitrate reductase in the cytoplasm and then further to ammonium by nitrite reductase in the plastids and glutamine synthetase (GS) in the plastids and cytoplasm (**Figure 1**; 84). The ammonium derived from nitrate or directly from ammonium uptake by ammonium transporters (AMTs) is further assimilated into amino acids via the GS/glutamine-2-oxoglutarate aminotransferase (GOGAT) cycle. The predominant GS/GOGAT isoenzymes are chloroplastic GS2 and Fd-GOGAT and cytosolic GS1 and NADH-GOGAT.

The glutamate (Glu) amino group can be transferred to amino acids by a number of different aminotransferases (84). Asparagine synthetase (AS) catalyzes the formation of asparagine (Asn) and Glu from glutamine (Gln) and aspartate. Together with GS, AS is believed to play a crucial role in primary N metabolism. In addition, the mitochondrial NADH–glutamate dehydrogenase (GDH) can alternatively incorporate ammonium into Glu in response to high levels of ammonium under stress (105).

RuBisCO accounts for 50% of the total soluble protein in the leaves of C_3 plants and 20% in the leaves of C_4 plants (120). In C_3 plants, oxygenation by RuBisCO leads to the release of CO_2 and photorespiratory ammonia (19). In addition, various catabolic biochemical processes in plants, such as protein degradation and amino acid deamination, release ammonia (NH_3) (1, 84). The C skeletons produced by photosynthesis are required to assimilate inorganic N into amino acids (84).

Nitrogen Transportation and Remobilization

Long-distance nitrate transport to different parts of a plant can be finely tuned. For example, AtNRT1.5 and AtNRT1.8, the two closely related low-affinity nitrate transporters (NRT1s) in *Arabidopsis*, are involved in loading and unloading into the root stele or from the shoot vasculature (89, 94). AtNRT1.9 in root companion cells facilitates the loading of nitrate into the root phloem and enhances downward nitrate transport in roots (165).

During the vegetative stage, the leaves are a sink for N; later, during senescence, this N is remobilized for reuse in the developing seeds, mainly as amino acids (114; **Figure 1**). Up to 95% of seed protein is derived from amino acids that are exported to the seed after the degradation of existing proteins in leaves (155). Increases of both Asn and Gln concentrations during senescence in the phloem sap suggests their key role in rendering N available for remobilization from the senescing leaves (105).

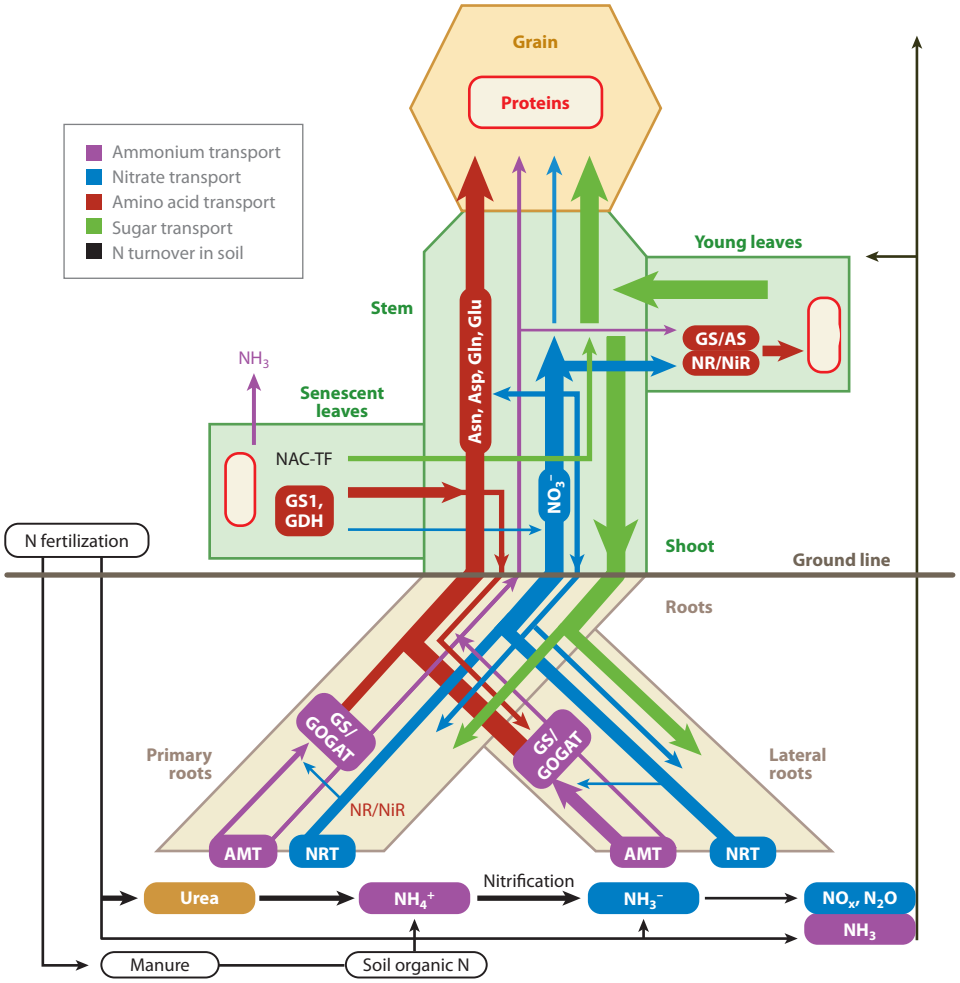

Figure 1

Schematic routes of N uptake from the rhizosphere including the source of fertilizer N to be acquired, mainly in the form of ammonium and nitrate by roots, transportation and assimilation, and remobilization inside the plant. The thicknesses of the arrows schematically represent the relative amounts of nitrogen and sugar inside the plant. Abbreviations: AMT, ammonium transporter; AS, asparagine synthetase; Asn, asparagine; Asp, aspartate; GDH, glutamate dehydrogenase; Gln, glutamine; Glu, glutamate; GOGAT, glutamine-2-oxoglutarate aminotransferase; GS, glutamine synthetase; NAC-TF, certain transcription factors belonging to the NAC family; NiR, nitrite reductase; NR, nitrate reductase; NRT, nitrate transporter.

Nitrogen Efflux from Roots

Nitrate and ammonium efflux to the external media are a component of their net uptake (43, 44). A nitrate excretion transporter belonging to the NRT1 family, NAXT1, has been identified in *Arabidopsis* (141). NAXT1, electrically coupled to the ATP-dependant H^+-pumping activity, has passive low-affinity nitrate efflux transport activity (K_m = 5 mM). NAXT1 expression is upregulated at the posttranscriptional level (141). The precise physiological role of the nitrate efflux transporter(s) needs to be characterized.

Ammonium efflux in roots occurs even in plants with nitrate as the only source of N

Harvest index (HI): the proportion of the biomass of the grains (seeds) to that of the whole plant [grain weight/(vegetative organ weight + grain weight)]

(34), suggesting that substantial futile cycling of ammonium occurs during net transport of ammonium into the root tissue of these plants. Ammonium efflux from the root elongation zone is linked with an inhibitory effect of ammonium on primary root development, mainly through repression of cell elongation (90).

Volatile Nitrogen Losses from Aboveground Parts

During leaf photorespiration, ammonium is released during methylene tetrahydrofolate synthesis from glycine (125). The main factor for volatilization loss of nitrogenous compounds (NH_3 as the prevalent form) from aboveground parts is the imbalance between N accumulation and N assimilation in plants. Differences in NH_3 emission rates among rice cultivars are related to the activity of GS involved in photorespiratory NH_3 recycling (78). Accumulated gaseous N losses in excess of 40 kg of N per hectare have been documented in soybean and maize (127). Failure to include direct plant N losses when calculating N budget leads to an overestimation of N losses in soil and underestimation of plant NUpE.

