

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Nitrogen Use Efficiency in Rice

---

Shuangjie Huang, Chunfang Zhao, Yali Zhang and  
Cailin Wang

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.69052>

---

## Abstract

Food security is a major global issue because of the growing population and decreasing land area. Rice (*Oryza sativa* L.) is the most important staple cereal crop in the world. Application of nitrogen (N) fertilizer has improved crop yield in the world during the past five decades but with considerable negative impacts on the environment. New solutions are therefore urgently needed to simultaneously increase yields while maintaining or preferably decreasing applied N to maximize the nitrogen use efficiency (NUE) of crops. Plant NUE is inherently complex with each step (including N uptake, translocation, assimilation, and remobilization) governed by multiple interacting genetic and environmental factors. Based on the current knowledge, we propose some possible approaches enhancing NUE, by molecular manipulation selecting candidate genes and agricultural integrated management practices for NUE improvement. Developing an integrated research program combining approaches, mainly based on whole-plant physiology, quantitative genetics, forward and reverse genetics, and agronomy approaches to improve NUE, is a major objective in the future.

**Keywords:** rice, nitrogen use efficiency, nitrate, ammonium, N uptake, N assimilation, N remobilization

---

## 1. Introduction

The global population is predicted to reach 9 billion, and food supplies are projected to increase by 70–100% by 2050 [1, 2]. Given the limited capacity for arable land expansion, it requires sustaining yield improvement in existing land to meet the increasing food demand [3]. Rice is one of the staple food crops for approximately half of the global population. Therefore, rice production must be increased significantly to satisfy the requirements of the growing world population. However, we are facing challenges in increasing rice production under

the pressures of decreased arable land area, global climate change, intensified natural disasters, and frequent occurrence of diseases and pests [4]. Nitrogen (N) is one of the essential macrolelements required for plant growth and development. Soil N availability usually limits plant yields in most agricultural cropping systems [5]. Thus, application of N fertilizer has become an important, cost-effective strategy to increase crop yields in intensive agricultural systems worldwide [6]. However, traditionally adding N fertilizer to improve crop yields may have reached a plateau. Excessive application of nitrogen fertilizer may not result in yield improvements but will lead to serious environmental problems [7, 8]. From 1960 to 2012, the global N fertilizer consumption increased by 800%, and the annual N consumption in China increased from 8 to 35% of the world's N consumption [4]. Although the rate of cereal grain yield increased by 65% between 1980 and 2010, the consumption of chemical fertilizers increased by 512% [9]. High N fertilizer input leads to low nitrogen use efficiency (NUE) due to the rapid N losses from ammonia volatilization, denitrification, surface runoff, and leaching in the soil-flood water system. As a consequence, significant environmental problems (i.e., soil acidification, air pollution, water eutrophication) occurred [10–12]. To achieve further high crop productivity and high NUE under well-fertilized conditions, new solutions are urgently needed to increase yields while maintaining or preferably decreasing applied N [13].

In this chapter, we outlined the definition of NUE, the genes related to NUE, as well as the effect of the factors on the expression of those genes, with an emphasis on rice research. Based on the current knowledge, we proposed some possible strategies enhancing NUE, by breeding, molecular manipulation selecting candidate genes, and developing a range of optimized crop management practices for NUE improvement.

## 2. Defining nitrogen use efficiency

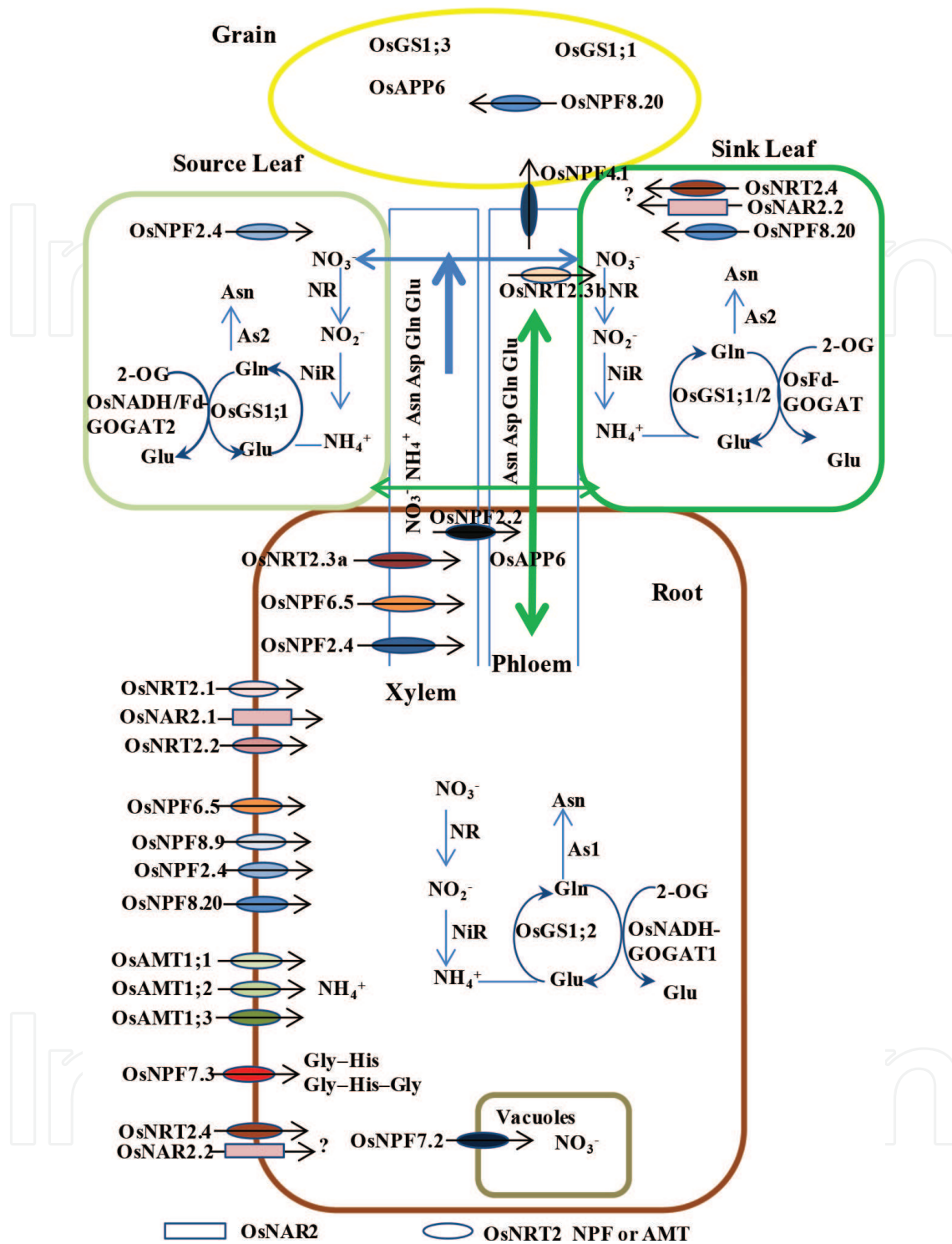
NUE is inherently complex determined by the interaction of multiple genes with the environment factors. A number of different definitions and calculations of NUE include N utilization, N content, and N availability as NUE equation components (**Table 1**) [13, 14]. In general, plant NUE comprises two key components: N uptake efficiency (NUpE), which is the efficiency of absorption/uptake of supplied N, and N utilization efficiency (NUtE), which is the efficiency of assimilation and remobilization of plant N to ultimately produce grain [13, 14]. The simplest definition of plant NUE is the grain yield per unit of supplied N, also an integration of NUpE and NUtE. Another method to describe NUE is the utilization index (UI), which means the absolute amount of biomass produced per unit of N. NUE can also be described as NUEg, which is grain production per unit of N available, and HI, which is grain production of the total plant biomass. However, a crop plant could produce large amounts of biomass per unit N (high UI) without converting the acquired N to seed production and therefore have a low NUEg and HI. There are other NUE calculations taking various agronomic and physiological variations into account described elsewhere [14–16]. In summary, improving NUE could be achieved by improving either NUpE, NUtE, or both. However, owing to the fluctuations in the rhizosphere that influenced by microorganism, root exudates, and the volatile loss of gaseous N from the soil/plant canopy, it is difficult to quantify the “real” amount of N fertilizer available or actually acquired by plants.

