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Improving Crop Nitrogen Use Efficiency Toward Sustainable Green Revolution

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Abstract

The Green Revolution of the 1960s improved crop yields in part through the widespread cultivation of semidwarf plant varieties, which resist lodging but require a high-nitrogen (N) fertilizer input. Because environmentally degrading synthetic fertilizer use underlies current worldwide cereal yields, future agricultural sustainability demands enhanced N use efficiency (NUE). Here, we summarize the current understanding of how plants sense, uptake, and respond to N availability in the model plants that can be used to improve sustainable productivity in agriculture. Recent progress in unlocking the genetic basis of NUE within the broader context of plant systems biology has provided insights into the coordination of plant growth and nutrient assimilation and inspired the implementation of a new breeding strategy to cut fertilizer use in high-yield cereal crops. We conclude that identifying fresh targets for N sensing and response in crops would simultaneously enable improved grain productivity and NUE to launch a new Green Revolution and promote future food security.

Contents

1. INTRODUCTION

A key part of the agricultural Green Revolution was the development of semidwarf plant varieties, which were later found to have mutations in gibberellin (GA) metabolism and signaling pathways (106, 114, 120). Normally, GA promotes plant growth by stimulating the destruction of growth-repressing DELLA proteins (DELLAs) as regulated by the GA receptor GIBBERELLIN-INSENSITIVE DWARF1 (GID1), the F-box proteins SLEEPY1(SLY1)/GA-INSENSITIVE DWARF2 (GID2), and the SCF (Skp1, Cullin, F-box-containing) ubiquitin ligase complex (26, 91, 96, 115, 135). The beneficial semidwarfism is conferred in wheat by *Reduced height-1* (*Rht-1*) and in rice by *semi-dwarf1* (*sd1*) alleles (106, 114, 120, 137). These alleles cause the accumulation of DELLAs (74, 82, 106), and resultant semidwarf Green Revolution varieties (GRVs) exhibit an increased harvest index (the ratio of harvested grain to total shoot dry matter) and reduced risk of yield loss due to the flattening of plants by wind and rain (known as lodging). However, GRVs have relatively poor nitrogen use efficiency (NUE) [yield per unit of nitrogen (N) fertilizer input] (35, 74), a property conferred by DELLAs, which also confer the characteristic semidwarfism. GRVs thus require a high-N fertilizer input to obtain high yields, and attempts to further increase crop yields have been largely frustrated by rapidly diminishing returns and the risk of environmental pollution. These problems highlight an urgent need for the evergreen revolution and sustainable

Nitrogen use efficiency (NUE): the overall grain yield per unit of applied nitrogen fertilizer in cereal crops

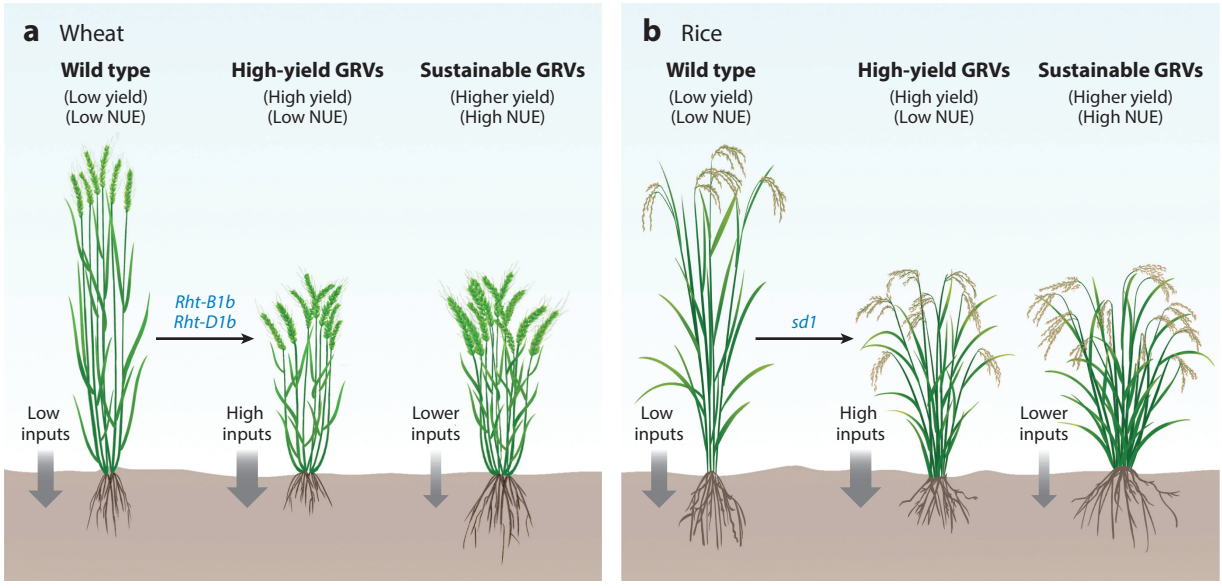


Figure 1

A proposed breeding strategy to enhance sustainable Green Revolution yields, depicting sustainable Green Revolution plant architecture of wheat (*a*) and rice (*b*). The traditional wild-type tall varieties (*left*) are relatively low-yielding and susceptible to lodging. The Green Revolution varieties (GRVs) (*center*) are semidwarf and more resistant to loading. They display the improved high-tillering plant type. However, the GRVs have relatively poor nitrogen use efficiency (NUE) and require a high-nitrogen fertilizer input to obtain high yield. A sustainable Green Revolution plant type (*right*) has a higher harvest index, higher tillering but sturdier stems, and more panicle branching but lower fertilizer inputs, without affecting beneficial semidwarfism.

food security that necessitate reduced N fertilizer use while boosting grain yield above what is currently achievable, with respect to enhanced panicle branching and tillering ability, improved root system architecture, and increased harvest index, without affecting the beneficial semidwarfism (**Figure 1**). Over the past several decades, extensive research efforts to understand how the model plant *Arabidopsis thaliana* regulates N uptake, transport, reduction, and assimilation have provided ample opportunities to use this knowledge to enhance sustainable Green Revolution yields (**Supplemental Table 1**). However, to date, only a few genes have been shown to reduce N fertilizer use while improving cereal crop yields (**Table 1**). The efficiency of N use is a measure of plants' ability to use soil-available N, and cereal NUE can be simply defined as grain yield per unit of applied N fertilizer (38). New knowledge of the molecular mechanisms related to how plants sense and respond to N availability has greatly expanded in model plants (30, 39, 44, 138, 145). The main purpose of this review is to summarize the understanding of N signaling networks that control plant developmental and metabolic adaptations to N availability, and although these advancements in knowledge can be exploited to improve NUE, more extensive research in crops is still needed.