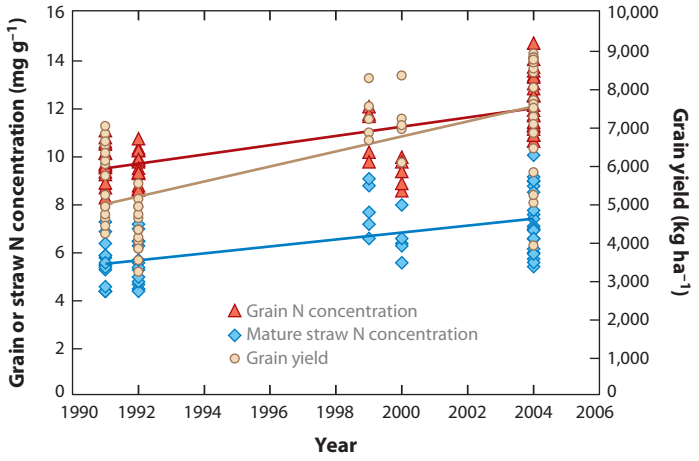

Figure 2

Relationship changes between grain N concentration, mature straw N concentration, and grain yield at harvest for a total of 62 rice cultivars grown in paddy cultivation from 1991 to 2004; changes are indicated in red triangles, blue diamonds, and black circles and their respective trend lines. Abbreviation: ha, hectare. Original data from Inhapanya et al. (64), Koutroubas & Ntanos (75), Ladha et al. (83), and Y.L. Zhang, G.H. Xu & Q.R. Shen, unpublished data.

GENETICALLY CONTROLLED DIFFERENCES IN NITROGEN USE EFFICIENCY

Natural Variation in Different Genotypes of the Same Plant Species

There is much genetic variation in traits that contribute to NUE, including total N uptake, postanthesis N uptake, N translocation, and N assimilation among different varieties of the same species (10, 16, 22; **Figure 2**). The total N uptake from soil is affected by the developmental stage of the plant. Therefore, for accurate fertilizer N recommendation, it is important to evaluate differences in NUE at several developmental stages besides that at harvest for different cultivars (22). Cultivars with more reproductive tillers and a higher harvest index (HI) demand more C and N during grain filling and thus may result in higher NpUE (127).

N uptake and remobilization appear to be independently inherited traits, so favorable alleles could be combined when breeding for high NUE (10, 22). Comparing different wheat genotypes showed that the protein ratio of leaf GS2 to GS1 was variable (2), suggesting that modulating the activities of these enzymes should be considered for future efforts at breeding for high NUE.

Variation of Nitrogen Use Efficiency at Limited and Sufficient Nitrogen Conditions

Plant responsiveness to N availability depends on both genotype and the interaction of genotype with N fertilization level (10). In general, NUE and NRE are higher at low N supplies than at high N supplies. Limiting steps in plant N metabolism are different under high and low N levels (16). At high N inputs, major variation in NUE is contributed mainly by differences in N uptake, particularly postanthesis N uptake; in low-N-input maize and *Arabidopsis*, in

contrast, NUE variation is determined largely by changes in N remobilization and NUtE (10, 104). This result appears to be the opposite of that in wheat, where NUE is related to NUpE at low N levels (88).

The evolutionary trade-off between high productivity and adaptation to low-nutrient environments presents a challenge to most current cultivars, which were selected in (and for) nutrient-rich environments (127). For example, high-yield breeding in Chinese maize hybrids has improved shoot growth at both N-sufficient and limited conditions, whereas root growth was improved only under N-sufficient conditions, indicating that root growth traits have been inadvertently selected to adapt to the increasing N supply in the environment (171). Therefore, breeding high-NUE cultivars should occur under conditions of moderate N supply, with the goal of maintaining high grain yield. Interestingly, the genetically controlled variation of NUE among a core collection of *Arabidopsis* accessions was largely unaffected by N supply levels at the vegetative stage (10). This surprising observation might be due to the lack of agronomic selection criteria for noncultivated plants to adapt to nutrient-rich soil conditions, unlike crops like rice, wheat, and maize.

AGRONOMY EFFICIENCY OF SOIL NITROGEN AND FERTILIZER NITROGEN

Soil and Fertilizer Nitrogen Use Efficiency

The major pathways of N losses from soil include leaching to surface and ground water, denitrification to N_2, volatilization of NH_3, fluxes of N_2O and NO_x to the atmosphere (**Figure 1**), and soil erosion. In most annual crop systems, uptake of N from soil at significant rates lasts for only 8–12 weeks, and the mismatching of N availability with crop needs is probably the single greatest contributor to excess N losses (132).

Fertilizer N management will continue to be the most important option for improving use efficiency in the short term. The adopted technologies of fertilizer application include deep placement, controlled release materials, and multiple-split applications based on leaf chlorophyll levels and N concentration in the plant (83). In addition, using biological sources of N, such as *Azolla* and legumes, as green manures (27) to replace or supplement fertilizer N becomes more attractive as chemical and energy costs increase.

Integrated Nutrient Management in Intensive Agriculture

Many technological approaches to improve N management in agricultural systems have been described (67, 132). The most comprehensive solution is to redesign the cropping system by making use of management tools such as rotations, intercropping, and perennial crops. This approach may require drastic changes to current systems, but may be necessary when considering agricultural sustainability over a longer time frame. Better prediction of soil-available N supplies, crop N, and water needs can improve NUE by tailoring applications of fertilizer N to site-specific conditions to decrease N losses and optimize crop performance (67). The crop N status can also be estimated in real time by remote sensing of the visible light reflected from the canopy and by satellite-derived hyperspectral images for the spatial and temporal variability of N in leaves (15). These new techniques are particularly helpful to improve midseason N management.

NITROGEN UPTAKE EFFICIENCY

Nitrogen-Regulated Root System

Breeding crop varieties that are more efficient at capturing soil N during the entire growing season can decrease N leaching and denitrification losses. Root architecture, morphology, and transporter activity for available forms of N in the rhizosphere determine N uptake rate. It is known that N form and concentrations regulate

Transceptor: a cell plasma membrane protein that has a dual nutrient transporter and receptor (signaling) function

root architecture (102). A localized supply of ammonium mainly stimulates lateral root initiation (93), whereas nitrate strongly promotes the elongation of lateral roots (177). Nitrate induces AFG3 (auxin signaling F-box 3) and N metabolite enhances miR393 levels to modulate root architecture (161). A dual-affinity nitrate transporter, CHL1 (NRT1.1), senses external nitrate concentration as a transceptor and activates the *ANR1* (a MADS-box gene)–mediated nitrate-signaling pathway to regulate nitrate-stimulated lateral root proliferation (56, 129, 177). Some AMTs (e.g., LjAMT1;3) and a GMPase (GDP mannose pyrophosphorylase) encoded by *HSN1* (*hypersensitive to* NH_4^+) play a role in ammonium-regulated root growth (93, 123).

The overall efficiency of the root system in taking up N depends not only on the root architecture but also on the availability of C provided by photosynthesis, and this efficiency is necessary to maintain root activity. Lateral root initiation, regulated by the high-affinity nitrate transporter NRT2.1, can be stimulated at low sucrose levels in the growth medium but suppressed by high sucrose levels (95, 130). The variability of some root morphophysiological traits could be directly dependent on genetic differences in total N uptake, remobilization, leaf greenness, and grain yield independent of the N fertilization supply (16, 17). However, larger roots take away more C from the shoots, limiting the plant's capacity to fix and store C in the harvested aboveground yield. Increased N uptake by large roots could decrease N store remobilization in plants, thus affecting NUE (17). This issue is complicated by the fact that larger roots provide more soil C storage capacity, an important way of countering increased atmospheric CO_2.

Function of Nitrate Transporters

Three families of transporters—NRT1, NRT2 (or NAR2/NRT2), and CLC—have been identified for uptake and translocation of nitrate in plants (24). Most NRT1 family members characterized so far are low-affinity nitrate transporters; an exception is NRT1.1 (CHL1), which operates over both ranges. Some NRT2 members require a partner protein, NAR2, for nitrate transport at relatively low concentration ranges (33; **Figure 3**). Among CLC members, CLCa mediates nitrate accumulation in the vacuole (23; **Figure 4**).

