Chapter 10

Nitrogen Use Efficiency in Rice

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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 macroelements 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	NUpE × NUtE = yield/N available
NUpE	N uptake efficiency	NUp/Nav (soil + fertilizer) = acquired N/N available
NUtE	N utilization efficiency	Yield/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	(NUp/N applied) × 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 (NO_3^-) in aerobic uplands and ammonium (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 NO_3^- at a rate comparable with that of NH_4^+ uptake [17, 18]. Direct molecular evidence for NO_3^- uptake in rice has been presented [19]. NH_4^+ or 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 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 NH_4^+ derived from NO_3^- or directly from 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.

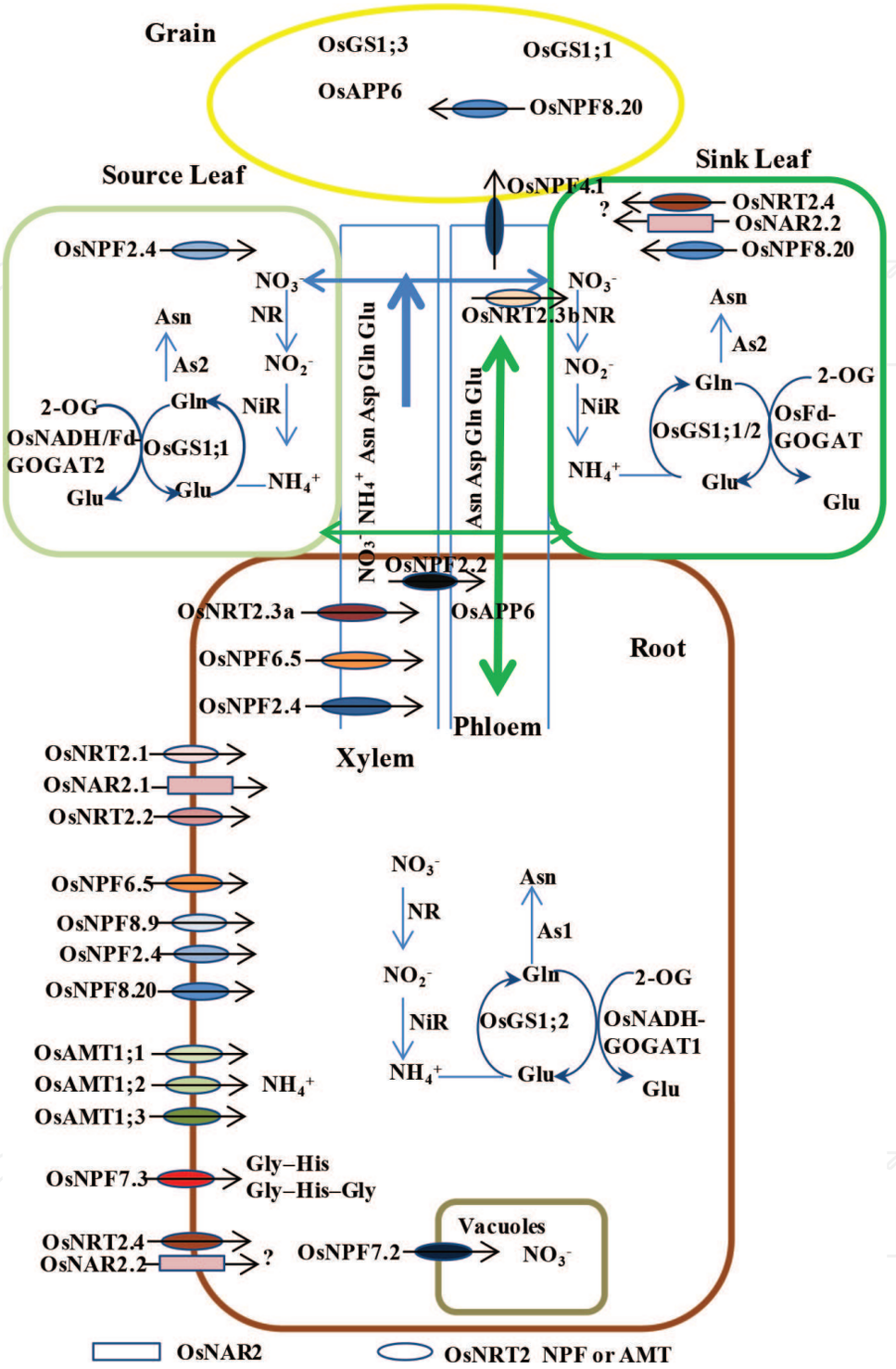

Figure 1. Schematic representation of characterized and predicted functions of the rice nitrate transporters of NRT and NPF families, ammonium transporters of AMT families, and nitrogen assimilation proteins of GS and GOGAT.