Abbreviation	Term	Definitions
NUE	N use efficiency	$\text{NUpE} \times \text{NUtE} = \text{yield}/\text{N available}$
NUpE	N uptake efficiency	$\text{NUp}/\text{N}_{\text{av}} (\text{soil} + \text{fertilizer}) = \text{acquired N}/\text{N available}$
NUtE	N utilization efficiency	$\text{Yield}/\text{NUp}$ (assimilation of plant N to produce grain)
NUEg	N use efficiency of grain	Grain production/available N
ANR	Apparent N recovery rate	Net increased total N uptake by the plant with and without N fertilization/total amount of fertilizer N
AE	Agronomy N efficiency	Net increased yield of the plant with and without N fertilization/total amount of fertilizer N
NpUE	N physiological use efficiency	Net increased yield/net increased N uptake with and without application of fertilizer N
NTE	N transport efficiency	Total N transported into the aboveground parts/total N in the whole plant
UI	Utilization index	Total plant biomass/total plant N
FUE	Fertilizer use efficiency	$(\text{NUp}/\text{N applied}) \times 100$
HI	Harvest index	Grain weight/(vegetative organ weight + grain weight)
NHI	Nitrogen harvest index	Grain N accumulation/total N accumulation in aboveground biomass (e.g., grain + straw)
NRE	Nitrogen remobilization efficiency	N remobilization from source or senescent leaves/that of sink leaves or developing grains (seeds)

**Table 1.** Some definitions of NUE mostly used with respect to nitrogen.

### 3. Genes responsible for nitrogen use efficiency

Generally, NUE can be divided into two parts: assimilation efficiency involved in N uptake and assimilation, and utilization efficiency involved in N remobilization. Understanding the mechanisms regulating these processes is crucial for improving crop NUE. In soil, inorganic N is available for plants as nitrate ( $\text{NO}_3^-$ ) in aerobic uplands and ammonium ( $\text{NH}_4^+$ ) in flooded wetland or acidic soils. Rice roots in paddy soils release oxygen via their aerenchyma, generate rapid nitrification on their surface, and thus absorb N as  $\text{NO}_3^-$  at a rate comparable with that of  $\text{NH}_4^+$  uptake [17, 18]. Direct molecular evidence for  $\text{NO}_3^-$  uptake in rice has been presented [19].  $\text{NH}_4^+$  or  $\text{NO}_3^-$  uptake by roots commonly results in acidification or alkalization of the rhizosphere, which in turn changes the soil N availability [14]. For many plants, some  $\text{NO}_3^-$  taken up by nitrate transporters (NAR2/NRTs) is assimilated in the roots, the other larger part transported to the shoots, where it is reduced to ammonium by a range of enzymes (**Figure 1**). The  $\text{NH}_4^+$  derived from  $\text{NO}_3^-$  or directly from  $\text{NH}_4^+$  uptake by ammonium transporters (AMTs) is assimilated into amino acids via the glutamine synthetase (GS)/glutamine-2-oxoglutarate aminotransferase (GOGAT) cycle and then is exported to sink organs [14]. Therefore, regulating gene function in N metabolism processes including N uptake, assimilation, compartmentation, translocation, and remobilization may be essential for improving NUE.



**Figure 1.** Schematic representation of characterized and predicted functions of the rice nitrate transporters of NRT and NPF families, ammonium transporters of AMT families, and nitrogen assimilation proteins of GS and GOGAT.

### 3.1. Nitrogen acquisition

Owing to the heterogeneity and dynamic variations of nitrate and ammonium concentrations, which range from lower than 100  $\mu\text{M}$  to higher than 10 mM in soil solutions, plants have

developed transporters for both nitrate and ammonium. These transporters are divided into high-affinity transporter system (HATS) and low-affinity transport system (LATS) [20]. Under low nitrogen concentrations (<1 mM), HATS mediates most of the N uptake, while under high concentrations of N (>1 mM), LATS plays roles in N uptake [21, 22]. Each high- and low-affinity nitrate transport system is composed of constitutive and nitrate-inducible components (cHATS and iHATS), respectively [20, 23]. So far, four families of nitrate transporters/channels have been identified: nitrate transporter 1/peptide transporter family (NPF, also known as the NRT1/PTR family), nitrate transporter 2 family (NRT2), the chloride channel family (CLC), and slow anion channel-associated homologues (SLAC/SLAH) [24].

In rice, two transporter families NPF and NRT2 (or NAR2/NRT2) for uptake and translocation of nitrate have been identified (**Table 2** and **Figure 1**) [14, 25, 26]. At least 80 genes belong to NPF family in rice genome [27]. Most NPF family members characterized so far are low-affinity nitrate transporters, except that OsNPF6.5 (NRT1.1b) showed dual-affinity nitrate transport activity, associated with enhancing nitrate uptake and root-to-shoot transport [28]. OsNPF6.5, considered as a putative mRNA splicing product of OsNPF8.9 (NRT1/NRT1.1/NRT1.1a), has a significant impact on both NUE and yield [26–29]. OsNPF8.9, mainly expressed in root epidermis and hairs, has been cloned contribution to N uptake [30]. The role of OsNPF4.1 (SP1) has been demonstrated to function in rice panicle elongation [31] and OsNPF8.20 (OsPTR9) function in ammonium uptake, promotion of lateral root formation, and increased grain yield [32]. However, their substrates are still unknown. Eight peptide transporters, OsPTR1 (OsNPF8.2), OsPTR2 (OsNPF2.2), OsPTR3 (OsNPF5.5), OsPTR4 (OsNPF7.1), OsPTR5 (OsNPF7.4), OsPTR6 (OsNPF7.3), OsPTR7 (OsNPF8.1), and OsPTR8 (OsNPF8.5), were investigated in a yeast ptr2 mutant strain, and their expression patterns were evaluated in plants. Only OsNPF7.3 transports Gly-His and Gly-His-Gly, showing substrate selectivity for di-/tripeptides. However, the other seven proteins did not transport the five tested di-/tripeptides [33]. Elevated expression of *OsNPF7.3* promoted rice growth through increasing ammonium transporter expression and glutamine synthetase activity [34]. Recently, OsNPF2.4 [35] and OsNPF2.2 [36] involved in long-distance root-to-shoot nitrate transport have been identified. Knockout of *OsNPF2.4* impaired potassium (K)-coupled nitrate upward transport and nitrate redistribution from old leaves to other organs [35]. OsNPF2.2 can unload nitrate from the xylem affecting the root-to-shoot nitrate transport and plant development [36]. In addition, a tonoplast-localized low-affinity nitrate transporter OsNPF7.2 has been characterized playing a pivotal role in intracellular allocation of nitrate in roots [37]. To date, five *NRT2s* (*OsNRT2.1/2.2/2.3a/2.3b/2.4*) and two *NAR2s* (*OsNAR2.1/2.2*) genes encoding HATS components have been identified in rice, each showing different expression and regulation patterns (**Table 2**) [19, 38]. Among the five *OsNRT2s* genes, *OsNRT2.1* and *OsNRT2.2* share an identical coding region sequence with different 5'- and 3'-untranscribed regions [38–40]. *OsNRT2.3a* and *OsNRT2.3b* are derived from the alternative splicing of *OsNRT2.3* [38]. *OsNRT2.3a* is mainly expressed in the xylem parenchyma of root participating in long-distance nitrate transport from root to shoot at low nitrate concentrations [41]. *OsNRT2.3b* is mainly expressed in the phloem of shoot, sensitive to pH. Elevated expression of *OsNRT2.3b* increased N, Fe, and P uptake and improved grain yield and NUE [42]. *OsNAR2.1*, *OsNRT2.1*, and *OsNRT2.2* were expressed abundantly throughout the primary and lateral roots. Overexpression of *OsNRT2.1*