Expression of the NRTs is regulated by nitrate, N metabolites, N starvation, circadian rhythm, sucrose, and pH (33, 77). Two nitrate-inducible kinases, CIPK8 and CIPK23 (calcineurin B-like interaction protein kinases 8 and 23), are either positive regulators for the low-affinity phase of NRT1.1 activity or negative regulators for the high-affinity phase (56, 60). Such genetically distinct regulation of low- and high-affinity primary nitrate transport responses indicates that there are likely to be differential regulators determining NUpE at deficient and sufficient N levels.

There are fundamental differences between *Arabidopsis* and grass species in the gene number and family structure of the NRTs (122). Significant separation in the NRT2 phylogenetic trees indicates that determination of function of the NRT2 genes in cereals based simply on sequence homology to functionally characterized *Arabidopsis* NRT2 genes may not be possible.

There are five NRT2 family members in rice, each showing different affinities and regulation patterns by N supply form (33, 173; **Figure 3**). Unlike its ortholog in *Arabidopsis*, the OsNAR2.1 accessory protein interacts with three NRT2 transporters (NRT2.1, NRT2.2, and NRT2.3a) at both the messenger RNA (mRNA) and protein levels and plays an important role in nitrate uptake over both high and low concentration ranges (**Figure 3**). In addition to comparing functions between mono- and eudicotyledonous plants, it is important to understand the contribution and regulation of NRT family members to NUE for nitrate- and ammonium-preferring plants.

Function of Ammonium Transporters

Ammonium uptake is carried out by plasma membrane (PM)–located AMT/MEP/Rh

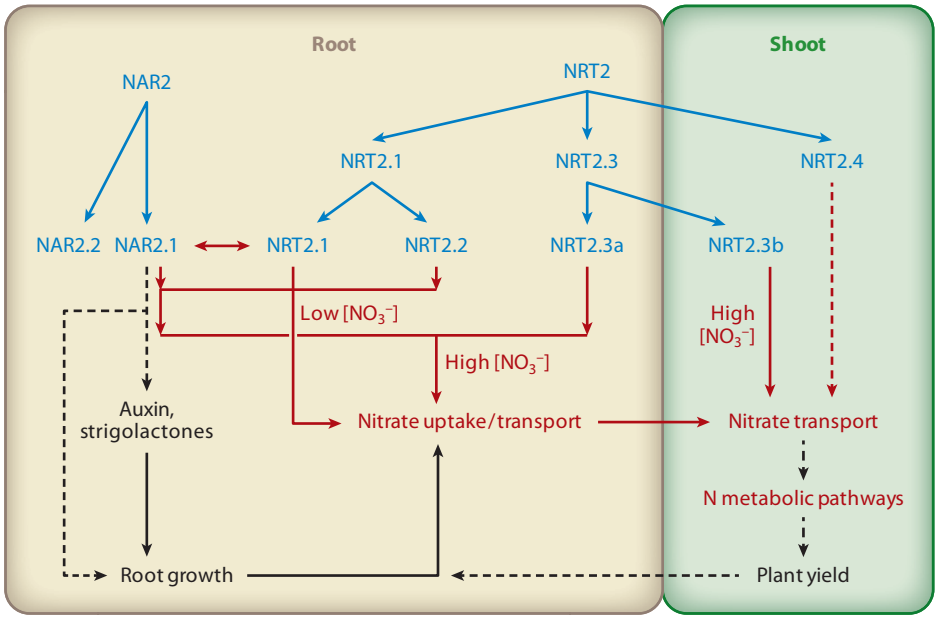

Figure 3

Schematic representation of proposed evolution and characterized and predicted functions for the rice NAR2/NRT2 nitrate transporters. OsNAR2.1, OsNAR2.2, OsNRT2.1, OsNRT2.2, and OsNRT2.3a are expressed mainly in roots; OsNRT2.3b and OsNRT2.4 are expressed mainly in shoots (33, 173). Both OsNRT2.1 and OsNRT2.2 associated with OsNAR2.1 transport nitrate in the high-affinity concentration range. OsNRT2.3a requires OsNAR2.1 for the nitrate transport function, and the protein has a 10-fold lower affinity for nitrate than OsNRT2.1 and OsNRT2.2. OsNAR2.1 can provide a switch, depending on the partner transporter, to enable a rapid response in uptake over the dynamic ranges of external nitrate concentrations (33, 173). In contrast, OsNRT2.3b can function in nitrate transport independently, mainly in the shoot, and its overexpression can greatly improve N use efficiency and grain yield in rice (33, 173; X.R. Fan, Z. Tang & G.H. Xu, unpublished data). The solid red arrows represent defined direct functions of the transporters in nitrate uptake and translocation; the dashed arrows represent presumed relationships based on the tissue localization of the genes in rice and functional expression in oocytes. The blue arrows indicate the proposed evolution of individual members of the NAR2 and NRT2 nitrate transporter families. Black arrows indicate the possible relationships between NAR2.1 and root growth and between the functions of NRT2 members and plant growth and development.

transporters (70). There are uncertainties regarding the exact chemical species transported by AMT, which can be in the form of either hydrophobic NH_3 or charged ammonium (70, 118). For example, PvAMT1;1 from bean (*Phaseolus vulgaris*) actually functions as an H^+/NH_4^+ symporter (118) mediating the high-affinity and rapidly saturating electrogenic transport of ammonium (**Figure 4**).

A phosphophorylation-dependent allosteric negative feedback mechanism of AMTs can prevent excess ammonium accumulation in plants (86, 98). In response to high external ammonium, conserved sites (a threonine residue) in the C-terminus of AtAMT1.1 and AtAMT1.2 are phosphorylated, leading to cooperative closure of all three subunits in the trimer complex (98, 111).

The activity of AMT members in the ammonium-preferring rice may play a more important role in NUpE than in nitrate-utilizing crops. Interestingly, artificial selection from wild progenitors to cultivated rice has dramatically decreased the genetic diversity of

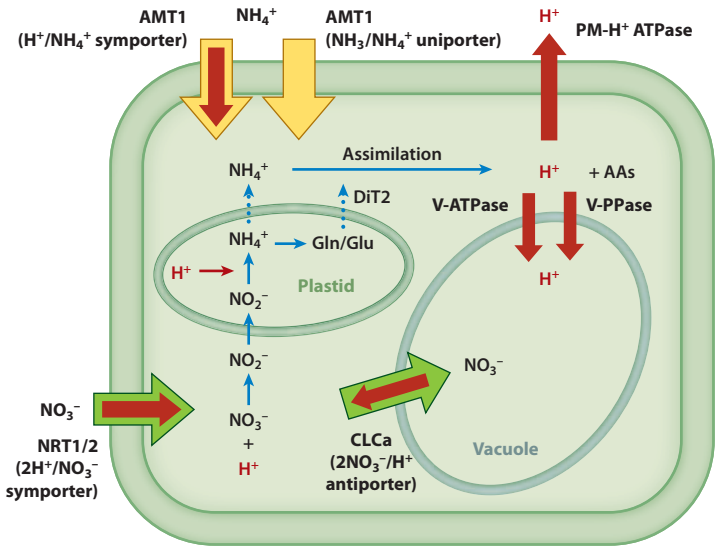

Figure 4

Relationship between ammonium and nitrate uptake and cytosolic pH. AMT1 is a plasma membrane (PM) ammonium transporter functioning either as an ammonia channel or as an ammonium uniporter or symporter with H^+ (70, 118), NRT1 and NRT2 family members are mostly PM-located proton nitrate symporters (30, 44), and CLCa is a nitrate proton antiporter on the tonoplast for transporting nitrate from the cytosol to the vacuole (23, 167, 180). The influxes of ammonium and nitrate via AMT1.1 and NRTs into the cytosol and nitrate into the vacuole via CLCa can result in a transient decrease in cytosolic pH. These cytosolic protons are pumped out by the PM H^+-ATPase under both ammonium nutrition (179) and nitrate nutrition (148), and are pumped into the vacuole by the vacuolar H^+-ATPase (V-ATPase) (76, 139) and the vacuolar PPase (V-PPase) (80, 166). The green, yellow, and red arrows represent nitrate, ammonium, and proton fluxes, respectively. Small blue arrows indicate the pathways of nitrate reduction and ammonium assimilation inside the cell. Small dotted blue arrows indicate the effluxes of ammonium ion and glutamine (Gln)/glutamate (Glu) from plastid to cytosol. Small red arrow indicates that proton is required for nitrite reduction in plastid. Additional abbreviation: AA, amino acid.

the *OsAMT1;1* gene, demonstrating a selective sweep caused by strong selection within or nearby the gene during the domestication process (29). As the *OsAMT1;1* alleles are fixed in cultivated rice, it is possible to discover novel alleles in wild relatives to broaden the genetic variation for improving NUpE (29).