3.1. Nitrogen acquisition

Owing to the heterogeneity and dynamic variations of nitrate and ammonium concentrations, which range from lower than 100 μM 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	*OsNPF8.9*	Unknown	Constitutively expressed in roots	NO_3^-	[29, 30]
AK066920	*OsNPF6.5*	NO_3^-	Root hairs, epidermis, and vascular tissues	NO_3^-	[28, 29]
AK099321.1	*OsNPF2.4*	NO_3^-	Root epidermis, xylem parenchyma, and phloem companion cells, leaf phloem cells	NO_3^-	[35]
AK068351	*OsNPF2.2*	NO_3^-, drought, salt	Parenchyma cells around the xylem	NO_3^-	[36]
XM_015767550	*OsNPF7.2*	NO_3^-	Root sclerenchyma, cortex, and stele cells	NO_3^-	[37]
AK101480	*OsNPF7.3*	NO_3^-	Root, seeds	Gly-His Gly-His-Gly	[33, 34]
AK064899	*OsNPF8.20*	N, light	Leaves, panicles, young root tips, cortical fiber cells of lateral roots, stems	Unknown	[32]
AK100802	*OsNPF4.1*	Unknown	Phloem of the branches of young panicles	Unknown	[31]
AK100112	*OsNPF8.2*	Drought, salt, cold	Seeds, leaf, panicle	Unknown	[33]
AK101055	*OsNPF5.5*	Unknown	Seeds, leaf	Unknown	[33]
AK101099	*OsNPF7.1*	Unknown	Constitutive expression	Unknown	[33]
AK070216	*OsNPF7.4*	Drought, salt	Root, panicle, node	Unknown	[33]
AK070036	*OsNPF8.1*	Drought, salt	Shoot, leaf, panicle, seeds	Unknown	[33]
AK072691	*OsNPF8.5*	Drought, salt	Constitutive expression	Unknown	[33]
AB008519	*OsNRT2.1*	NO_3^-, light, sucrose	Root tip, meristem	NO_3^-	[38–40]
AK109733	*OsNRT2.2*	NO_3^-, light, sucrose	Root tip, meristem	NO_3^-	[38–40]
AK109776	*OsNRT2.3a*	NO_3^-, light, sucrose	Root stele	NO_3^-	[38, 41]
AK072215	*OsNRT2.3b*	Light, sucrose, pH	Shoot phloem	NO_3^-	[38, 42]
NM_193361	*OsNRT2.4*	NO_3^-, light, sucrose, pH, NAA	Root, shoot	Unknown	[38–40]
NM_001053852.2	*OsNAR2.1*	NO_3^-, light, sucrose	Root epidermal cells	Unknown	[19, 38–40]
AK109571	*OsNAR2.2*	Light, sucrose	Root, shoot	None	[19, 38, 39]
AF289477	*OsAMT1;1*	NH_4^+, circadian rhythm	Constitutive expression	NH_4^+	[46, 48, 50, 52]

Accession no.	Gene	Regulation	Expression pattern	Substrates	References
AF289478	*OsAMT1;2*	NH_4^+	Root central cylinder and cell surface of root tips	NH_4^+	[46, 50]
AF289479	*OsAMT1;3*	Repressed, circadian rhythm	Root exodermis, sclerenchyma, endodermis, and pericycle cells of primary root	NH_4^+	[46, 47, 50, 53]
AB051864	*OsAMT2;1*	Unknown	Constitutive expression	NH_4^+	[46]
NM 190445	*OsAMT2;2*	NO_3^-, NH_4^+	Unknown	Unknown	[46, 55]
NM_001051237	*OsAMT2;3*	Unknown	Unknown	Unknown	[46]
AB083582	*OsAMT3;1*	Unknown	Roots, shoots	NH_4^+	[46]
AC104487	*OsAMT3;2*	Unknown	Unknown	Unknown	[46]
AP004775	*OsAMT3;3*	Unknown	Unknown	Unknown	[46]
AC091811	*OsAMT4*	Unknown	Unknown	Unknown	[46]
AB037664	*OsGS1;1*	NH_4^+	Leaves	NH_4^+, Glu	[58, 59]
AB180688	*OsGS1;2*	NH_4^+	Roots	NH_4^+, Glu	[58, 59, 64]
AB180689	*OsGS1;3*	Unknown	Spikelets	NH_4^+, Glu	[58, 59]
X14246	*OsGS2*	Unknown	Leaves	NH_4^+, Glu	[58, 60]
AB024716	*OsFd-GOGAT*	Light	Shoots	Gln, 2-OG	[60, 61]
AB008845	*OsNADH-GOGAT1*	NH_4^+, Gln	Developing tissues: root tip, premature leaf blade, spikelet at the early stage of ripening	Gln, 2-OG	[60, 61]
AB274818	*OsNADH-GOGAT2*	NH_4^+	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 NH3 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 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 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 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 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 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 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 β-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 NH_4^+ nutrition, a mixture of NH_4^+ and 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 reassimilation. As mentioned above, changes in the expression and activity of GS and GOGAT would have an effect on N assimilation, recycle, reassimilation, 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_2, 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_4^+ 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_3 and C_4 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_3 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_4^+ [108].

Growth and yield of rice plants are markedly affected by increased CO_2 concentration and temperature [109, 110]. Numerous studies have indicated that an increase in CO_2 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_2 under future climate change is associated with an increase in air temperature, many studies about plant response to the interaction of CO_2 and temperature have been reported [109, 110, 116]. Increases in CO_2 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.

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