Supplemental Material >

2. THE TRADE-OFF BETWEEN YIELD AND NITROGEN USE EFFICIENCY

In the past 50 years, global crop production has significantly increased due to more cultivation of GRVs, together with advances in agricultural mechanization, irrigation, and fertilizer use (59). Today, the world's most productive varieties of wheat and rice retain beneficial semidwarfing

Table 1 The genes associated with improvements in grain yield and nitrogen use efficiency in cereal crops

Gene name	Gene ID	Target crop	Biological function(s)	Reference(s)
OsNRT1.1A	Os08g0155400	Rice	Heading-date; grain yield; NUE	143
OsNRT1.1B	Os10g0554200	Rice	N uptake; grain yield; NUE	48
OsNPF6.1	Os01g0103100	Rice	Grain yield; NUE	86
OsNRT2.1	Os02g0112100	Rice	N uptake; biomass; grain yield; NUE	14
OsNRT2.3b	Os01g0704100	Rice, barley	Biomass; grain yield; NUE	19, 87
OsNAR2.1	Os02g0595900	Rice	Biomass; grain yield; NUE	12
OsAMT1;1	Os04g0509600	Rice	N uptake; grain yield; NUE	110
OsAMT1;2	Os04g0509600	Rice	N uptake; grain yield; NUE	69
OsGOGAT1	Os01g0681900	Rice	N uptake; grain yield; NUE	69
OSA1	Os03g0689300	Rice	Biomass; photosynthesis; NUE	176
AAP1	Os07g0134000	Rice	Biomass; grain yield; NUE	56, 107
TaGS2-2Ab	TraesCS2A02g500400	Wheat	Biomass; grain yield; NUE	49
OsNR2	Os02g0770800	Rice	N assimilation; grain yield; NUE	29
GRF4	Os02g0701300	Rice; wheat	N uptake; photosynthesis; grain yield; NUE	74
MYB61	Os01g0285300	Rice	Biomass; grain yield; NUE	28
NGR5	Os05g0389000	Rice	Tillering; plant height; grain yield; NUE	151
OsTCP1	Os06g0226700	Rice	Tillering; grain yield; NUE	84
OsNLP1	Os03g0131100	Rice	Biomass; grain yield; NUE	1
OsNLP4	Os09g0549450	Rice	Tillering; grain yield; NUE	150, 168
NAC42	Os09g0493700	Rice	N uptake; grain yield; NUE	128
TaNAC2-5A	TraesCS5A02g468300	Wheat	Root growth; grain yield; NUE	40
ZmNAC7	Zm00001d034277	Maize	Senescence; grain yield; NUE	173
TabZIP60	TraesCS6A02g333600	Wheat	Root growth; grain yield; NUE	162
TaNFYA-B1	TraesCS6B02g366100	Wheat	Root growth; grain yield; NUE	109
OsDof2	Os01g0264000	Rice	N uptake; grain yield; NUE	54
Z'mDof1	Zm00001d031278	Wheat, sorghum	Biomass; grain yield; NUE	104
OsMADS1	Os03g0215400	Rice	Biomass; grain yield; NUE	81
Ghd7	Os07g0261200	Rice	Biomass; grain yield; NUE	142
ARE1	Os08g0224300	Rice	Senescence; grain yield; NUE	141
DEP1	Os09g0441900	Rice	Plant height; grain yield; NUE	122
NAL1	Os04g0615000	Rice	Tillering; grain yield; NUE	158
Ms44	Zm00001eb198610	Maize	Ear growth; grain yield; NUE	24
DNR1	Os01g0178000	Rice	N uptake; grain yield; NUE	177

Abbreviations: N, nitrogen; NUE, nitrogen use efficiency.

genotypes. Indeed, GRVs have several advantages over tall traditional varieties: (*a*) They are highly resistant to lodging and thus improve overall productivity, quality of production, and mechanical harvesting efficiency (58); (*b*) they have an increased harvest index, which enables crops to allocate relatively more energy and resources to seed production; (*c*) they are relatively insensitive to N-mediated growth promotion (74), which allows farmers to apply excessive fertilizer to obtain high yields without incurring the risk of lodging from N-promoted stem elongation; and (*d*) they display increased shoot branching (tillering), which makes it possible to increase grain yield per unit land area through increasing plant density (151). However, despite these obvious advantages, the molecular mechanisms underlying the semidwarfing alleles that regulate N responses remain unknown.

2.1. Identifying the Dwarfing Genes Underlying the Green Revolution Phenotypes

The wheat dwarfing genes of the Green Revolution originated from a Japanese variety called Daruma (41); its derivative cultivar Norin 10 has been successfully used to develop the semidwarf spring wheat varieties that helped Norman Borlaug at the CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo) triple the average yield in the 1960s. After that, a succession of high-yielding semidwarf varieties of both spring and winter wheat were developed and widely distributed in many countries, resulting in spectacular wheat yield improvements (41, 106). Genetic analysis reveals that two mutant alleles, *Rht-B1b* (formerly *Rht1*) and *Rht-D1b* (formerly *Rht2*) at the *Rht-1* loci (**Figure 1**), are now present in more than 70% of modern wheat varieties worldwide (41, 82).