Function of Urea Transporters

Urea is the major N form supplied as fertilizer, including both soil and foliar applications in agriculture worldwide. In soils, urea is rapidly degraded to ammonium and CO_2 by urease. The addition of urease inhibitors to urea fertilizers to prevent or at least slow down urea cleavage has been confirmed as a strategy to minimize N losses from soil (102). PM-localized major intrinsic proteins (MIPs) and the DUR3 ortholog have been shown to play roles in low- and high-affinity urea transport, respectively (107). The MIPs mediate passive urea fluxes in heterologous expression systems (97); however, their *in planta* functions in urea acquisition need to be examined, particularly for urea capture at the high soil concentrations after fertilization. AtDUR3 is the main high-affinity urea transporter at the PM of N-deficient *Arabidopsis* roots (73).

Besides acquisition from the environment, urea can also accumulate in plant cells as a consequence of secondary N metabolism (107). However, it is unclear how and to what extent urea is transported across intracellular membranes (73). Enhancing uptake of urea applied both in soil and on leaves by improving urea transport pathways might offer a strategy for improving NUpE.

Crosstalk with Phytohormones

It is generally assumed that auxin (AUX) is transported basipetally and mediates N signals from shoot to root (71). The C and N gene network contains dozens of genes encoding AUX responsive factors, receptors, and transporters (49). Links for AUX to N-regulated root development are well characterized. Gln and some downstream metabolites of N assimilation suppress expression of *miR167a* and then *ARF8* (*AUX responsive factor 8*) (42). Nitrate itself can directly induce the expression of an AUX receptor (*AFB3*) whose mutation failed to respond to nitrate-regulated root growth (161). NRT1.1/CHL1 as a nitrate transceptor has also been identified as a basipetal AUX transporter in roots, explaining how NRT1.1 is involved in regulation of lateral root growth (77).

Cytokinins (CKs) may function as both a local and long-distance signal of N status in plants in both directions between root and shoot (71). Nitrate-inducible IPT3 (adenosine

phosphate iso-pentenyl-transferase 3) is a key determinant of nitrate-dependent CK biosynthesis (154). Interestingly, CKs enhance NRT expression in the shoot and thus also enhance nitrate distribution and translocation in the shoot. However, CKs repress NRT expression in roots, although expression of CK receptors AHK4 and/or AHK3 is independent of N status, indicating that CKs act as an N satiety signal to decrease nitrate uptake in roots (71). Both abscisic acid and brassinosteroids are also involved in N-regulated root growth and N acquisition (71). Trying to improve crop NUpE by directly modulating phytohormone balance to coordinate root architecture and transporter activity is likely too challenging.

NITROGEN PHYSIOLOGICAL USE EFFICIENCY

Nitrogen Assimilation Efficiency

Light-dependent nitrate reductase expression is induced by nitrate and repressed by amino acids and particularly C starvation; the enzyme is subject to complex regulation at the level of translation, protein degradation, and protein phosphorylation (92). The importance of GS activity in N remobilization, growth rate, yield, and grain filling has been emphasized by functional genomics and quantitative trait loci (QTL) approaches and by using cultivars exhibiting contrasting NUE (1). GS1, functioning primarily in assimilating ammonia generated from the various processes involved during the remobilization of assimilate, is encoded by multiple genes in plants: three in rice and five in maize and *Arabidopsis* (1, 84, 103). These genes are not regulated in a similar manner, and GS1 isoenzymes are located in various plant tissues and have different kinetic properties, suggesting that each plays important roles in N assimilation (66, 103).

GS2 has been implicated in assimilating the ammonia that originates from nitrate reduction or photorespiration in chloroplasts (2, 84), and is encoded by a single gene in rice and *Arabidopsis* (140, 151). In *Medicago truncatula*, a second plastid-located GS2 gene product (MtGS2b, sharing 94% amino acid identity with MtGS2a) has been identified that shows seed-specific expression (140), and this may be specific to legume seed metabolism.

Expression of GS isozymes in leaves is developmentally regulated. GS2 is the predominant isozyme in leaf mesophyll cells of wheat, and it might be the major contributor to green leaf GS activity (2). In wheat, the cytosolic GS1 and GSr (putatively orthologous of OsGln1; 2) are the predominant forms during leaf senescence, suggesting their major roles in assimilating NH_3 during N remobilization from leaves to the grain (2). In roots there are ammonium-enhanced low-affinity GS1 isoenzymes located mainly in laterals. GS1 can provide sustained Gln biosynthesis at high ammonium levels and may represent an efficient system of NH_3 detoxification (117). In addition, Glu or other Glu-derived signals act as inputs to the N-assimilatory pathway circadian clock, which is directly regulated by a master clock controller, CCA1, providing a link between plant N nutrition and circadian rhythms (50).

Nitrogen Translocation and Remobilization Efficiency

The regulatory targets for improving NUE during early vegetative growth are different from those at senescence. The role of a "stay-green" phenotype has been underlined in favoring N uptake capacity and thus grain yield and quality (58). A number of senescence-induced marker genes encoding proteases and some isoforms of GS1, GDH, and AS are strongly activated during N remobilization (105; **Figure 1**). The nature of the amino acid transporters, which are encoded by a large number of genes belonging to several families, is poorly understood in phloem loading for N redistribution during senescence (114).

The QTLs for N remobilization detected by ^{15}N tracer methods mainly coincide with QTLs for leaf senescence (17). However, the benefit of using leaf senescence as a

Quantitative trait locus (QTL): a region of DNA associated with a particular phenotypic trait

selection criterion to improve grain protein concentration largely depends on soil N availability during the postanthesis period (4). N remobilization during leaf senescence is tightly regulated by chloroplastic and vacuolar protease activities as well as by the various long-distance transport pathways. For example, the downregulation of BnD22, a protease inhibitor, parallels the increase of numerous proteases in senescent oilseed rape leaf (28). Overexpressing leaf senescence–associated PPDK (orthophosphate dikinase) under the control of a senescence-inducible promoter accelerates N remobilization from leaves and thereby increases rosette growth rate and seed weight as well as N content (155). PPDK activity may be a target for crop improvement of NUE.

Crosstalk with Carbon Metabolism and Transportation

It has long been recognized that N assimilation requires energy and C skeletons (112). In plants, starch has been found to correlate with protein content as an integrator of overall biomass production (149). Nitrate reduction requires parallel C oxidation. Production of 2OG (2-oxoglutarate) requires oxidation through respiratory pathways involving the cytosol and mitochondria (36). Photorespiration can enhance redox transfer to the cytosol through the chloroplast envelope or mitochondrial malate/oxaloacetate shuttles, and thus links to N assimilation rates (36, 125). Double labeling ($^{13}C/^{15}N$) together with nuclear magnetic resonance analyses indicated that the 2OG used for GS/GOGAT during the day originates from stored organic acids (probably malate or citrate) produced during the night, and therefore the day/night cycle seems important for N assimilation (41). In pea seeds, 2OG/malate translocator (PsOMT) affects sucrose and glycolytic metabolism, plastid differentiation and amino acid biosynthesis, and seed sink strength (131).