Accession no.	Gene	Regulation	Expression pattern	Substrates	References
AF140606	<i>OsNPF8.9</i>	Unknown	Constitutively expressed in roots	$\text{NO}_3^-$	[29, 30]
AK066920	<i>OsNPF6.5</i>	$\text{NO}_3^-$	Root hairs, epidermis, and vascular tissues	$\text{NO}_3^-$	[28, 29]
AK099321.1	<i>OsNPF2.4</i>	$\text{NO}_3^-$	Root epidermis, xylem parenchyma, and phloem companion cells, leaf phloem cells	$\text{NO}_3^-$	[35]
AK068351	<i>OsNPF2.2</i>	$\text{NO}_3^-$ , drought, salt	Parenchyma cells around the xylem	$\text{NO}_3^-$	[36]
XM_015767550	<i>OsNPF7.2</i>	$\text{NO}_3^-$	Root sclerenchyma, cortex, and stele cells	$\text{NO}_3^-$	[37]
AK101480	<i>OsNPF7.3</i>	$\text{NO}_3^-$	Root, seeds	Gly-His Gly-His-Gly	[33, 34]
AK064899	<i>OsNPF8.20</i>	N, light	Leaves, panicles, young root tips, cortical fiber cells of lateral roots, stems	Unknown	[32]
AK100802	<i>OsNPF4.1</i>	Unknown	Phloem of the branches of young panicles	Unknown	[31]
AK100112	<i>OsNPF8.2</i>	Drought, salt, cold	Seeds, leaf, panicle	Unknown	[33]
AK101055	<i>OsNPF5.5</i>	Unknown	Seeds, leaf	Unknown	[33]
AK101099	<i>OsNPF7.1</i>	Unknown	Constitutive expression	Unknown	[33]
AK070216	<i>OsNPF7.4</i>	Drought, salt	Root, panicle, node	Unknown	[33]
AK070036	<i>OsNPF8.1</i>	Drought, salt	Shoot, leaf, panicle, seeds	Unknown	[33]
AK072691	<i>OsNPF8.5</i>	Drought, salt	Constitutive expression	Unknown	[33]
AB008519	<i>OsNRT2.1</i>	$\text{NO}_3^-$ , light, sucrose	Root tip, meristem	$\text{NO}_3^-$	[38–40]
AK109733	<i>OsNRT2.2</i>	$\text{NO}_3^-$ , light, sucrose	Root tip, meristem	$\text{NO}_3^-$	[38–40]
AK109776	<i>OsNRT2.3a</i>	$\text{NO}_3^-$ , light, sucrose	Root stele	$\text{NO}_3^-$	[38, 41]
AK072215	<i>OsNRT2.3b</i>	Light, sucrose, pH	Shoot phloem	$\text{NO}_3^-$	[38, 42]
NM_193361	<i>OsNRT2.4</i>	$\text{NO}_3^-$ , light, sucrose, pH, NAA	Root, shoot	Unknown	[38–40]
NM_001053852.2	<i>OsNAR2.1</i>	$\text{NO}_3^-$ , light, sucrose	Root epidermal cells	Unknown	[19, 38–40]
AK109571	<i>OsNAR2.2</i>	Light, sucrose	Root, shoot	None	[19, 38, 39]
AF289477	<i>OsAMT1;1</i>	$\text{NH}_4^+$ , circadian rhythm	Constitutive expression	$\text{NH}_4^+$	[46, 48, 50, 52]

Accession no.	Gene	Regulation	Expression pattern	Substrates	References
AF289478	<i>OsAMT1;2</i>	NH <sub>4</sub> <sup>+</sup>	Root central cylinder and cell surface of root tips	NH <sub>4</sub> <sup>+</sup>	[46, 50]
AF289479	<i>OsAMT1;3</i>	Repressed, circadian rhythm	Root exodermis, sclerenchyma, endodermis, and pericycle cells of primary root	NH <sub>4</sub> <sup>+</sup>	[46, 47, 50, 53]
AB051864	<i>OsAMT2;1</i>	Unknown	Constitutive expression	NH <sub>4</sub> <sup>+</sup>	[46]
NM 190445	<i>OsAMT2;2</i>	NO <sub>3</sub> <sup>-</sup> , NH <sub>4</sub> <sup>+</sup>	Unknown	Unknown	[46, 55]
NM_001051237	<i>OsAMT2;3</i>	Unknown	Unknown	Unknown	[46]
AB083582	<i>OsAMT3;1</i>	Unknown	Roots, shoots	NH <sub>4</sub> <sup>+</sup>	[46]
AC104487	<i>OsAMT3;2</i>	Unknown	Unknown	Unknown	[46]
AP004775	<i>OsAMT3;3</i>	Unknown	Unknown	Unknown	[46]
AC091811	<i>OsAMT4</i>	Unknown	Unknown	Unknown	[46]
AB037664	<i>OsGS1;1</i>	NH <sub>4</sub> <sup>+</sup>	Leaves	NH <sub>4</sub> <sup>+</sup> , Glu	[58, 59]
AB180688	<i>OsGS1;2</i>	NH <sub>4</sub> <sup>+</sup>	Roots	NH <sub>4</sub> <sup>+</sup> , Glu	[58, 59, 64]
AB180689	<i>OsGS1;3</i>	Unknown	Spikelets	NH <sub>4</sub> <sup>+</sup> , Glu	[58, 59]
X14246	<i>OsGS2</i>	Unknown	Leaves	NH <sub>4</sub> <sup>+</sup> , Glu	[58, 60]
AB024716	<i>OsFd-GOGAT</i>	Light	Shoots	Gln, 2-OG	[60, 61]
AB008845	<i>OsNADH-GOGAT1</i>	NH <sub>4</sub> <sup>+</sup> , Gln	Developing tissues: root tip, premature leaf blade, spikelet at the early stage of ripening	Gln, 2-OG	[60, 61]
AB274818	<i>OsNADH-GOGAT2</i>	NH <sub>4</sub> <sup>+</sup>	Mature leaf blade and sheath: phloem companion and parenchyma cells	Gln, 2-OG	[60, 61, 68]

**Table 2.** Literature summary of the tissue expression and regulation of genes responsible for NUE.

gene alone did not increase nitrate uptake in rice [43], owing to that the nitrate uptake activity of *OsNRT2.1*, *OsNRT2.2*, and *OsNRT2.3a* requires a partner protein, *OsNAR2.1* [19, 38, 44]. The transcripts of *OsNAR2.2* and *OsNRT2.4* were detected in roots and shoots, accumulation induced by nitrate [38–40]. However, their functions remain unknown.

Ammonium uptake is mainly mediated by proteins of the ammonia transport protein (AMT)/ transports methylammonium (MEP)/rhesus (RH) superfamily [45]. There are uncertainties regarding the exact chemical species transported by AMT, which can be in the form of either hydrophobic NH<sub>3</sub> or charged ammonium [14, 45]. The activity of AMT members may play a more important role in NUpE in ammonium-preferring rice than in nitrate-utilizing crops. In rice, there are at least ten putative *OsAMT*-like genes grouped into four subfamilies (i.e., three each for *OsAMT1*, *OsAMT2*, and *OsAMT3*, respectively, and one for *OsAMT4*) (**Table 2**) [46]. So far, studies on expression regulation of *AMT* genes in rice are mainly focused on *OsAMT1* gene family, which displayed different spatiotemporal expression patterns in response to changes in N levels or daily irradiance (**Table 2**) [47, 48]. *OsAMT1;1* is constitutively expressed



in rice roots and shoots showing a positive feedback regulation by endogenous glutamine [49]. It has been reported that *OsAMT1;1*, showing a higher expression level in roots under ammonium supply, contributes to  $\text{NH}_4^+$  uptake and plays an important role in NK homeostasis [48, 50–52]. *OsAMT1;2* showed root-specific expression, is induced by ammonium, and may function as a nitrogen assimilator [49, 53]. Root-specific and nitrogen-derepressible expression for *OsAMT1;3* may function as a nitrogen sensor [49, 53]. Overexpression *OsAMT1;3* displayed significant decreases in growth but with poor nitrogen uptake ability, accompanied with a higher leaf C/N ratio [54]. *OsAMT2;1* showed constitutive expression in both roots and shoots, and *OsAMT3;1* showed very weak expression in roots and shoots [46]. *OsAMT2;2* is evenly expressed in roots and shoots and is induced by nitrogen [55].

### 3.2. Nitrogen assimilation

After taken up by the roots, nitrate is assimilated in the roots, the other larger part transported to the shoots, where it is first reduced to nitrite catalyzed by nitrate reductase (NR) in the cytoplasm and then further to ammonium by nitrite reductase (NiR) in the plastids. The ammonium derived from nitrate or directly from ammonium uptake by AMTs is finally assimilated into amino acids via the GS/GOGAT cycle (**Figure 1**) [14, 22]. GOGAT catalyzes the transfer of the amide group of glutamine (Gln) formed by GS to 2-oxoglutarate (2-OG) to yield two molecules of glutamate (Glu). One of the Gln molecules can be cycled back as a substrate for the GS reaction, and the other can be used for many synthetic reactions [56, 57].