The *Rht-B1b* and *Rht-D1b* alleles are semidominant, and their additive effect is thought to confer semidwarfism by inhibiting GA-promoted stem elongation (106). The wild-type Rht-B1a and Rht-D1a proteins are characterized by the presence of a DELLA motif at the N-terminal region, which is conserved in land plants (105, 106, 167), including *Arabidopsis* [REPRESSOR OF *ga1-3* (RGA), GA INSENSITIVE (GAI), RGA-LIKE1 (RGL1), RGL2, RGL3], maize [dwarf8 (d8)], barley [SLENDER1 (SLN1)], and rice [SLENDER RICE1 (SLR1)] (25, 53, 106, 124). The mutant *Rht-B1b* and *Rht-D1b* alleles are caused by single-nucleotide substitutions that create premature stop codons within the DELLA motif, resulting in N-terminally truncated DELLAs from a translational reinitiation after the mutant stop codon (82, 106, 137), thus conferring the characteristic semidwarfism. By contrast, recessive mutations in the C-terminal domain of wheat *Rht-B1b*, barley *SLN1*, and rice *SLR1* alleles confer a slender phenotype as a result of exaggerated elongation growth (25, 53, 94).

The mutant *sd1* allele has been used extensively and is still being used to develop high-yielding semidwarf rice varieties, closely paralleling wheat development in the Green Revolution (41, 114, 120). Historically, the rice semidwarfing strains have been independently developed and produced. The three most common alleles in the *SD1* locus originated from Chinese semidwarf landraces Ai-zi-zhan, Ai-jiao-nan-te, and Dee-geo-woo-gen (**https://www.ricedata.cn/variety**). In the 1950s, Ai-zi-zhan was used to breed the first outstanding semidwarf variety Guang-chang-ai in China (155). In the 1960s, Dee-geo-woo-gen was used at the International Rice Research Institute (IRRI) to produce the first so-called miracle variety, IR8, which served as a key driver of the rice Green Revolution (41). The semidwarf trait of rice GRVs is controlled by a recessive *sd1* allele, a gene encoding GA 20-oxidase 2 (GA20ox2), which is involved in the GA biosynthesis pathway (114, 120). Unlike wheat GRVs, the rice plants carrying the mutant *sd1* allele exhibit GA-sensitive semidwarf phenotypes, and therefore plant height can be rescued by exogenous GA treatment. Thus, the GA-DELLA regulatory system is central in the regulation of semidwarfism and high-yield traits during the Green Revolution (41, 85, 124).

2.2. The Negative Impact of DELLA Accumulation on Nitrogen Use Efficiency

In recent decades, a huge amount of synthetic fertilizer has been used to increase cereal crop production. This leads to an increase in the cost of agricultural production and to environmental and health problems. Therefore, it is necessary and important to find a so-called evergreen breeding strategy to improve sustainable productivity in agriculture. However, a major challenge for agricultural sustainability and food security is whether improvement of NUE through the reduction of fertilizer use can be achieved without the corresponding yield penalty.

Although they lack a DNA-binding motif, DELLAs act as transcriptional repressors and/or activators through their interaction with various transcription factors, cofactors, and chromatin

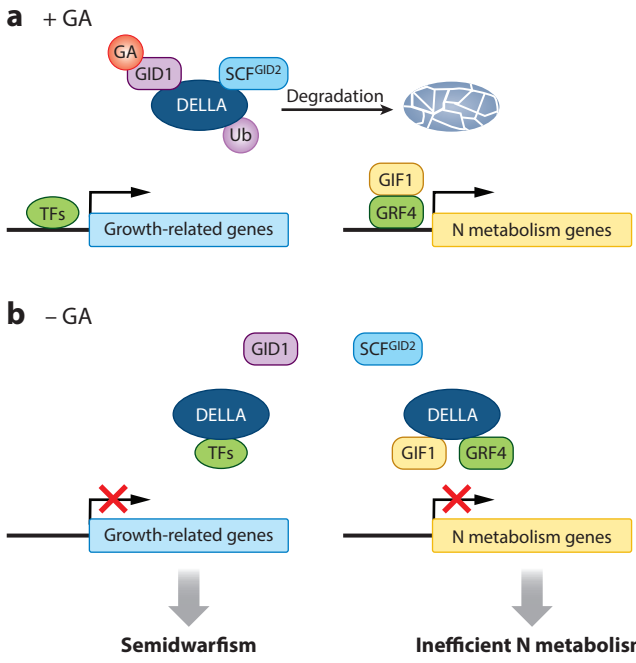


Figure 2

A DELLA-mediated trade-off between growth and N metabolism. (*a*) GA lifts DELLA repression of plant growth and N metabolism by directing the destruction of DELLA via the ubiquitin-proteasome pathway. Bioactive GA is perceived by the GA receptor GID1, which in turn promotes the formation of the GID1-DELLA-SCF^{GID2} complex that catalyzes DELLA Ub, causing DELLA to be degraded by the 26S proteasome, thus releasing its inhibitory interaction with TFs. (*b*) In the absence of GA, DELLA accumulation restrains plant growth and N metabolism by altering the activity of TFs and/or cofactors through protein-to-protein interaction. High-level accumulation of DELLA inhibits the transcriptional activation activity of GRF4 through disruption of the interaction between GRF4 and its transcriptional coactivator, GIF1, resulting in a DELLA-dependent homeostatic trade-off mechanism that explains how reduced plant growth requires less N assimilation. Abbreviations: GA, gibberellin; GID1, GA-INSENSITIVE DWARF1; GIF1, GRF-INTERACTING FACTOR1; GRF4, GROWTH-REGULATING FACTOR4; N, nitrogen; SCF, Skp1, Cullin, F-box-containing; TF, transcription factor; Ub, polyubiquitination.

regulators (42, 74, 82, 151, 152). Normally, GA stimulates the destruction of DELLAs, thereby releasing their inhibitory interaction with transcription factors and promoting plant growth and N metabolism (**Figure 2*a***). It is well known that wheat *Rht-1* and rice *sd1* semidwarfing alleles inhibit N-promoted increases in plant height, but less well known are reports that DELLA accumulation is associated with the reduced efficiencies of N acquisition and assimilation (35). Recently, the negative impact of DELLAs on the efficiency of N fertilizer use was investigated using near-isogenic line (NIL) plants grown under different N conditions (74). While the uptake rate of either ammonium (NH_4^+) or nitrate (NO_3^-) is itself repressed by high N supply, both *sd1* and *Rht-B1b* alleles confer a significant reduction in N uptake. Compared with the wild-type NJ6 plants, the NJ6-*sd1* plants exhibit reduced uptake rates of NH_4^+ and NO_3^- under low- and high-N conditions. Furthermore, the activities of glutamine synthase (GS; NH_4^+ assimilation) and nitrate reductase (NR; NO_3^- assimilation) are, at varying N-supply levels, consistently lower in NJ6-*sd1* than in NJ6 (74).