The partitioning of assimilated C between synthesis of organic acids, starch, and sucrose is noticeably affected by N availability (36). It is tempting to explore whether there are plant-specific advantages to storing C as organic acids rather than as carbohydrates when it is to be subsequently used for the assimilation or use of N. Interestingly, ammonium-preferring rice plant has a unique plant-type phospho*enol*pyruvate carboxylase (PEPC), Osppc4, located in its chloroplasts that accounts for approximately one-third of total PEPC protein (106). Knockdown of *Osppc4* suppresses ammonium assimilation and subsequent amino acid synthesis by decreasing organic acids, which are C-skeleton donors for these processes, suggesting that rice has a unique route for organic acid synthesis and that primary ammonium assimilation is not necessarily the same in all vascular plants (106).

Nitrogen Use Efficiency Under Elevated CO_2 and Temperature

The atmospheric CO_2 concentration has been rising, increasing from 280 to 379 ppm since the Industrial Revolution, and it is predicted to double in this century (144). Long-term elevated atmospheric [CO_2] may result in stomatal adjustments and therefore decreased leaf transpiration rate. There is the possibility that lower carbohydrate supply to the roots at later growth stages limits the capacity of plant roots to acquire N from the rhizosphere, and in turn counters an improvement in NUE (144). Therefore, changing the capacity of root systems with the stage of growth to take up nitrate and ammonium could be important for plant acclimation to elevated [CO_2]. In addition, elevating atmospheric [CO_2] inhibits the photorespiration-dependent nitrate assimilation in the shoots of many species (125). Rising atmospheric [CO_2] could increase the net primary productivity of ammonium-preferring plants like pine and rice or plants that assimilate nitrate primarily in their roots (125).

Seed Quality and Storage Proteins

Increasing grain sink strength by improving assimilate uptake capacity may be a promising approach for improving yields and N harvest

index (NHI). In cereal crops, grain protein content (GPC) and grain yield commonly show a negative relationship (4, 54). However, total N concentrations in grains are not associated with yield productivity among wild emmer wheat (12). The trend of increasing both grain yield and N concentration in rice cultivars is obvious during the past several decades (**Figure 2**). Overexpression of a barley sugar transporter gene (*HvSUT1*) under the control of an endosperm-specific promoter in wheat increases sucrose flux into the grain, storage prolamin synthesis, and total N accumulation without any effects on grain yield (168). These results suggest that increasing seed C import may be an interesting potential target for future breeding efforts to improve yield and GPC simultaneously (4). However, little is known about the regulation of the accumulation of storage proteins during seed development.

The QTLs for GPC and N remobilization are not colocalized in barley (108). *FLO2* (*FLOURY ENDOSPERM2*) may play a pivotal regulatory role in rice grain size and accumulation of storage starch and proteins (143). Overexpression of *FLO2* could increase grain size enormously, together with upregulation of the *GluA1* (*glutelin A1*) gene encoding storage protein and the *RA16* gene encoding a 16-kD rice allergenic protein (143).

APPROACHES TO IMPROVE NITROGEN USE EFFICIENCY

With the aim of improving NUE, researchers have used various promoters (mainly CaMV 35S) to manipulate the expression of many candidate genes involved in N uptake and metabolism. Many transgenic approaches based on either overexpressing or using knockout mutations in candidate genes to improve NUE have also been used during past decade (see **Table 1**).

Root Architecture and Maintaining Activity

The several positive correlations between QTLs for N uptake and root architecture traits suggest that one way of increasing NUE is to simply breed for a root system that is more efficient at taking up N (17). However, better root architecture on its own is insufficient; enhancing NUpE by maintaining root activity during the entire growing season is also important. Maintaining root activity during the grain-filling period can increase grain N content and NUE (4).

Enhanced expression of *CKX1* in roots of both *Arabidopsis* and tobacco enhanced root-specific degradation of CK, a negative regulator of root growth, resulting in up to 60% increases in primary root elongation, root branching, and root biomass formation, whereas growth and development of the shoot were unaltered (169). This result indicates that a complex genetically controlled trait like root growth could be regulated by a single dominant gene. In addition, *ANR1* overexpression appears to be necessary but not sufficient to stimulate lateral root growth, probably owing to a specific requirement for nitrate and/or posttranslational regulation of *ANR1* (129, 163). Moreover, some NRT1 and NAR2/NRT2 family members (such as NRT1.1, NRT2.1, and NAR2.1) have been found to be involved in nitrate-regulated root development (40). Root-based traits can offer great opportunities for future improvements in NUE for cereals, but direct evidence that manipulating genes regulating root growth and activity will improve NUE is still lacking.

Nitrogen harvest index (NHI): the proportion of N content in the grains (seeds) to that of the whole plant [grain N/(vegetative organ N + grain N)]

Overexpression of Nitrate and Ammonium Transporters

Some plant N transporters facilitate root N losses under N-replete and low carbohydrate supplies by increasing N efflux and down-regulating some NRTs and AMTs involved in uptake (44, 141). Several lines of evidence demonstrate that it is nitrate itself inside the plant that directly regulates the expression of genes involved in nitrate uptake and assimilation, the synthesis of 2OG, the generation of NADPH in the oxidative pentose phosphate pathway, the regulation of shoot-root allocation, and the proliferation of lateral roots (112).

Table 1 Transgenic approaches to improve plant nitrogen use efficiency (NUE)

Gene source (accession number)	Gene family	Transgenic approach	Host plant(s)	Characteristic of NUE: Growth condition	Characteristic of NUE: Grain yield/biomass N uptake/metabolism	Reference(s)
Nitrogen transporters						
AtNRT1.1 (At1g12110)	Nitrate transporter	*CaMV 35S*	*Arabidopsis*	HS	U_{Ni} ↑	96
NpNRT2.1 (CAA69387)	High-affinity nitrate transporter	*CaMV 35S*, *rolD*	Tobacco, *Arabidopsis*	MS	U_{Ni} → (both LN and HN), root $^{15}NO_3^-$ ↑	37
OsNRT2.1 (Os01g50820)		*CaMV 35S*	*Arabidopsis*	Agar	shoot DW ↑, U_N →	69
OsAMT1-1 (*At4g13510*)	Ammonium transporter	*Ubiquitin*	Rice	HS	Shoot and root DW ↓, U_{Am} ↑ under LA and HA	57, 79
Nitrate reductase, nitrite reductase						
NpNia2	Nitrate reductase	*CaMV 35S*	Potato	Pots	TN ↓ 98%	25, 26
LsNia	Nitrate reductase	*CaMV 35S*	Lettuce	MS	NR and nitrate content ↑ in leaves	20
NpNR	Nitrate reductase	*CaMV 35S*	Tobacco	MS	High nitrite excretion and NO emission from leaf and root tissue	87
SoNiR (EC 1.7.7.1)	Nitrite reductase	*CaMV 35S*	*Arabidopsis*	Pots	NO_2 assimilation ↑	153
Amino acid transporters, aminotransferases, and dehydrogenases						
PmAspAT (EC 2.6.1.1)	Aspartate aminotransferase	*CaMV 35S*	Tobacco	MS	Endogenous PEPC polypeptides ↑	142
ASN1/DglnAS1	Asparagine synthetase	*CaMV 35S*	Tobacco	MS	Free asparagine in leaves ↑, growth rate ↑	6
AtLHT1 (*At5g40780*)	Lysine histidine transporter	*CaMV 35S*, T-DNA insertion	*Arabidopsis*	MS	Asp, Glu, and Gln uptake ↑; improved growth under LN	55
HvAlaAT (Z26322)	Alanine aminotransferase	*btg26*	*Arabidopsis*	Soil-less mixture	Seed yield ↑ 32.7%, DW ↑ 55%–64% under LN	45
				HS	DW ↑ 30%–75% under LN	
HvAlaAT (Z26322)	Alanine aminotransferase	*OsAnt1*	Rice	Soil-less mixture	Spikelet yield ↑ 31%–54%, DW ↑ 30%–34%	145
				HS	TN ↑ 36%–61%	
AtASN1 (At3g47340)	Asparagine synthetase	*CaMV 35S*	*Arabidopsis*	MS	Seeds TN ↑ under LN	85