Rice possesses three homologous but distinct genes for cytosolic glutamine synthetase (i.e., *OsGS1;1*, *OsGS1;2*, and *OsGS1;3*) and one chloroplastic gene (*OsGS2*). *OsGS1;1* and *OsGS1;2* both showed a high substrate affinity for ammonium and were induced by ammonium within the central cylinder of rice-elongating zone [58]. *OsGS1;1* was constitutively expressed, with higher expression profile in leaf blade and participated in rice normal growth and grain filling [59, 60]. *OsGS1;1* also functions in coordinating the global metabolic network in rice plants grown using ammonium as the nitrogen source [60] and is important for remobilization of nitrogen during natural senescence [61, 62]. *OsGS1;2* is constitutively expressed in surface cells of roots responsible for the primary assimilation of ammonium, and knockout of *OsGS1;2* showed severe reduction in active tiller number [63]. However, Ohashi et al. thought that the reduction in tiller number is an  $\text{NH}_4^+$ -specific event and the outgrowth of the axillary buds was severely suppressed caused by metabolic disorder in *OsGS1;2* mutants [64]. *OsGS1;3* is exclusively expressed in spikelet [59], indicating that it is probably important in grain ripening and/or germination. The *OsGS2* subunit protein was present in leaves but was hardly detectable in roots [58]. There is also a small gene family for GOGAT: one ferredoxin (Fd)-dependent type and two NADH-dependent types [65]. *OsFd-GOGAT* is highly abundant in mesophyll cells and other chloroplast-containing cells regulated by light [56] and is important in reassimilation of ammonium generated by photorespiration in chloroplasts [65]. Recently, participating in nitrogen assimilation, C/N balance, [66], leaf senescence, and the nitrogen remobilization has been reported [67]. *OsNADH-GOGAT1* is mainly expressed in surface cells of rice roots in an  $\text{NH}_4^+$ -dependent manner and is important for primary ammonium assimilation in roots at the seedling stage and development of active tiller number until the harvest [62, 65]. *OsNADH-GOGAT2* is mainly expressed in vascular tissues of mature leaf blades and is important in the process

of glutamine generation in senescing leaves for the remobilization of leaf nitrogen through phloem to the panicle during natural senescence. *OsNADH-GOGAT2* mutants had marked reduction in spikelet number per panicle [62, 68].

Although these observed phenotypes and those observed for GS enzymes have been identified, the interaction between isozymes of GOGAT and the GS isozymes, how they affect NUE, as well as posttranscriptional regulation of these enzymes needs to be further investigated.

### 3.3. Nitrogen remobilization and reassimilation

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 (**Figure 1**) [69]. 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 [14], and the rest is supplemented from the soil and late top-dressed fertilizers [70]. Gln and asparagine (Asn) are major forms of total amino acids in phloem and xylem sap of rice plants [14, 71]. Increases of both Asn and Gln concentrations during senescence in the phloem sap suggest their key role in rendering N available for remobilization from the senescing leaves. Some isoforms of GS1, NADH-glutamate dehydrogenase (GDH), and asparagine synthetase (AS) are strongly activated during N remobilization [72]. The nature of the amino acid transporters, belonging to complex multigene families, is poorly understood in phloem loading for N redistribution during senescence [69].

The importance of GS/GOGAT activity in N remobilization, reassimilation, growth rate, yield, and grain filling has been emphasized previously. *OsGS1;1* and *OsNADH-GOGAT2* are important in remobilization of nitrogen during natural senescence [62]. *GS1;2* is also important in the development of active tillers through the assimilation of  $\text{NH}_4^+$  generated during lignin synthesis [64]. Together with GS, AS is believed to play a crucial role in primary N metabolism, catalyzing the formation of Asn and Glu from Gln and aspartate [14, 64]. There are two genes (i.e., *OsAS1* and *OsAS2*) identified encoding AS in rice. *OsAS1* is mainly expressed in root surface (epidermis, exodermis, and sclerenchyma) in an  $\text{NH}_4^+$ -dependent manner, which are very similar with *OsGS1;2* and *NADH-GOGAT1* in rice roots. Thus, AS1 is apparently coupled with the primary assimilation of  $\text{NH}_4^+$  in rice roots. *OsAS2* detected in phloem companion and parenchyma cells [71, 73] is abundant in leaf blades and sheathes, along with the *GS1;1* protein [61]. These suggest that AS2 in rice leaves is probably important in the long-distance transport of asparagine from rice leaves during natural senescence. In addition, the mitochondrial GDH plays a major role in reassimilation of photorespiratory ammonia and can alternatively incorporate ammonium into Glu in response to high levels of ammonium under stress [72]. Although there are a large number of amino acid permeases (AAPs) presented in rice [74, 75], no transporters have been functionally characterized with an exception for *OsAAP6*, which is mainly expressed in seeds for grain protein content [76]. Recently, the transport function of four rice AAP genes (*OsAAP1*, *OsAAP3*, *OsAAP7*, and *OsAAP16*) has been analyzed by expression in *Xenopus laevis* oocytes, electrophysiology, and cellular localization. *OsAAP1*, *OsAAP7*, and *OsAAP16* functioned as general AAPs and could transport all amino acids well except aspartate and  $\beta$ -alanine. While *OsAAP3* had a distinct substrate specificity transporting the basic amino acids lysine and arginine well but selected against aromatic amino acids [77].

## 4. Enhancing nitrogen use efficiency

As mentioned above, molecular studies have provided a general validation of the physiological conceptual framework of NUE in rice. However, besides genetics, there are other factors needed to consider such as the interactions between N uptake and water availability, the interaction between N utilization and carbon metabolism, and the interaction between different macronutrients and micronutrients [13]. Understanding the mechanisms regulating nitrogen movement in rice is crucial for improvement of NUE. Improvements in NUE result from NUpE, NUtE, or both. We describe approaches for increasing NUE with special consideration to genetics and agricultural management.

### 4.1. Increasing uptake capacity

Increased nitrogen uptake capacity may be achieved through better nitrogen transporters, more effective regulation of the transport systems, or better storage and assimilation. A simple example to improve NUpE would be to increase uptake by overexpressing more efficient transporters or all the transporters using transgenic methods [28, 42, 48, 78]. However, only increasing the uptake capacity of roots is not simple because of the tight regulation of N uptake, N taken up surplus to requirements increasing plant N status, which, in turn, leads to feedback regulation and reduction in uptake capacity [20].

Physiological traits that may also affect NUpE including root architecture and any other characteristic play a pivotal role in extracting available N from the soil [13, 79]. The capacity of the root for uptake depends on the degree to which the root extends and its absorption area, which is determined by complex root morphology. A common example is to target genes related to root morphology through a mapping approach, whereby traits are identified through genetic crosses using distinct populations, and then quantitative trait loci (QTLs) can be cloned by positional cloning [79–81]. To date, studies have been carried out to identify root morphological features such as root mass and depth, root axis length, and lateral branching related to NUE [82–84].

However, ammonium or nitrate uptake by rice roots commonly results in acidification or alkalization of the rhizosphere, which in turn changes the soil N availability for plants. In the rhizosphere, rice roots can also release oxygen and exudates that greatly influence local redox potential and the density and activity of microbial populations, which in turn can interconvert soil N forms, including those derived from fertilizer [14]. Thus, soil N availability fluctuating greatly in both space and time affects root morphology, which could make plants uptake N efficiently [14]. Studies in rice have been confirmed that compared to sole  $\text{NH}_4^+$  nutrition, a mixture of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  promoted root growth as well as N absorption and assimilation [85, 86]. In the course of agricultural management, fertilizer type (i.e., controlled N release fertilizers, new potential N sources), methods of applying N fertilizers (e.g., the 4R nutrient stewardship framework: right source, right rate, right time, and right placement), soil types, tillage, transplanting density, cropping system, and microorganisms are governed to avoid nitrogen loss increasing fertilizer nitrogen use efficiency [87, 88].

Water is another key factor determining crop yield and NUE. Without sufficient water, plants cannot extract nutrients from the soil. Yield is constrained by moisture availability, not N availability, especially in maize [89]. In contrast to upland crops, alternate wetting and drying (AWD, flooding the soil and then allowing to dry down before being reflooded) to reduce total water for irrigation in rice has been developed for a number of decades. A number of studies have shown that AWD increases grain yield when compared to continuous flooding (CF) [90, 91].

On the base of current knowledge, scientists have developed a range of optimized crop management practices, such as site-specific nutrient management (SSNM) [92], real-time N management (RTNM) [93], and preliminary integrated precision rice management (PRM) system combining SSNM with alternate drying and wetting irrigation and optimized transplanting density [94]. Only integrated N management strategies are allowed for the achievement of production goals while minimizing the risk of environmental pollution. Sources of N and timing of application determine the most suitable method for application. The interest in implementing new knowledge about the methods of application is to develop sensors to diagnose the N status of crops in real time throughout large areas and decision support systems to help determine N fertilizer recommendations [88].

#### **4.2. Increasing utilization efficiency**

A number of physiological traits can affect the NUtE in crops, including the effect of N on carbohydrate partitioning, the storage of N, and the remobilization of N from senescent tissues, and these have been subdivided into a number of components by researchers [95, 96].

Increasing nitrogen utilization capacity can be achieved through overexpression of candidate genes in the pathways relating to N assimilation, translocation, remobilization, and re-assimilation. As mentioned above, changes in the expression and activity of GS and GOGAT would have an effect on N assimilation, recycle, re-assimilation, C/N balance, and senescence in rice, potentially affecting grain filling, yield, and NUE [62, 64, 66]. Identifying candidate genes cosegregate with NUE in genetic crosses is another efficient method. One of the first QTL studies conducted analyzing NUE in rice was carried out [97]. They looked at QTLs associated with NUE and determined whether they cosegregated with GS1 and NADH-GOGAT. The analysis identified seven loci that cosegregated with GS1 activity and six loci that cosegregated with NADH-GOGAT activity. A number of QTLs for agronomic traits related to N use and yield have been mapped to the chromosomal regions containing GS2 in rice [97, 98], suggesting that the genomic region surrounding GS2 may be valuable for breeding rice with improved agronomic performance and NUE. However, to date, no one has been able to introduce a GS gene into a NUE-inefficient background and show either enhanced NUE or yield.