The rice transcription factor GROWTH-REGULATING FACTOR4 (GRF4) was shown to bind to DNA and activate the transcription of target genes involved in N uptake and assimilation. The rice DELLA protein SLR1 inhibits GRF4-mediated gene activation through disruption of functional interaction between GRF4 and its transcriptional coactivator, GRF-INTERACTING FACTOR1 (GIF1), and explains a DELLA-dependent homeostatic trade-off mechanism (i.e., reduced plant growth requires less N capture) (**Figure 2*b***). The GRF4–DELLA interaction balance thus enables homeostatic regulation of N metabolism: An increased GRF4 abundance activates N uptake, transport, and assimilation, while an increased DELLA abundance inhibits these processes (74). Taken together, DELLA accumulation causes reduction of N uptake, assimilation, and use efficiency in high-yield GRVs.

2.3. Enhancing Sustainable Green Revolution Yield by Modulating DELLA Proteins

Accordingly, the manipulation of DELLA stability and activity provides an efficient method for signal amplification and rapid response to endogenous and/or environmental cues (9, 68, 74, 82, 151), which might explain the widespread adoption of semidwarf GRVs. Increasing DELLA accumulation improves lodging resistance and is coupled with reduced plant height and NUE (27, 74). The different types of *Rht-1* mutations (**Figure 3*a***) display a broad range of inflorescence architecture; for example, the *Rht-B1b* and *Rht-D1b* alleles produce only about a 20% reduction in plant height, while *Rht-B1c* and *Rht-D1c* are about half the size of the wild-type *Rht-A1a* control (103). Plant height, grain yield, and NUE of wheat GRVs depend on the combinations of different *Rht-1* alleles and varieties of different genetic backgrounds (23, 35). The discovery of excellent gene allelic variations in the GA-DELLA regulatory system may provide a new method to improve GRV yield and NUE simultaneously.

A total of twenty new *Rht-1* allelic variations (e.g., *Rht-A1b*, *Rht-B1h*, and *Rht-D1e*) have been identified using a modified EcoTILLING method (**Figure 3*a***). The mutant Rht-D1e [A395P; converts an alanine codon to a proline codon in the leucine heptad repeat II (LHRII) motif of Rht-D1a] and Rht-D1h (L391R) proteins interact with GID1 in the presence of GA, but GA-dependent interaction among mutant DELLAs, GID1, and GID2 is lost (71). Compared with the semidwarf plants carrying *Rht-B1b* and *Rht-D1b* alleles, the NIL plants carrying the combination of *Rht-B1i* and *Rht-D1b* alleles exhibit increased plant height, which is positively correlated with N uptake and assimilation. In addition, 35 intragenic *Rht-B1c* suppressors, termed *overgrowth* alleles (**Figure 3*a***), have been created in the spring wheat cultivar Maringá (10, 136). To validate the effects of *Rht-B1c* variants on stem elongation, *overgrowth* alleles were introgressed into the spring wheat cultivar Faller that contains the mutant *Rht-B1b* allele. In greenhouse-grown Faller, the stem lengths of two semidwarf lines carrying *Rht-B1c.23* and *Rht-B1c.26* alleles were either up to 18% shorter than or not significantly different from *Rht-B1b*, and the reduction effects were further confirmed in field experiments (136). Importantly, the mutant *Rht-B1c.23* and *Rht-B1c.26* alleles confer the beneficial semidwarfism that is correlated with improved harvest index, without negative effects on the overall grain yield in field experiments (136). Similarly, several *Slr-d* alleles (**Figure 3*b***) caused by gain-of-function mutations in the N-terminal domains of SLR1 have been identified (6, 153). Strikingly, the plant height of *Slr1-d6* heterozygous lines in the genetic background of the high-yield GRV 93-11 is equivalent to that of control plants carrying the homozygous *sd1* allele, but with an increase in grain yield of 25% under the same levels of N fertilization, suggesting that *Slr1-d6* enables enhanced sustainable Green Revolution yield in GRVs (153). Although experimental evidence supporting the effects of the newly identified *DELLA* alleles on NUE has not yet been forthcoming, particularly at relatively low levels of