(*Continued*)

Table 1 (*Continued*)

Gene source (accession number)	Gene family	Transgenic approach	Host plant(s)	Characteristic of NUE		Reference(s)
				Growth condition	Grain yield/biomass N uptake/metabolism	
AtASN2 (At5g65010)		*CaMV 35S*	*Arabidopsis*	MS	Effective use of N mediated under HA conditions	21
VfAAP1	Amino acid permease	*LeB4*	Pea	Pots	TN and protein in seeds ↑	135
AtAAP1 (At1g58360)	Amino acid transporter	T-DNA insertion	*Arabidopsis*	MS	TN and C in seeds ↓, TAA ↑	137
AtCAT6 (At5g04770)	Amino acid transporter	T-DNA insertion	*Arabidopsis*	MS	Amino acids supplied to sink tissues	52
Glutamine synthetase/glutamine-2-oxoglutarate aminotransferase (GS/GOGAT)						
PsGS1 (EC 6.3.1.2)	Glutamine synthetase	*CaMV 35S*	Tobacco	MS	Growth improved, leaf TAA ↓	116
PsGS1 (EC 6.3.1.2)	Glutamine synthetase	*CaMV 35S*	Poplar	HS	Leaf DW ↑ (112% under LN and 26% under HN)	100
PvGS1	Glutamine synthetase	*Rubisco small subunit*	Wheat	Peat-based compost	Root and grain DW ↑, enhanced capacity to accumulate N, mainly in grain	51
MsGS1 (EC 6.3.1.2)	Glutamine synthetase	*CaMV 35S*	Tobacco	MS	Shoot DW ↑ 70% and root DW ↑ 100% under LN	38
GmGS1	Glutamine synthetase	*CaMV 35S*	Lotus	MS	DW →	162
OsGS1;1 (AB037595)	Glutamine synthetase	*CaMV 35S*	Rice	Field	Yield ↓ 25%–33%	8
				HS	TN ↑ under both LN and HN	
OsGS1;2 (AB180688)	Glutamine synthetase	*CaMV 35S*	Rice	Field	Yield ↓ 7%–25%	
				HS	TN ↑ under both LN and HN	
OsGS1;2 (AB180688)	Glutamine synthetase	*Ubiquitin*	Rice	Soil (growth chambers)	Spikelet yield ↑ 29%–35% under HN	5
					NUE ↑ 30%–33% under HN	
				Soil	→	
					→	
OsGS2 (*X14246*)	Glutamine synthetase	*CaMV 35S*	Rice	MS medium	Soluble protein and free NH_4^+ →	7, 59

(*Continued*)

Table 1 (*Continued*)

Gene source (accession number)	Gene family	Transgenic approach	Host plant(s)	Characteristic of NUE		Reference(s)
				Growth condition	Grain yield/biomass N uptake/metabolism	
ZmGS1	Glutamine synthetase	*Ubiquitin*	Maize	Soil	Shoot DW →, grain yield ↑ 45% under LN	103
		T-DNA insertion			Leaf TAA and TN ↑, grain yield ↓ 85% under LN	
MsNADH-GOGAT	NADH-dependent glutamate synthase	*CaMV 35S*	Tobacco	HS	Total C and TN in shoots ↑, DW ↑	13
OsNADH-GOGAT (AB008845)	NADH-dependent glutamate synthase	*OsNADH-GOGAT*	Rice	HS	Grain filling ↑	172
MsNADH-GOGAT	NADH-dependent glutamate synthase	*Ibc3*	Alfalfa	Pots (verculite, nutritive solution)	Shoot fresh mass ↓ 29%–41%, N content ↓ 37%–38%, nodule TAA ↓ 50%–70%	18
Regulatory and transcription factors						
AtANR1	MADS TF	*CaMV 35S*	*Arabidopsis*	Agar	Insensitive to nitrate	177
ZmDof1 (X66076)	Dof TF	*35SC4PPDK*	*Arabidopsis*	MS medium	Growth rate ↑ under LN	174
ZmDof1 (X66076)	Dof TF	*Ubiquitin*	Rice	HS	C and N metabolites modulated, N assimilation and growth ↑ under LN	81
TsNAM-B1 (DQ869673)	NAC TF	*RNAi*	Wheat	Field	Senescence delayed by more than 3 weeks; grain protein, zinc, and iron content ↓ by more than 30%.	158
Others						
OsENOD93-1 (Os06g05010)	Early nodulin	*Ubiquitin*	Rice	Soil	Grain yield ↑ 10%–20%, shoot DW ↑ 10%–20%;	3
				HS	TAA and TN in xylem sap ↑ under LN	
APO1 (AP003628)	Aberrant panicle organization	*OsAPO1*	Rice	Field	Grain yield per plant ↑ 5%–7%	156
AtSTP13 (At5g26340)	Monosaccharide transporter	*CaMV 35S*	*Arabidopsis*	Agar	TN ↑ 90% and FW ↑ 75% under HN	138
AtMKK9-MPK6 (At1g73500 At2g43790)	Mitogen-activated protein kinase	T-DNA insertion, *CaMV 35S*	*Arabidopsis*	MS	Leaf senescence controlled	178

(*Continued*)

Table 1 (*Continued*)

Gene source (accession number)	Gene family	Transgenic approach	Host plant(s)	Characteristic of NUE: Growth condition	Characteristic of NUE: Grain yield/biomass N uptake/metabolism	Reference(s)
AtPPDK (At4g15530)	Pyruvate orthophosphate dikinase	*pSAG12*	*Arabidopsis*	Pots in growth chamber	N remobilization from leaves accelerated, thereby increasing rosette growth rate and seed weight and TN in *Arabidopsis*	155
			Tomato	Pots in greenhouse		

Abbreviations: *35SC4PPDK*, CaMV 35S promoter with TATA box and the transcription site of the maize *C4PPDK* gene; Asp, aspartate; *btg26*, canola root-specific promoter; *CaMV 35S*, cauliflower mosaic virus 35S promoter; DW, dry weight; FW, fresh weight; Gln, glutamine; Glu, glutamate; HA, high ammonium concentration; HN, high nitrogen concentration; HS, hydroponic solution; *Ibc3*, soybean leghemoglobin promoter; LA, low ammonium concentration; *LeB4*, legumin B4 promoter, which controls seed-specific expression; LN, low nitrogen concentration; MS, Murashige and Skoog medium; NR, nitrate reductase activity; *OsAnt1*, aldehyde dehydrogenase promoter; *OsNADH-GOGAT*, NADH-dependent glutamate synthase promoter; PEPC, phospho*enol*pyruvate carboxylase; *pSAG12*, senescence associated gene 12 promoter; RNAi, RNA interference; rolD, *Agrobacterium* rhizogenes rolD promoter; TAA, total amino acids; T-DNA, transfer DNA; TF, transcription factor; TN, total nitrogen content; *ubiquitin*, maize ubiquitin promoter; U_{am}, ammonium uptake; U_N, nitrogen uptake; U_{Ni}, nitrate uptake; ↑, increase; ↓, decrease; →, no change.

In *Arabidopsis*, overexpression of a seed vacuole–localized nitrate transporter, At-NRT2.7, increased nitrate accumulation in the seed and improved germination (14). In rice, increased expression of OsNRT2.1 slightly improved seedling growth, but did not have any effect on N uptake (69), probably owing to the missing required interaction with OsNAR2.1 for functional nitrate transport (33, 173). In contrast, overexpression of OsNRT2.3b could significantly increase rice yield and total N uptake (**Figure 3**; G. Xu, X. Fan & Z. Tan, unpublished data).