C and N metabolisms are tightly linked with each other in plants. N assimilation requires carbon metabolism to provide adenosine triphosphate (ATP), reductants, and C skeletons through photosynthesis, photorespiration, and respiration. Large amounts of N are used in photosynthesis, particularly during ribulose 1,5-bisphosphate carboxylase-oxygenase

(Rubisco) and light-harvesting complexes to support the light-dependent use of CO<sub>2</sub>, inorganic N, and water to produce sugars, amino acids, and organic acids [99]. Photorespiration, a side reaction of photosynthesis, has crucial implications in N reassimilation, which is catalyzed by the Rubisco. During photorespiration, NH<sub>4</sub><sup>+</sup> is produced during methylenetetrahydrofolate synthesis from glycine [100]. Respiration is a third fundamental process of energy metabolism in the dark and in nonphotosynthetic tissues, as well as in the light. In the respiratory pathways, the C skeletons for N assimilation are generated in different sectors, such as the oxidative pentose phosphate pathway (OPPP), glycolysis, and TCA cycle [101]. The operation of the TCA cycle in illuminated leaves is critical for the provision of 2-OG, which is necessary for glutamate and glutamine production [101–103]. Evidence has shown that the synthesis of 2-OG is induced by the activity of phosphoenolpyruvate carboxylase (PEPC), citrate synthase, isocitrate dehydrogenase, and aconitase, while the subsequent conversion of 2-OG to fumarate may be repressed in the light [101].

Thus, exploiting candidate genes involved in C/N metabolism is another approach to improve NUE. To date, there are two key genes identified to contribute to NUE in rice. Chloroplastic proteins are known to make up approximately 80% of the stored N in leaf tissues, with Rubisco accounting for up to 50% and 20% of the stored N in C<sub>3</sub> and C<sub>4</sub> plants, respectively [104]. Thus, Rubisco is an excellent N storage molecule, and its autophagic degradation in rice leaves may contribute to an efficient and rapid N remobilization by facilitating protein degradation for N mobilization in senescent leaves [70]. Rubisco is also involved in photorespiratory losses which can be as high as 20% of the total carbon fixation in C<sub>3</sub> plants and also liberates ammonia, which is required for reassimilation [105]. However, when rice plants overexpressing the Rubisco (*rbcS*) gene were analyzed, Rubisco-N to leaf-N increased, but there was no change in the rate of photosynthesis [106]. PEPC is a component of primary metabolism in plants and has a nonphotosynthetic role as one of its products is OAA, a component of the TCA cycle [107]. RNAi knockdown experiments of the chloroplastic isoform in rice have indicated that PEPC plays an important role in N assimilation, specifically when the main N source is NH<sub>4</sub><sup>+</sup> [108].

Growth and yield of rice plants are markedly affected by increased CO<sub>2</sub> concentration and temperature [109, 110]. Numerous studies have indicated that an increase in CO<sub>2</sub> generally stimulates photosynthesis, reduces stomatal conductance, and changes the rhizosphere conditions of plants, leading to increases in biomass and yield of crops [111–113], whereas an increase in temperature accelerates crop phenological development and shortens grain-filling period of crops, leading to decrease grain yield and reduce crop production in many regions of the world [114, 115]. Furthermore, high temperature, if occurring at critical stages of crop development (such as meiosis and flowering stages), reduces spikelet fertility [115]. Owing to elevated CO<sub>2</sub> under future climate change is associated with an increase in air temperature, many studies about plant response to the interaction of CO<sub>2</sub> and temperature have been reported [109, 110, 116]. Increases in CO<sub>2</sub> were unable to compensate for the negative impact of increases in temperature on biomass and yield in rice [109, 110]. Thus, selecting high-temperature-tolerant germplasm will be required to realize yield benefits in the future.

## 5. Conclusions

Plant NUE is a complex trait determined by quantitative trait loci and influenced by environmental changes and is the integration of NUpE and NUtE. There is a complex regulation of N uptake, assimilation, and remobilization.

Enhanced NUE can be achieved by genetically modifying plants and integrated agricultural management practices. The former is the most effective biotechnological method for increasing NUE. This can be achieved by overexpression of nitrate and ammonium transporters responsible for N uptake by roots and by manipulation of key genes controlling the balance of N and C metabolism.

Developing an integrated research program combining approaches, mainly based on whole-plant physiology, quantitative genetics, forward and reverse genetics, and agronomy approaches to improve NUE, is a major objective in the future.

## Acknowledgements

We thank Dr. Chang Li for his comments on **Figure 1** and for his helpful discussions on this timely topic. We apologize to all colleagues whose work could not be cited owing for space limitations. This work is supported by grants from China's Agriculture Research System (#CARS-01-47) and the National Key Technology Support Program (2015BAD01B02).

## Author details

Shuangjie Huang<sup>1\*</sup>, Chunfang Zhao<sup>1</sup>, Yali Zhang<sup>2</sup> and Cailin Wang<sup>1</sup>

\*Address all correspondence to: [huangdeifan@163.com](mailto:huangdeifan@163.com)

<sup>1</sup> Institute of Food Crops of Jiangsu Academy of Agricultural Sciences, Nanjing, China

<sup>2</sup> State Key Laboratory of Crop Genetics and Germplasm Enhancement, Key Laboratory of Plant Nutrition and Fertilization in Low-Middle Reaches of the Yangtze River, Ministry of Agriculture, Nanjing Agricultural University, China

## References

- [1] Godfray HCJ, Beddington JR, Crute IR. Food security: The challenge of feeding 9 billion people. *Science*. 2010;**327**:812-818. DOI: 10.1126/science.1185383
- [2] Tilman D, Balzer C, Hill J. Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;**108**:20260-20264. DOI: 10.1073/pnas.1116437108

- [3] Cassman KG. Ecological intensification of cereal production systems: Yield potential, soil quality, and precision agriculture. *Proceedings of the National Academy of Sciences*. 1999;**96**: 5952-5959. DOI: 10.1073/pnas.96.11.5952
- [4] Wu LL, Yuan S, Huang LY. Physiological mechanisms underlying the high-grain yield and high-nitrogen use efficiency of elite rice varieties under a low rate of nitrogen application in china. *Frontiers in Plant Science*. 2016;**7**:1024. DOI: 10.3389/fpls.2016.01024
- [5] Robertson GP, Vitousek PM. Nitrogen in agriculture: Balancing the cost of an essential resource. *Annual Review of Environment and Resources*. 2009;**34**:97-125. DOI: 10.1146/annurev.environ.032108.105046
- [6] Andrews M, Raven JA, Lea PJ. Do plants need nitrate? The mechanisms by which nitrogen form affects plants. *Annals of Applied Biology*. 2013;**163**:174-199. DOI: 10.1111/aab.12045
- [7] Vitousek PM, Naylor R, Crews T. Nutrient imbalances in agricultural development. *Science*. 2009;**324**:1519-1520. DOI: 10.1126/science.1170261
- [8] Good AG, Beatty PH. Fertilizing nature: A tragedy of excess in the commons. *PLoS Biology*. 2011;**9**:e1001124. DOI: 10.1371/journal.pbio.1001124
- [9] Zhang FS, Cui ZL, Fan MS. Integrated soil-crop system management: Reducing environmental risk while increasing crop productivity and improving nutrient use efficiency in china. *Journal of Environmental Quality*. 2011;**40**:1051-1057. DOI: 10.2134/jeq2010.0292
- [10] Smil V. Nitrogen in crop production: An account of global flows. *Global Biogeochemical Cycles*. 1999;**13**:647-662. DOI: 10.1029/1999gb900015
- [11] Diaz RJ, Rosenberg R. Spreading dead zones and consequences for marine ecosystems. *Science*. 2008;**321**:926-929. DOI: 10.1126/science.1156401
- [12] Guo JH, Liu XJ, Zhang Y. Significant acidification in major Chinese croplands. *Science*. 2010;**327**:1008-1010. DOI: 10.1126/science.1182570
- [13] Han M, Okamoto M, Beatty PH. The genetics of nitrogen use efficiency in crop plants. *Annual Review of Genetics*. 2015;**49**:269-289. DOI: 10.1146/annurev-genet-112414-055037
- [14] Xu GH, Fan XR, Miller AJ. Plant nitrogen assimilation and use efficiency. *Annual Review of Plant Biology*. 2012;**63**:153-182. DOI: 10.1146/annurev-arplant-042811-105532
- [15] Good AG, Shrawat AK, Muench DG. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production?. *Trends in Plant Science*. 2004;**9**:597-605. DOI: 10.1016/j.tplants.2004.10.008
- [16] Foulkes MJ, Hawkesford MJ, Barraclough PB. Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. *Field Crops Research*. 2009;**114**:329-342. DOI: 10.1016/j.fcr.2009.09.005
- [17] Kirk GJD, Kronzucker HJ. The potential for nitrification and nitrate uptake in the rhizosphere of wetland plants: A modelling study. *Annals of Botany*. 2005;**96**:639-646. DOI: 10.1093/aob/mci216