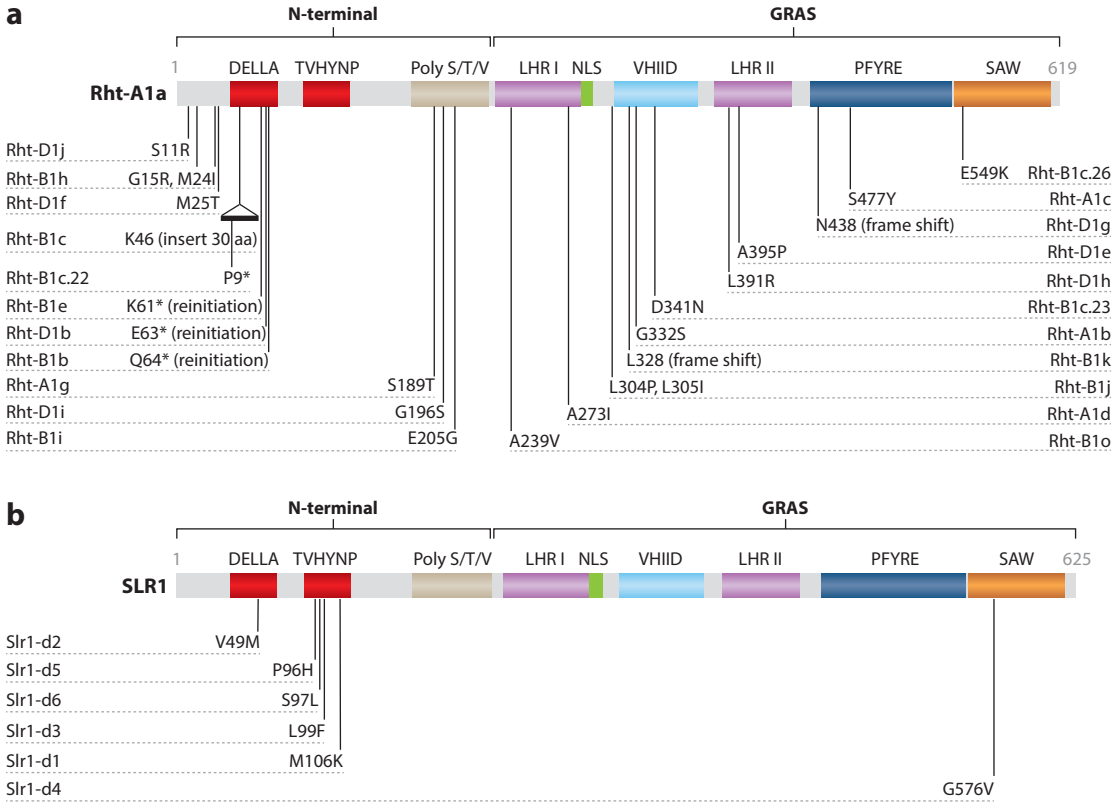


Figure 3

Natural variations and artificial mutations of the DELLA proteins. (*a*) The wheat wild-type Rht-A1a protein contains DELLA and TVHYNP domains in the N-terminal region, and LHR, VHIID, PFYRE, and SAW motifs in the C-terminal GRAS domain. The mutant *Rht-B1b* and *Rht-D1b* alleles create a premature stop codon that converts glutamine (Q64) codon and glutamic acid (E63) codon, respectively, to a translation stop codon. The N-terminally truncated Rht-B1b, Rht-B1e, and Rht-D1b proteins are generated through translational reinitiation, conferring the yield-enhancing semidwarfism characteristic of wheat GRVs. The severely dwarfing *Rht-B1c* allele encodes a mutant protein containing a 30-aa insertion in the DELLA domain. (*b*) Schematic representation of nonsynonymous mutations in rice DELLA protein SLR1. A dominant *Slr-d6* allele caused by a single nucleotide substitution in the TVHYNP domain confers an increase in grain yield without affecting the semidwarfism characteristic of rice GRVs. Abbreviations: aa, amino acid; GRAS, GA INSENSITIVE, REPRESSOR of *ga1-3*, and SCARECROW; GRV, Green Revolution variety; LHR, leucine heptad repeat; SLR1, SLENDER RICE1.

N supply, CRISPR/Cas9-based high-throughput targeted mutagenesis of DELLAs [e.g., an appropriate mutation in the LHR I domain responsible for the protein–protein interaction] provides a new method to improve NUE and grain yield in GRVs.

3. GENETIC MANIPULATION OF NITROGEN METABOLISM GENES

Plant roots possess multiple transport systems for N uptake, ranging from inorganic N compounds such as NH_4^+ and NO_3^- to polymeric N forms such as amino acids. NO_3^- from soil is the main source of N for most plants grown in an aerobic (with oxygen) environment, whereas NH_4^+ is the main source of N for plants grown in flooded conditions or acidic soils (80, 145, 157). Normally, NO_3^- is mainly translocated from roots to shoots and leaves, where it is reduced

to nitrite by NR in the cytosol, and then translocated to the plastids and chloroplasts, where it is further reduced to NH_4^+ by nitrite reductase (NiR). Because of NH_4^+ toxicity, NH_4^+ must be assimilated in roots and then translocated through the xylem to shoots. Recently, the molecular mechanisms affecting N uptake, transport, and assimilation have been extensively investigated in model plants (138, 145, 157), which present new approaches to potentially overcome the inhibitory effect of DELLAs on NUE.

3.1. Improving Nitrogen Use Efficiency by Regulating Nitrate Transporter Activity

The soil NO_3^- concentration can vary over a wide range. The high-affinity NO_3^- uptake system (HATS) and low-affinity NO_3^- uptake system (LATS) are transcriptionally regulated by N supply and thereby enable plants to adapt to fluctuating environmental N availability (157). In higher plants, the NO_3^- transport systems consist of four transmembrane protein families: NITRATE TRANSPORTER 1 (NRT1)/PEPTIDE TRANSPORTER (PTR) (referred to as NPF), NRT2, CHLORIDE CHANNEL (CLC), and SLOWLY ACTIVATING ANION CHANNEL (SLAC)/SLAC-ASSOCIATED 1 HOMOLOG (SLAH) (145, 157). The *Arabidopsis* CHLORIDE RESISTANT 1 (CHL1, also termed AtNPF6.3 or AtNRT1.1) was the first NO_3^- transporter to be identified by genetic selection and screening for chlorate resistance (131). With the exception of AtNRT1.1, which serves as a dual-affinity transporter, most members of the AtNRT1 family are characterized as a LATS (77). AtNRT1.1 and AtNRT1.2 play major roles in root NO_3^- uptake at high soil NO_3^- concentrations (50), some members of the AtNRT1 family (e.g., AtNRT1.5, AtNRT1.6, AtNRT1.7, AtNRT1.8, AtNRT1.9, AtNRT1.11, and AtNRT1.12) are mainly involved in long-distance translocation and remobilization of NO_3^- (2, 15, 18, 46, 73, 76, 147). In addition to the conventional transport of NO_3^-, some NRT1 proteins can transport various other substrates, including chloride, dipeptides, amino acids, glucosinolates, and phytohormones (17, 57, 97, 126).