Overexpressing *AMT1* genes could enhance ammonium uptake capacity, but it decreases shoot and root biomass at relatively high ammonium supplies, probably owing to toxicity and the inability of ammonium assimilation to cope (57). This result suggests that overexpressing AMT1 family members might be helpful to improve N acquisition in low-ammonium soils. However, it should be noted that for legumes, some AMT1 family members (like LjAMT1;3) not directly involved in ammonium acquisition from the external solution may function as an intracellular ammonium sensor (133).

Manipulation of Key Genes Controlling Balance of Nitrogen and Other Metabolism

Overexpression of either the nitrate reductase or nitrite reductase gene increased N uptake, but did not seem to increase the yield or growth of plants regardless of N availability, probably owing to regulation occurring at post-transcriptional and translational levels (92). Overexpression of the *GS1* gene could increase GS activity, growth rate, yield, and biomass at low N supplies but not always at high N supplies (46). Expression of a barley *AlaAT* (*alanine aminotransferase*) gene in rice, driven by a rice tissue-specific promoter (OsAnt1), significantly increased NUpE, biomass, and grain yield at high N supplies (145), whereas its overexpression driven by a root-specific promoter (btg26) in *Brassica napus* increased only the biomass and seed yield at low N (45). In *Arabidopsis*, constitutively overexpressing a hexose transporter, STP13, increased expression of NRT2.2 and total N uptake as well as plant growth (138). Genetic engineering of *Arabidopsis* with a Dof1 transcription factor not only allowed better growth under N-limiting

conditions, but also enhanced net N assimilation, including upregulation of *PEPC* genes both in *Arabidopsis* and rice (81, 174).

Manipulating mitochondrial metabolism is a potential target for enhancing NUE. In potato, constitutive overexpression of a mutated *PEPC* gene carrying both N-terminal and internal modifications fixed more CO_2 into malate and redirected C flow from sugars to organic acids and amino acids (126). In rice, overexpression of a mitochondria-located N-responsive early nodulin gene, *OsENOD93-1*, led to increased shoot biomass and seed yield, enhanced N translocation, and higher concentrations of amino acids in the xylem sap (3).

The C-N regulated network occurs at multiple levels, including potential post-transcriptional control by microRNAs and a C-regulated bZIP transcription factor (bZIP1). Several primary miR169 species as well as pri-miR398a have been found to be repressed during N limitation, and can move in the phloem (119), indicating that small RNAs play a role in N systemic signaling. Because bZIP1 induces expression of *ASN1* encoding Gln-dependent Asn synthetase, it may be an integrator of C and N signaling for N assimilation (49, 50). NLA (N limitation adaptation), a RING-type ubiquitin ligase, has been found to be a positive regulator of plant acclimation to N limitation (121). Interestingly, NLA also plays a key role in the maintenance of plant phosphate homeostasis in a nitrate-dependent fashion (68). The transcription factors NLP7 (NIN-LIKE PROTEINS 7) and LBD37/38/39 have been demonstrated as positive and negative regulators of the primary nitrate response (9, 136), indicating complex feedback regulation of N use. In wheat, a NAC transcription factor, NAM-B1, coordinately regulates whole-plant senescence and transport of N, zinc, and iron from vegetative organs to the grains (158).

Cytosolic pH Balance

The N form taken up by plants influences pH homeostasis (128). In rice, ammonium enters cells in much greater quantities than nitrate, causing alkalinization in the cytoplasm, which in turn enhances proton-coupled nitrate transport for cytosolic pH balance and results in a synergism of ammonium and nitrate uptake. **Figure 4** schematically shows how plants maintain cytosolic pH balance by functions of AMT, NRT, and ATPase in the PM, together with CLCa, V-ATPase, and V-PPase in the tonoplast. The H^+ or OH^- produced during ammonium and nitrate assimilation in excess of that required to maintain cytoplasmic pH are exported from the cell in energy-requiring steps (**Figure 4**). Indirect evidence for this homeostatic activity is provided by the demonstration that the adaptation of rice roots to low pH is associated with careful regulation of PM H^+-ATPase genes (179).

To test whether cytosolic pH balance is critical in both N uptake and long-distance transport, the relationship between the rate of nitrate, amino acid transport to developing leaves or seeds, and pH in phloem sap can be measured at different N supply forms and concentrations. The role of pH balance in the regulation of C-N metabolism is an important topic that requires more investigation (112). Cellular carboxylate metabolism, especially malate metabolism, is important for the regulation of cytosolic pH (63). A tonoplast dicarboxylate (malate and fumarate) transporter (AttDT) is required for full cytosolic pH homeostasis, and its expression is tightly regulated by external pH (63). These findings provide new tools to allow a molecular understanding of the interaction between N nutrition, pH balance, and organic acid metabolism. Enhancing cellular pH balance through transgenic approaches might be a new target for improving NUE.

Increasing Yield and Nitrogen Harvest Index to Drive Nitrogen Acquisition and Utilization

Increasing plant NUpE can decrease N losses from soil, whereas increasing NUtE or NpUE can decrease the N concentration in a plant. Thus, NUE can be increased by improving

the grain yield per unit of N application. Because most of the N taken up by cereals is distributed into grains and the N concentration in the vegetative organs at later developmental stages is commonly much lower than it is in the seeds, relatively lower protein content (a low seed N concentration) represents a higher NpUE. Single-seed dry weight and N concentration are robust traits, highly heritable (104), whereas HI and NHI are highly correlated and affected largely by N supply level and availability, particularly at the seed-filling stage (104). Therefore, lowering total N concentration in high-yield seeds has the advantage of improving NUE if adequate essential protein components can be maintained.

Several genes that influence grain weight and N remobilization (thereby improving HI and NHI) have been identified in several plant species (**Figure 5**). For example, overexpression of a cytosolic GS1-encoding gene (*Gln1-3*) constitutively in leaves increased maize grain yield by 30%, but did not increase shoot biomass (103), suggesting that the effect of Gln1-3 is specific to grain production. The *NAC* gene (*Gpc-B1*) might be another good candidate for enhancing N remobilization from source leaves to the seeds, diminishing the amount of N lost in residual dry plant material at harvest, thus increasing NHI (158). Asn synthetase 1 might have a role in enhancing HI and N remobilization from vegetative tissues to the seeds (105). Vacuolar stored nitrate can also be remobilized, and this remobilization is important to sustain vigorous growth during short-term N deficiency via a phloem-regulated mechanism (32).

Molecular Marker–Assisted Breeding for Crops with High Nitrogen Use Efficiency

QTLs for NUE have now been identified in mapping populations of barley (108), maize (39), rice (113), *Arabidopsis* (99), and wheat (124). Some QTLs for grain yield and for less complex traits, such as root architecture and GS activity, might be determinants for grain yield regardless of the level of N fertilization in these species. Accessions or genotypes of the same species with large differences in NUE and growth performance can be used as parent lines of recombinant inbred line populations to perform QTL mapping of traits linking to the components of NUE and yield potential (10). Furthermore, applying cross-genome map-based dissection of the NUE ortho-metaQTL can be considered for functional validation (or at least as a source) of accurate molecular markers or conserved orthologous sets (124).

GS1 might be a key component of plant NUE and yield, whereas the physiological function of GS2 associated with NUE needs to be identified (1, 2). The NUE QTL and *GOGAT* genes are conserved at orthologous loci in the cereal genomes of wheat, rice, sorghum, and maize, which diverged from a common ancestor some 50–70 million years ago, suggesting that some traits underlying NUE have been conserved during evolution, at least in cereals (124). In wheat, 11 genes were mapped within the confidence intervals of 10 NUE metaQTLs that colocalize with key developmental genes such as *Ppd* (photoperiod sensitivity), *Vrn* (vernalization requirement), and *Rht* (reduced height) (124). These genes can be considered robust markers from a molecular breeding perspective.