- [18] Li YL, Fan XR, Shen QR. The relationship between rhizosphere nitrification and nitrogen-use efficiency in rice plants. *Plant Cell & Environment*. 2008;**31**:73-85. DOI: 10.1111/j.1365-3040.2007.01737.x
- [19] Yan M, Fan XR, Feng HM. Rice OsNAR2.1 interacts with OsNRT2.1, OsNRT2.2 and OsNRT2.3a nitrate transporters to provide uptake over high and low concentration ranges. *Plant Cell & Environment*. 2011;**34**:1360-1372. DOI: 10.1111/j.1365-3040.2011.02335.x
- [20] Miller AJ, Fan XR, Orsel M. Nitrate transport and signalling. *Journal of Experimental Botany*. 2007;**58**:2297-2306. DOI: 10.1093/jxb/erm066
- [21] Williams LE, Miller AJ. Transporters responsible for the uptake and partitioning of nitrogenous solutes. *Annual Review of Plant Physiology & Plant Molecular Biology*. 2011;**52**:659-688. DOI: 10.1146/annurev.arplant.52.1.659
- [22] Neeraja CN, Subramanyam D, Surekha K. Advances in genetic basis of nitrogen use efficiency of rice. *Indian Journal of Plant Physiology*. 2016;**21**:504-513. DOI: 10.1007/s40502-016-0254-z
- [23] O'Brien JA, Vega A, Bouguyon E. Nitrate transport, sensing, and responses in plants. *Molecular Plant*. 2016;**9**:837-856. DOI: 10.1016/j.molp.2016.05.004
- [24] Krapp A, David LC, Chardin C. Nitrate transport and signalling in Arabidopsis. *Journal of Experimental Botany*. 2014;**65**:789-798. DOI: 10.1093/jxb/eru001
- [25] Dechorgnat J, Nguyen CT, Armengaud P. From the soil to the seeds: The long journey of nitrate in plants. *Journal of Experimental Botany*. 2011;**62**:1349-1359. DOI: 10.1093/jxb/erq409
- [26] L eran S, Varala K, Boyer J. A unified nomenclature of nitrate transporter 1/peptide transporter family members in plants. *Trends in Plant Science*. 2013;**19**:5-9. DOI: 10.1016/j.tplants.2013.08.008
- [27] Tsay YF, Chiu CC, Tsai CB. Nitrate transporters and peptide transporters. *FEBS Letters*. 2007;**581**:2290-2300. DOI: 10.1016/j.febslet.2007.04.047
- [28] Hu B, Wang W, Ou SJ. Variation in *NRT1.1B* contributes to nitrate-use divergence between rice subspecies. *Nature Genetics*. 2015;**47**:834-838. DOI: 10.1038/ng.3337
- [29] Fan XR, Feng HM, Tan YW. A putative 6-transmembrane nitrate transporter *OsNRT1.1b* plays a key role in rice under low nitrogen. *Journal of Integrative Plant Biology*. 2016;**58**:590-599. DOI: 10.1111/jipb.12382
- [30] Lin CM, Koh S, Stacey G. Cloning and functional characterization of a constitutively expressed nitrate transporter gene, *OsNRT1*, from rice. *Plant Physiology*. 2000;**122**:379-388. DOI: 10.1104/pp.122.2.379
- [31] Li SB, Qian Q, Fu ZM. Short panicle1 encodes a putative PTR family transporter and determines rice panicle size. *Plant Journal*. 2009;**58**:592-605. DOI: 10.1111/j.1365-313X.2009.03799.x



- [32] Fang ZM, Xia KF, Yang X. Altered expression of the PTR/NRT1 homologue *OsPTR9* affects nitrogen utilization efficiency, growth and grain yield in rice. *Plant Biotechnology Journal*. 2013;**11**:446-458. DOI: 10.1111/pbi.12031
- [33] Ouyang J, Cai ZY, Xia KF. Identification and analysis of eight peptide transporter homologs in rice. *Plant Science*. 2010;**179**:374-382. DOI: 10.1016/j.plantsci.2010.06.013
- [34] Fan XR, Xie D, Chen JG. Over-expression of *OsPTR6*, in rice increased plant growth at different nitrogen supplies but decreased nitrogen use efficiency at high ammonium supply. *Plant Science*. 2014;**227**:1-11. DOI: 10.1016/j.plantsci.2014.05.013
- [35] Xia XD, Fan XR, Wei J. Rice nitrate transporter *OsNPF2.4* functions in low-affinity acquisition and long-distance transport. *Journal of Experimental Botany*. 2015;**66**:317-331. DOI: 10.1093/jxb/eru425
- [36] Li Y, Ouyang J, Wang YY. Disruption of the rice nitrate transporter *OsNPF2.2* hinders root-to-shoot nitrate transport and vascular development. *Scientific Reports*. 2015;**5**:9635. DOI: 10.1038/srep09635
- [37] Hu R, Qiu DY, Chen Y. Knock-down of a tonoplast localized low-affinity nitrate transporter *OsNPF7.2* affects rice growth under high nitrate supply. *Frontiers in Plant Science*. 2016;**7**:1529. DOI: 10.3389/fpls.2016.01529
- [38] Feng HM, Yan M, Fan XR. Spatial expression and regulation of rice high-affinity nitrate transporters by nitrogen and carbon status. *Journal of Experimental Botany*. 2011;**62**:2319-2332. DOI: 10.1093/jxb/erq403
- [39] Araki R, Hasegawa H. Expression of rice (*Oryza sativa* L.) genes involved in high-affinity nitrate transport during the period of nitrate induction. *Breeding Science*. 2006;**56**:295-302. DOI: 10.1270/jsbbs.56.295
- [40] Cai C, Wang JY, Zhu YG. Gene structure and expression of the high-affinity nitrate transport system in rice roots. *Journal of Integrative Plant Biology*. 2008;**50**:443-451. DOI: 10.1111/j.1744-7909.2008.00642.x
- [41] Tang Z, Fan XR, Li Q. Knockdown of a rice stelar nitrate transporter alters long-distance translocation but not root influx. *Plant Physiology*. 2012;**160**:2052-2063. DOI: 10.1104/pp.112.204461
- [42] Fan XR, Tang Z, Tan, YW. Overexpression of a pH-sensitive nitrate transporter in rice increases crop yields. *Proceedings of the National Academy of Sciences*. 2016;**113**:7118-7123. DOI: 10.1073/pnas.1525184113
- [43] Katayama H, Mori M, Kawamura Y. Production and characterization of transgenic rice plants carrying a high-affinity nitrate transporter gene (*OsNRT2.1*). *Breeding Science*. 2009;**59**:237-243. DOI: 10.1270/jsbbs.59.237
- [44] Liu XQ, Huang DM, Tao JY. Identification and functional assay of the interaction motifs in the partner protein *OsNAR2.1* of the two-component system for high-affinity nitrate transport. *New Phytologist*. 2014;**204**:74-80. DOI: 10.1111/nph.12986