NRT1 homologs of rice and maize have been found to exhibit NO_3^- transport activity, suggesting a conserved function in NO_3^- uptake and transport across species (48, 149). Rice contains three putative homologs of AtNRT1.1: OsNRT1.1A/OsNPF6.3, OsNRT1.1B/OsNPF6.5, and OsNRT1.1C/OsNPF6.4 (143). *OsNRT1.1B* is expressed in the root hairs, epidermis, and vascular tissues and is predominantly involved in NO_3^- uptake and translocation. Interestingly, the NO_3^- transport activity of the OsNRT1.1B variant from *indica* rice varieties is higher than that from *japonica* rice varieties; this difference is caused by a single amino acid substitution (48). Either overexpression of *OsNRT1.1B* or introduction of the elite $OsNRT1.1B^{indica}$ allele into *japonica* rice varieties facilitates the uptake and transport of NO_3^-, resulting in the enhancement of aboveground biomass, plant height, and grain yield (48). Moreover, OsNRT1.1B not only integrates N and phosphate signaling pathways that have been shown to be involved in regulating the N and phosphorous balance but also coordinates recruitment of the root microbiota to optimize uptake of the soil-available N contents (47, 174). OsNRT1.1A is predominantly localized to the tonoplast. Overexpression of *OsNRT1.1A* enhances the uptake rates of NO_3^- and NH_4^+ and thus improves NUE and grain yield in the *japonica* rice varieties (143). OsNPF2.2 and OsNPF2.4 have been shown to be involved in acquisition and long-distance transport of NO_3^- (148); mutants lacking either *OsNPF2.2* or *OsNPF2.4* exhibit impaired NO_3^- translocation from roots to shoots and leaves, unloading from the xylem, and redistribution from old leaves to young leaves (75, 148, 154, 157). A natural variant of *OsNPF6.1* originally found in wild rice, whose allele-specific expression of *OsNPF6.1* tends to be more transactivated by the transcription factor OsNAC42, can result in increased NUE and grain yield in rice (128). Furthermore, overexpression of

NRT1/NPF family genes, such as *OsNPF7.1*, *OsNPF7.3*, *OsNPF7.4*, and *OsNPF8.20/OsPTR9*, increases N uptake, transport, and assimilation, consequently improving grain yield (20, 21, 51).

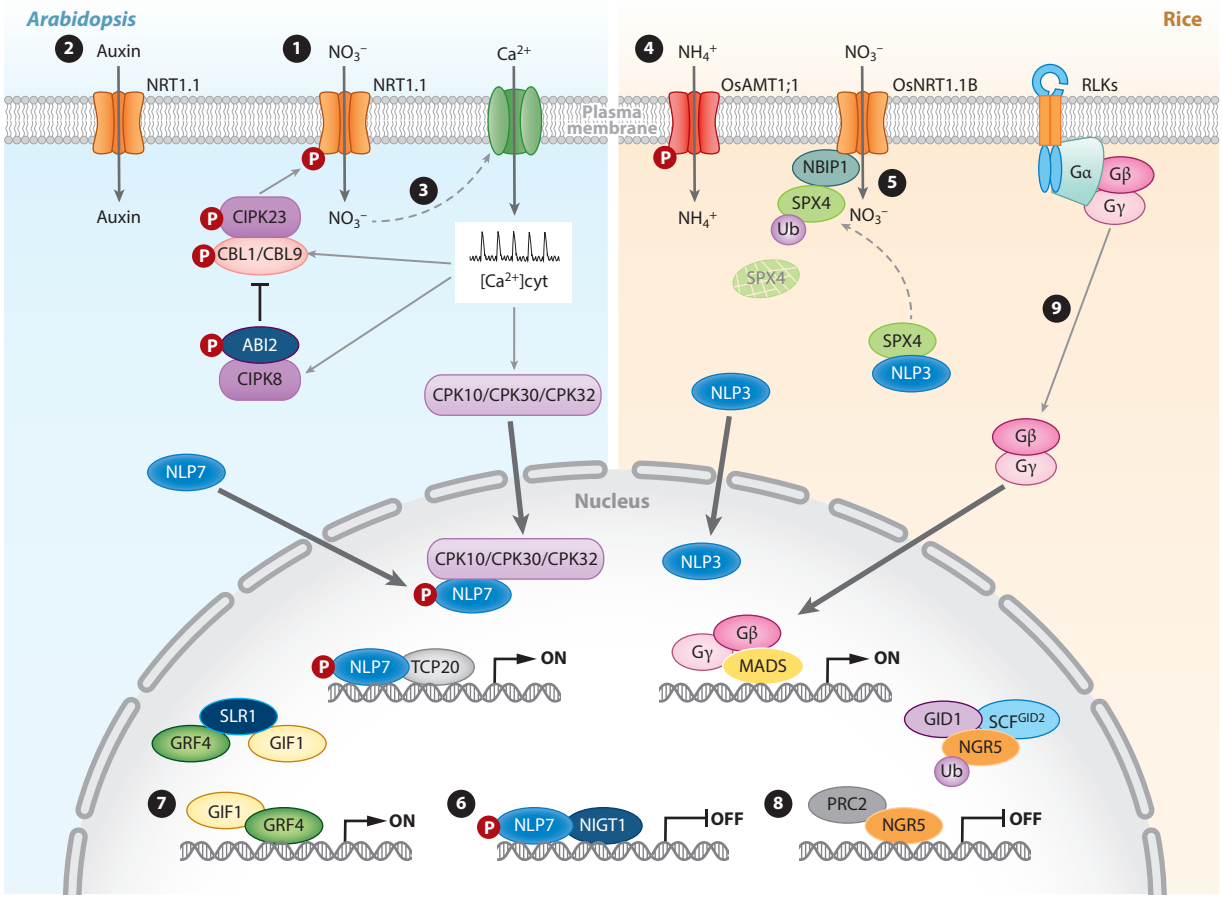
The NRT2 family is suggested to function as the HATS (138, 145, 157), and its high-affinity NO_3^- transport activities require partner protein NITRATE ASSIMILATION RELATED PROTEIN2 (NAR2) (83, 131). The NRT2–NAR2 interaction stabilizes NRT2 protein and enhances its plasma membrane localization (83). Four *OsNRT2* and two *OsNAR2* genes exist in rice. With the exception of OsNRT2.4, which appears to have a dual affinity for NO_3^- (148), the high-affinity NO_3^- transport activities of OsNRT2.1, OsNRT2.2, and OsNRT2.3a are fully dependent on the interaction with OsNAR2.1 (83, 160). *OsNRT2.3* mRNA is alternatively spliced into *OsNRT2.3a* and *OsNRT2.3b* isoforms (19). Unlike OsNRT2.3a, which is involved in root-to-shoot translocation of NO_3^-, OsNRT2.3b functions as a sensor that switches NO_3^- transport activity on or off by a pH-sensing mechanism (19). Overexpression of *OsNRT2.3b* enhances the pH-buffering capacity of the transgenic rice plants in response to changes in N availability, improving gain productivity and NUE in modern high-yield rice (19). Moreover, higher expression of *OsNRT2.1*, *OsNRT2.3a*, or *OsNAR2.1* alone increases NUE and grain yield, and co-overexpression of *OsNAR2.1* and *OsNRT2.3a* could further enhance these beneficial traits in rice (12, 13, 14). Taken together, modulating the expression levels and pyramiding multiple alleles of NO_3^- transporter genes, which enhance activities of uptake, root-to-shoot translocation, and remobilization of NO_3^- (15, 19, 48, 143), play an important role in improving NUE of cereal crops (**Table 1**), although the molecular mechanisms underlying the coordination between N acquisition and biomass accumulation remain to be identified.