CONCLUDING REMARKS AND FUTURE ISSUES

For economically and environmentally friendly use of valuable N resources, developing high-NUE cultivars is more challenging than targeting N applications as part of an integrated nutrient management. Complex multigene traits for NUE are the integration of genotype and environmental conditions, particularly N supply. The proper evaluation of plant NUE to identify the main bottlenecks for maximizing NUE has to be considered for crop improvement. The most important aspect of the different NUE components is the N requirement for producing the highest potential yield, which is an integration of NUpE and NUtE.

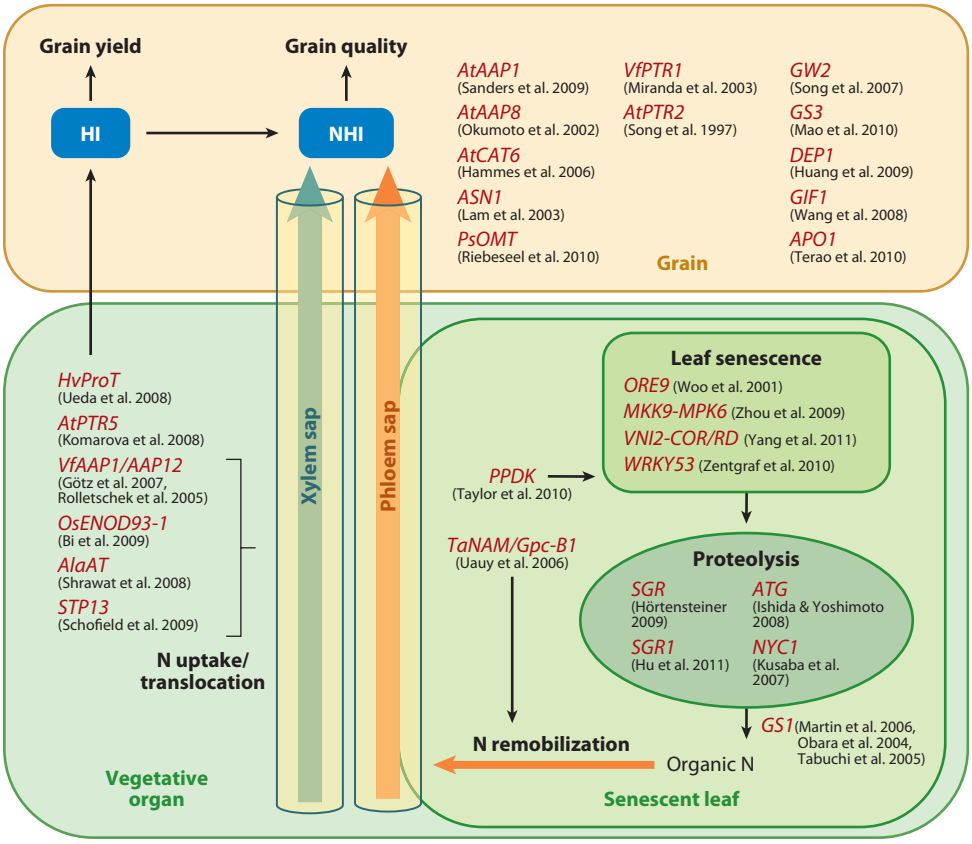

Figure 5

The genes involved in regulating N remobilization in senescing leaves, grain (seed) development, harvest index (HI), N harvest index (NHI), and grain yield. *AtAAP1*, *AtAAP8*, *AtCAT6*, *ASN1*, and *PsOMT* play a role in supplying amino acids to sink tissues of plants and are important for storage protein synthesis and seed yield; *VfPTR1* and *AtPTR2* are important during embryo development and seed development; *GW2*, *GS3*, *DEP1*, and *GIF1* are major QTLs for grain width, length, thickness, weight, and yield; and *APO1* is responsible for the number of grains per panicle. Reduction in biomass production was observed in aerial parts of *35Sp-HvProT* plants; overexpression of *AtPTR5* resulted in enhanced shoot growth and increased N content; and manipulation of *VfAAP1/AAP12*, *OsENOD93-1*, *AlaAT*, and *STP13* can increase both N percentage and plant biomass by improving the N uptake efficiency of the plant. *PPDK* and *TaNAM/Gpc-B1* function in N remobilization during leaf senescence and regulate seed growth and N content; *ORE9*, *MKK9-MPK6*, *VNI2-COR/RD*, and *WRKY53* regulate leaf senescence; *SAG*, *ATG*, *SGR1*, and *NYC1* regulate chlorophyll and protein degradation during senescence; and *GS1* functions in N assimilation in the senescence leaves.

The most striking advances in understanding the regulation of N use in plants during the past decade have been in identifying transporters for nitrate and ammonium along with the functions of plant-specific sensors and transcription factors. Several reports show that changing the expression of a single transgene can significantly improve NUE, particularly the NUpE of crops. However, NUpE is genetically governed by both N-regulated root architecture and the activities of N transporters. In addition, enhanced N acquisition must be consumed by being efficiently transported and assimilated to drive growth and development; otherwise, the increased N pools might actually decrease net N uptake

through feedback effects on the transporter activity and/or through increased root efflux. To fully assess the impact and yield potential of the resulting plants, researchers must evaluate the effectiveness of NUE improvement by single-gene transformation in large field experiments as well as in different genetic backgrounds and environmental conditions.

Delay of leaf senescence at the grain-filling stage in cereals prolongs leaf photosynthesis and thus increases grain yield and HI; however, such leaves commonly maintain high N contents and result in lower NRE and GPC. In contrast, rapid senescence increases N remobilization from the vegetative parts and thus results in relatively higher NRE and GPC and particularly high NHI, but also high N volatilization through photorespiratory pathways. Because photorespiration has been reported to be necessary for optimal rates of nitrate assimilation, maintaining photosynthesis and enhancing the reassimilation of photorespiratory ammonia in relatively low-N-content leaves at the grain-filling stage is a potential avenue for improving NUE in agriculture.

Altering the storage protein content in cereal grains has demonstrated the feasibility using transgenic approaches to improve seed components and therefore nutritional quality. Because most of the N in cereal crops is transported into grain, decreasing the content of nonessential seed protein components without affecting yield could be an alternative strategy for improving NUE.

Most transgenic approaches for improving NUE by overexpression of relevant genes have been carried out using various constitutive gene promoters. Given the complexity of plant systems, different engineering approaches that include novel genes and the selection of tissue-specific promoters to drive the expression might result in better improvements in NUE. For example, enhancing N uptake by overexpression of nitrate and ammonium transporters driven by low-N-induced promoters might improve N uptake at low soil N concentrations. In the future, direct gene transfer together with marker-assisted selection to breed the high-NUE cultivars will be highly feasible. Increasing costs of fertilizer and pollution are driving the demand for this new generation of crops.

SUMMARY POINTS

1. Plant NUE is the integration of NUpE and NUtE, and is governed by multiple interacting genetic and environmental factors. There is complex feedback regulation of N uptake and assimilation from transcription to posttranslational levels.
2. Enhanced N uptake by overexpression of nitrate and ammonium transporters must be consumed to drive growth in order to avoid feedback effects on the transporter activity and increase of N efflux by roots.
3. Manipulation of key genes controlling the balance of N and C metabolism (particularly the flexibility of respiratory pathways) and the balance of cytosolic pH can be key targets for NUE improvement.
4. Breeding cultivars with high NUE should combine direct gene transfer with marker-assisted selection approaches to increasing both yield and NHI in order to drive N acquisition and utilization.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Dr. Yali Zhang for providing the data for **Figure 2**, Dr. Yiyong Zhu for comments on **Figure 4**, Mr. Zhong Tan for the drawing of **Figure 5**, Ms. Huimin Feng for preparing **Table 1**, and Professor Uzi Kafkafi at Hebrew University of Jerusalem for critical comments on this article. We apologize to all colleagues whose work could not be cited owing to space limitations. Work in the Xu laboratory is supported by the China 973 Program, the Crop Transgenic Project, the National Natural Science Foundation, 111 project (No. B12009) and PAPD in Jiangsu Province of China.

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