- [45] Khademi S, O'Connell J, Remis J. Mechanism of ammonia transport by amt/mep/rh: Structure of amtb at 1.35 Å. *Science*. 2004;**305**:1587-1594. DOI: 10.1126/science.1101952
- [46] Suenaga A, Moriya K, Sonoda Y. Constitutive expression of a novel-type ammonium transporter *OsAMT2* in rice plants. *Plant & Cell Physiology*. 2003;**44**:206-211. DOI: 10.1093/pcp/pcg017
- [47] Kumar A, Silim SN, Okamoto M. Differential expression of three members of the AMT1, gene family encoding putative high-affinity NH<sub>4</sub><sup>+</sup> transporters in roots of *oryza sativa* subspecies indica. *Plant Cell & Environment*. 2003;**26**:907-914. DOI: 10.1046/j.1365-3040.2003.01023.x
- [48] Ranathunge K, El-kereamy A, Gidda S. *AMT1;1* transgenic rice plants with enhanced NH<sub>4</sub><sup>+</sup> permeability show superior growth and higher yield under optimal and suboptimal NH<sub>4</sub><sup>+</sup> conditions. *Journal of Experimental Botany*. 2014;**65**:965-979. DOI: 10.1093/jxb/ert458
- [49] Sonoda Y, Ikeda A, Saiki S. Feedback regulation of the ammonium transporter gene family *AMT1* by glutamine in rice. *Plant & Cell Physiology*. 2003;**44**:1396-1402. DOI: 10.1093/pcp/pcg169
- [50] Sonoda Y, Ikeda A, Saiki S. Distinct expression and function of three ammonium transporter genes (*OsAMT1;1-1;3*) in rice. *Plant & Cell Physiology*. 2003;**44**:726-734. DOI: 10.1093/pcp/pcg083
- [51] Hoque MS, Masle J, Udvardi MK. Over-expression of the rice *OsAMT1-1* gene increases ammonium uptake and content, but impairs growth and development of plants under high ammonium nutrition. *Functional Plant Biology*. 2006;**33**:153-163. DOI: 10.1071/FP05165
- [52] Li C, Tang Z, Wei J. The *OsAMT1.1* gene functions in ammonium uptake and ammonium-potassium homeostasis over low and high ammonium concentration ranges. *Journal of Genetics & Genomics*. 2016;**43**:639-649. DOI: 10.1016/j.jgg.2016.11.001
- [53] Yao SG, Sonoda Y, Tsutsui T. Promoter analysis of *OsAMT1;2* and *OsAMT1;3* implies their distinct roles in nitrogen utilization in rice. *Breeding Science*. 2008;**58**:201-207. DOI: 10.1270/jsbbs.58.201
- [54] Bao A, Liang ZJ, Zhao ZQ. Overexpressing of *OsAMT1-3*, a high affinity ammonium transporter gene, modifies rice growth and carbon-nitrogen metabolic status. *International Journal of Molecular Sciences*. 2015;**16**:9037-9063. DOI: 10.3390/ijms16059037
- [55] Li SM, Shi WM. Quantitative characterization of nitrogen regulation of *OsAMT1;1*, *OsAMT1;2*, and *OsAMT2;2*, expression in rice seedlings. *Russian Journal of Plant Physiology*. 2006;**53**:837-843. DOI: 10.1134/S102144370606015X
- [56] Ishiyama K, Hayakawa T, Yamaya T. Expression of NADH-dependent glutamate synthase protein in the epidermis and exodermis of rice roots in response to the supply of ammonium ions. *Planta*. 1998;**204**:288-294. DOI: 10.1007/s004250050258

- [57] Forde BG, Lea PJ. Glutamate in plants: Metabolism, regulation, and signalling. *Journal of Experimental Botany*. 2007;**58**:2339-2358. DOI: 10.1093/jxb/erm121
- [58] Ishiyama K, Inoue E, Tabuchi M. Biochemical background and compartmentalized functions of cytosolic glutamine synthetase for active ammonium assimilation in rice roots. *Plant & Cell Physiology*. 2004;**45**:1640-1647. DOI: 10.1093/pcp/pch190
- [59] Tabuchi M, Sugiyama K, Ishiyama K. Severe reduction in growth rate and grain filling of rice mutants lacking OsGS1;1, a cytosolic glutamine synthetase1;1. *Plant Journal for Cell & Molecular Biology*. 2005;**42**:641-651. DOI: 10.1111/j.1365-313X.2005.02406.x
- [60] Kusano M, Tabuchi M, Fukushima A. Metabolomics data reveal a crucial role of cytosolic glutamine synthetase 1;1 in coordinating metabolic balance in rice. *Plant Journal*. 2011;**66**:456-466. DOI: 10.1111/j.1365-313X.2011.04506.x
- [61] Tabuchi M, Abiko T, Yamaya T. Assimilation of ammonium ions and reutilization of nitrogen in rice (*Oryza sativa* L.). *Journal of Experimental Botany*. 2007;**58**:2319-2327. DOI: 10.1093/jxb/erm016
- [62] Yamaya T, Kusano M. Evidence supporting distinct functions of three cytosolic glutamine synthetases and two NADH-glutamate synthases in rice. *Journal of Experimental Botany*. 2014;**65**:5519-5525. DOI: 10.1093/jxb/eru103
- [63] Funayama K, Kojima S, Tabuchi-kobayashi M. Cytosolic glutamine synthetase1;2 is responsible for the primary assimilation of ammonium in rice roots. *Plant & Cell Physiology*. 2013;**54**:934-943. DOI: 10.1093/pcp/pct046
- [64] Ohashi M, Ishiyama K, Kusano M. Lack of cytosolic glutamine synthetase1;2 in vascular tissues of axillary buds caused severe reduction in their outgrowth and disorder of metabolic balance in rice seedlings. *Plant Journal*. 2015;**81**:347-356. DOI: 10.1111/tbj.12731
- [65] Tamura W, Hidaka Y, Tabuchi M. Reverse genetics approach to characterize a function of NADH-glutamate synthase1 in rice plants. *Amino Acids*. 2010;**39**:1003-1012. DOI: 10.1007/s00726-010-0531-5
- [66] Yang XL, Nian JQ, Xie QJ. Rice ferredoxin-dependent glutamate synthase regulates nitrogen-carbon metabolomes and is genetically differentiated between *japonica* and *indica* subspecies. *Molecular Plant*. 2016;**9**:1520-1534. DOI: 10.1016/j.molp.2016.09.004
- [67] Zeng DD, Qin R, Li M. The ferredoxin-dependent glutamate synthase (OsFd-GOGAT) participates in leaf senescence and the nitrogen remobilization in rice. *Molecular Genetics & Genomics*. 2017;**292**:385-395. DOI: 10.1007/s00438-016-1275-z
- [68] Tamura W, Kojima S, Toyokawa A. Disruption of a novel NADH-glutamate synthase2 gene caused marked reduction in spikelet number of rice. *Frontiers in Plant Science*. 2011;**2**:57. DOI: 10.3389/fpls.2011.00057
- [69] Okumoto S, Pilot G. Amino acid export in plants: A missing link in nitrogen cycling. *Molecular Plant*. 2011;**4**:453-463. DOI: 10.1093/mp/ssr003

- [70] Yoneyama T, Tanno F, Tatsumi J. Whole-plant dynamic system of nitrogen use for vegetative growth and grain filling in rice plants (*Oryza sativa* L.) as revealed through the production of 350 grains from a germinated seed over 150 days: A review and synthesis. *Frontiers in Plant Science*. 2016;**7**:1151. DOI: 10.3389/fpls.2016.01151
- [71] Ohashi M, Ishiyama K, Kojima S. Asparagine synthetase1, but not asparagine synthetase2, is responsible for the biosynthesis of asparagine following the supply of ammonium to rice roots. *Plant & Cell Physiology*. 2015;**56**:769-778. DOI: 10.1093/pcp/pcv005
- [72] Masclaux-daubresse C, Danielvedele F, Dechorgnat J. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. *Annals of Botany*. 2010;**105**:1141-1157. DOI: 10.1093/aob/mcq028
- [73] Nakano K, Suzuki T, Hayakawa T. Organ and cellular localization of asparagine synthetase in rice plants. *Plant & Cell Physiology*. 2000;**41**:874-880. DOI: 10.1093/pcp/pcd006
- [74] Tegeder M, Ward JM. Molecular evolution of plant AAP and LHT amino acid transporters. *Frontiers in Plant Science*. 2012;**3**:21. DOI: 10.3389/fpls.2012.00021
- [75] Zhao HM, Ma HL, Yu L. Genome-wide survey and expression analysis of amino acid transporter gene family in rice (*Oryza sativa* L.). *PLoS ONE*. 2012;**7**:e49210. DOI: 10.1371/journal.pone.0049210
- [76] Peng B, Kong HL, Li YB. *OsAAP6* functions as an important regulator of grain protein content and nutritional quality in rice. *Nature Communications*. 2014;**5**:4847. DOI: 10.1038/ncomms5847
- [77] Taylor MR, Reinders A, Ward JM. Transport function of rice amino acid permeases (AAPs). *Plant & Cell Physiology*. 2015;**56**:1355-1363. DOI: 10.1093/pcp/pcv053
- [78] Chen JG, Zhang Y, Tan YW. Agronomic nitrogen-use efficiency of rice can be increased by driving *OsNRT2.1* expression with the *OsNAR2.1* promoter. *Plant Biotechnology Journal*. 2016;**14**:1705-1715. DOI: 10.1111/pbi.12531
- [79] Garnett T, Conn V, Kaiser BN. Root based approaches to improving nitrogen use efficiency in plants. *Plant Cell & Environment*. 2009;**32**:1272-1283. DOI: 10.1111/j.1365-3040.2009.02011.x
- [80] Zhang HM, Forde BG. An Arabidopsis mads box gene that controls nutrient-induced changes in root architecture. *Science*. 1998;**279**:407-409. DOI: 10.1126/science.279.5349.407
- [81] McAllister CH, Beatty PH, Good AG. Engineering nitrogen use efficient crop plants: The current status. *Plant Biotechnology Journal*. 2012;**10**:1011-1025. DOI: 10.1111/j.1467-7652.2012.00700.x
- [82] Yadav R, Courtois B, Huang N. Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. *Theoretical and Applied Genetics*. 1997;**94**:619-632. DOI: 10.1007/s001220050459