3.2. Regulation and Characterization of Ammonium Transporters

In dryland soils, application of N fertilizer can lead to short-term NH_4^+ dominance. Although NH_4^+ may move across the plasma membrane through nonspecific transport systems, such as potassium transporters and aquaporins, NH_4^+ transporter (AMT)-mediated high-affinity uptake is essential for plants grown in flooded and acidic soils. AMTs belong to the AMT/methylamine permease (MEP)/Rhesus-type (Rh) protein superfamily, which can be divided into two subtypes, AMT1 and AMT2. *Arabidopsis* contains six AMTs: AtAMT1;1, AtAMT1;2, AtAMT1;3, AtAMT1;4, AtAMT1;5, and AtAMT2;1. With the exception of *AtAMT1.4*, the five *AtAMT* genes are highly expressed in roots (32, 169). *AtAMT1;1* and *AtAMT1;3* are expressed in the epidermal and cortical cells and responsible for high-affinity uptake of NH_4^+ from soil into the root cells and then for symplasmic transport within the root (86). *AtAMT1;2* is expressed in the endodermal and cortical cells, and that plays an important role in not only NH_4^+ uptake and retrieval from the root apoplast but also NH_4^+ translocation into the vasculature (170). *AtAMT2;1* is expressed in the marginal epidermis, and overexpression of *AtAMT2;1* in the background of *AtAMT1;2* and *AtAMT1;3* expression lines facilitates the root-to-shoot translocation of NH_4^+ (34). In addition, AtAMT1s can be phosphorylated in the cytosolic C-terminal region (CTR) in response to elevated external NH_4^+, thereby shutting off their transport activity and consequently inhibiting NH_4^+ uptake to prevent NH_4^+ toxicity (67).

Physiological studies show that NH_4^+ uptake in *Arabidopsis* roots increases under NH_4^+ limitation and rapidly declines when plants are resupplied with NH_4^+ (32, 67, 171). In contrast, an increasing NH_4^+ supply increases NH_4^+ uptake when N-deficient rice plants are resupplied with NH_4^+ (63), suggesting that regulatory mechanisms of NH_4^+ uptake may vary widely depending on plant species. The rice OsAMT family comprises five subfamilies (OsAMT1–OsAMT5). The OsAMT1 subfamily consists of three members: OsAMT1;1, OsAMT1;2, and OsAMT1;3. *OsAMT1;1* is constantly expressed in roots and shoots, whereas *OsAMT1;2* and *OsAMT1;3* are

more generally root specific (119). In contrast to N-induced expression patterns of *OsAMT1;1* and *OsAMT1;2*, *OsAMT1;3* is N repressible (119). Although the regulatory mechanisms underlying the temporal, spatial, and abundance patterns of *OsAMT1* family genes have not been characterized, the rice transcription factors GRF4 and INDERTERMINATE DOMAIN 10 (OsIDD10) have been shown to bind to and activate their transcriptions of *OsAMT1;1* and *OsAMT1;2* (74, 159). Recent studies have shown that the rice serine/threonine/tyrosine protein kinase OsACTPK1 induces the phosphorylation of the corresponding Thr460 in OsAMT1;2 (8), which has been shown to be critical for the functional switch of AtAMT1;1 in response to NH_4^+ availability (85). An increasing NH_4^+ supply increases the transcript abundance of *OsACTPK1*, which in turn mediates the phosphorylation and subsequent inactivation of OsAMT1;2 transport activity, thereby preventing NH_4^+ toxicity (8). Fine-tuned regulation of the NH_4^+ transport activity of OsAMT1 controlled by a balanced phosphorylation and dephosphorylation switch may be able to improve the NH_4^+ uptake efficiency in crops (**Figure 4**). Furthermore, mutations of *OsAMT1;1* cause decreases in the uptake and translocation of NH_4^+, growth of roots and shoots, and



(*Caption appears on following page*)

Figure 4 (*Figure appears on preceding page*)