- [83] Shen L, Courtois B, McNally KL. Evaluation of near-isogenic lines of rice introgressed with QTLs for root depth through marker-aided selection. *Theoretical and Applied Genetics*. 2011;**103**:75-83. DOI: 10.1007/s001220100538
- [84] Horii H, Nemoto K, Miyamoto N. Quantitative trait loci for adventitious and lateral roots in rice. *Plant Breeding*. 2006;**125**:198-200. DOI: 10.1111/j.1439-0523.2006.01124.x
- [85] Duan YH, Zhang YL, Ye LT. Responses of rice cultivars with different nitrogen use efficiency to partial nitrate nutrition. *Annals of Botany*. 2007;**99**:1153-1160. DOI: 10.1093/aob/mcm051
- [86] Song WJ, Makeen K, Wang DS. Nitrate supply affects root growth differentially in two rice cultivars differing in nitrogen use efficiency. *Plant and Soil*. 2011;**343**:357-368. DOI: 10.1007/s11104-011-0723-0
- [87] Chen D, Suter H, Islam A. Prospects of improving efficiency of fertilizer nitrogen in Australian agriculture: A review of enhanced efficiency fertilizers. *Australian Journal of Soil Research*. 2008;**46**:289-301. DOI: 10.1071/SR07197
- [88] Herrera JM, Rubio G, Häner LL. Emerging and established technologies to increase nitrogen use efficiency of cereals. *Agronomy*. 2016;**6**:25. DOI: 10.3390/agronomy6020025
- [89] Bänziger M, Edmeades GO, Lafitte HR. Selection for drought tolerance increases maize yields across a range of nitrogen levels. *Crop Science*. 1999;**39**:1035-1040. DOI: 10.2135/cropsci1999.0011183x003900040012x
- [90] Yang JC, Huang DF, Duan, H. Alternate wetting and moderate soil drying increases grain yield and reduces cadmium accumulation in rice grains. *Journal of the Science of Food & Agriculture*. 2009;**89**:1728-1736. DOI: 10.1002/jsfa.3648
- [91] Zhang H, Xue YG, Wang ZQ. An alternate wetting and moderate soil drying regime improves root and shoot growth in rice. *Crop Science*. 2009;**49**:2246-2260. DOI: 10.2135/cropsci2009.02.0099
- [92] Dobermann A, Witt C, Dawe D. Site-specific nutrient management for intensive rice cropping systems in Asia. *Field Crops Research*. 2002;**108**:37-66. DOI: 10.1016/S0378-4290(01)00197-6
- [93] Peng SB, Buresh RJ, Huang JL. Strategies for overcoming low agronomic nitrogen use efficiency in irrigated rice systems in china. *Field Crops Research*. 2006;**96**:37-47. DOI: 10.1016/j.fcr.2005.05.004
- [94] Zhao GM, Miao YX, Wang HY. A preliminary precision rice management system for increasing both grain yield and nitrogen use efficiency. *Field Crops Research*. 2013;**154**:23-30. DOI: 10.1016/j.fcr.2013.07.019
- [95] Hirel B, Le Gouis J, Ney B. The challenge of improving nitrogen use efficiency in crop plants: Towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of Experimental Botany*. 2007;**58**:2369-2387. DOI: 10.1093/jxb/erm097

- [96] Lea PJ, Azevedo RA. Nitrogen use efficiency. 2. Amino acid metabolism. *Annals of Applied Biology*. 2007;**151**:269-275. DOI: 10.1111/j.1744-7348.2007.00200.x
- [97] Obara M, Kajiura M, Fukuta Y. Mapping of QTLs associated with cytosolic glutamine synthetase and NADH-glutamate synthase in rice (*Oryza sativa* L.). *Journal of Experimental Botany*. 2001;**52**:1209-1217. DOI: 10.1093/jexbot/52.359.1209
- [98] Yamaya T, Obara M, Nakajima H. Genetic manipulation and quantitative-trait loci mapping for nitrogen recycling in rice. *Journal of Experimental Botany*. 2002;**53**:917-925. DOI: 10.1093/jexbot/53.370.917
- [99] Zhu XG, Long SP, Ort DR. What is the maximum efficiency with which photosynthesis can convert solar energy into biomass?. *Current Opinion in Biotechnology*. 2008;**19**:153-159. DOI: 10.1016/j.copbio.2008.02.004
- [100] Guo SW, Yi Z, Gao YX. New insights into the nitrogen form effect on photosynthesis and photorespiration. *Pedosphere*. 2007;**17**:601-610. DOI: 10.1016/S1002-0160(07)60071-X
- [101] Nunesnesi A, Fernie AR, Stitt M. Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. *Molecular Plant*. 2010;**3**:973-996. DOI: 10.1093/mp/ssq049
- [102] Sweetlove LJ, Beard KF, Nunes-Nesi A. Not just a circle: Flux modes in the plant TCA cycle. *Trends in Plant Science*. 2010;**15**:462-470. DOI: 10.1016/j.tplants.2010.05.006
- [103] Foyer CH, Noctor G, Hodges M. Respiration and nitrogen assimilation: Targeting mitochondria-associated metabolism as a means to enhance nitrogen use efficiency. *Journal of Experimental Botany*. 2011;**62**:1467-1482. DOI: 10.1093/jxb/erq453
- [104] Kant S, Bi YM, Rothstein SJ. Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. *Journal of Experimental Botany*. 2011;**62**:1499-1509. DOI: 10.1093/jxb/erq297
- [105] Bauwe H, Hagemann M, Fernie AR. Photorespiration: Players, partners and origin. *Trends in Plant Science*. 2010;**15**:330-336. DOI: 10.1016/j.tplants.2010.03.006
- [106] Suzuki YJ, Ohkubo M, Hatakeyama H. Increased Rubisco content in transgenic rice transformed with the 'sense' *rbcS* gene. *Plant & Cell Physiology*. 2007;**48**:626-637. DOI: 10.1093/pcp/pcm035
- [107] Doubnerová V, Ryšlavá H. What can enzymes of C<sub>4</sub> photosynthesis do for C<sub>3</sub> plants under stress. *Plant Science*. 2011;**180**:575-583. DOI: 10.1016/j.plantsci.2010.12.005
- [108] Masumoto C, Miyazawa SI, Ohkawa H. Phosphoenolpyruvate carboxylase intrinsically located in the chloroplast of rice plays a crucial role in ammonium assimilation. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;**107**:5226-5231. DOI: 10.1073/pnas.0913127107
- [109] Wang JY, Wang C, Chen NN. Response of rice production to elevated [CO<sub>2</sub>] and its interaction with rising temperature or nitrogen supply: A meta-analysis. *Climatic Change*. 2015;**130**:529-543. DOI: 10.1007/s10584-015-1374-6

- [110] Cai C, Yin XY, He SQ. Responses of wheat and rice to factorial combinations of ambient and elevated CO<sub>2</sub> and temperature in face experiments. *Global Change Biology*. 2016;**22**:856-874. DOI: 10.1111/gcb.13065
- [111] Ainsworth EA, Rogers A. The response of photosynthesis and stomatal conductance to rising [CO<sub>2</sub>]: Mechanisms and environmental interactions. *Plant Cell & Environment*. 2007;**30**:258-270. DOI: 10.1111/j.1365-3040.2007.01641.x
- [112] Rajkumar M, Prasad MNV, Swaminathan S. Climate change driven plant-metal-microbe interactions. *Environment International*. 2013;**53**:74-86. DOI: 10.1016/j.envint.2012.12.009
- [113] Xu ZZ, Jiang YL, Jia BR. Elevated-CO<sub>2</sub> response of stomata and its dependence on environmental factors. *Frontiers in Plant Science* 2016;**7**:657. DOI: 10.3389/fpls.2016.00657
- [114] Wheeler TR, Craufurd PQ, Ellis RH. Temperature variability and the yield of annual crops. *Agriculture Ecosystems & Environment*. 2000;**82**:159-167. DOI: 10.1016/S0167-8809(00)00224-3
- [115] Jagadish SVK, Craufurd PQ, Wheeler TR. High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). *Journal of Experimental Botany*. 2007;**58**:1627-1635. DOI: 10.1093/jxb/erm003
- [116] Kimball BA. Crop responses to elevated CO<sub>2</sub> and interactions with H<sub>2</sub>O, N, and temperature. *Current Opinion in Plant Biology*. 2016;**31**:36-43. DOI: 10.1016/j.pbi.2016.03.006