Schematic representation of nitrogen sensing and its integrative signaling. (❶) NO_3^- is perceived and transported by the *Arabidopsis* transceptor NRT1.1. The switch between activation (high affinity) and inactivation (low affinity) of NRT1.1 by its P and dephosphorylation is controlled by CBL1/CBL9-CIPK23, CIPK8, and ABI2. (❷) NRT1.1 also transports auxin, and the NO_3^- influx prevents auxin from being imported into the cell. (❸) Cytoplasmic NO_3^- stimulates Ca^{2+} influx, which induces the feedback regulation of NRT1.1 and activation of Ca^{2+}-dependent kinases CPK10/CPK30/CPK32, thus promoting P of NLP7 and triggering its nuclear retention. (❹) P of high-affinity NH_4^+ transporter OsAMT1;1 is critical for the functional switch in response to NH_4^+ availability in rice, and subsequent inactivation of OsAMT1;1 transport activity, thereby preventing NH_4^+ toxicity. (❺) Under high-N conditions, OsNRT1.1B signaling results in the formation of a complex including OsNRT1.1B, SPX4, and the E3 ligase NBIP1 in rice, causing Ub and degradation of SPX4, resulting in the release of NLP3. (❻) The interaction of NLP7 and TCP20 facilitates the integration of local NO_3^- response with systemic N status, but the NLP7–NIGT1 interaction inhibits downstream target genes. (❼) N-regulated GRF4 interacts with its coactivator GIF1, triggering a transcriptional cascade. However, the rice DELLA protein SLR1 inhibits the activity of GRF4 through disruption of the interaction of GRF4 and GIF1. (❽) N-induced NGR5 facilitates recruitment of PRC2 to repress gene expression through H3K27me3 modification. NGR5 interacts with the GA receptor GID1, resulting in GID1-SCF^{GID2}-promoted proteasomal destruction. (❾) The Gα forms an inactive heterotrimer with Gβ and Gγ subunits, and the freely released G protein βγ dimer interacts with MADS transcription factors, consequently promoting the expression of target genes and developmental responses. Abbreviations: ABI2, ABSCISIC ACID INSENSITIVE 2; Ca^{2+}, calcium; $[Ca_2{}^+]$cyt, cytosolic free $Ca_2{}^+$; CBL, CALCINARIN B-LIKE PROTEIN; CIPK, CBL-INTERACTING PROTEIN KINASE; GA, gibberellin; Gα, G-protein alpha subunit; Gβ, G-protein beta subunit; Gγ, G-protein gamma subunit; GID1, GA-INSENSITIVE DWARF1; GIF1, GRF-INTERACTING FACTOR1; GRF4, GROWTH-REGULATING FACTOR4; N, nitrogen; NBIP1, NRT1.1B INTERACTING PROTEIN 1; NGR5, NITROGEN-MEDIATED TILLER GROWTH RESPONSE 5; NIGT1, NITRATE-INDUCIBLE, GARP-TYPE TRANSCRIPTIONAL REPRESSOR 1; NLP, NODULE-INCEPTION-LIKE PROTEIN; NO_3^-, nitrate; NRT1.1, NITRATE TRANSPORTER 1.1; P, phosphorylation; PRC2, POLYCOMB REPRESSIVE COMPLEX 2; RLKs, receptor-like kinases; SCF, Skp1, Cullin, F-box-containing; SPX4, SYG1, Pho81, XPR1 domain-containing protein 4; TCP20, TEOSINTE BRANCHED 1-CYCLOIDEA-PCF 20; Ub, polyubiquitination.

accumulation of biomass production under both low- and high-N conditions (72). Overexpression of *OsAMT1;1* improves both NH_4^+ uptake and rice yield under low-NH_4^+ conditions but exhibits stunted plant growth and yield penalty under high-NH_4^+ conditions (45, 110). To date, many efforts to improve NUE by modulating AMTs have had only limited success (45, 72, 110).

3.3. Modulation of Whole-Plant Nitrogen Assimilation and Remobilization

After uptake, inorganic N assimilation involves the reduction of NO_3^- to NH_4^+ by NR and NiR and its subsequent incorporation into amino acids. In many species, NO_3^- can be loaded into the xylem vessels of roots and transferred to shoots and leaves through the transpiration stream. The spatial and temporal regulation of NO_3^- uptake, transport, assimilation, and remobilization is tightly coordinated with developmental adaptations to N availability (74, 93). Most of the energy for NO_3^- assimilation in shoots and leaves is derived from photosynthesis, and NR is the rate-limiting step for efficiency of N acquisition and utilization. In *Arabidopsis*, the enzyme activity of NR is regulated by the phosphorylation of the serine residue (Ser534) by SUCROSE NONFERMENTING-1 (SNF1)-RELATED PROTEIN KINASE (SnRK1), and phosphorylation-dependent binding to 14-3-3 proteins results in the inhibition of NR activity (121). By contrast, the phosphorylation of the serine residue (Ser627) by MITOGEN-ACTIVATED PROTEIN KINASE6 (MPK6) is likely to be associated with an increase of NR activity (138, 140). The CALCINERIN B-LIKE PROTEIN (CBL)-INTERACTING PROTEIN KINASES (CIPKs, such as CIPK7, CIPK12, and CIPK14) and receptor-like kinase FERONIA (FER) can phosphorylate E3 ubiquitin ligases ATL31 and ATL6 and subsequently enhance their stability and activity, which, in turn, regulates NR activity by triggering the degradation of 14-3-3 proteins (156, 165, 166). Chlorate as a transport analog for NO_3^- is taken up by roots and further reduced to toxic chlorite by NR in shoots; chlorate resistance is therefore used to isolate NR-defective mutants in both model and crop species. A natural variant of *OsNR2* is identified as

a major quantitative trait locus (QTL) responsible for chlorate resistance in rice. The elite *OsNR2* allele from *indica* germplasm was shown to be associated with the increases in NR enzyme activity and NO_3^- uptake via a positive feed-forward loop regulating the expression of *OsNRT1.1B*, resulting in improvements of NUE and grain yield in *japonica* germplasm (29). In addition, overexpression of *OsNiR* is found to increase tiller number, grain number, and rice yield (168).

NH_4^+, whether taken directly from the soil or converted from NO_3^-, is first assimilated into glutamine and glutamate. In addition to the GS/glutamate synthase (GOGAT) cycle, asparagine synthetase (AS) can transfer the glutamine-amide group to aspartate, forming asparagine and glutamate (31). These compounds (glutamine, glutamate, asparagine, and aspartate) have been shown to be the major transported N carriers in the phloem of plants. The GS/GOGAT cycle together with AS play important roles not only for N assimilation and allocation within the plant but also for glutamate and glutamine recovery in both source and sink tissues (31). Indeed, glutamine is also sensed by plant PII proteins (11), while a deletion polymorphism is associated with the glutamine-binding domain in the *Arabidopsis* and *Brassicaceae* PII proteins. There are two groups of GS isoenzymes (cytosolic GS1 and plastid GS2) and two types of GOGAT (NADH-GOGAT and Fd-GOGAT) in vascular plants. Cytosolic GS1 is important for primary NH_4^+ assimilation in roots and for reassimilation of NH_4^+ generated during protein turnover in shoots. Previous studies have shown that cytosolic OsGS1;2 and plastid OsNADH-GOGAT1 take part in NH_4^+ assimilation in roots. The mutant *osgs1;2* allele causes a reduction of outgrowth of tiller buds and symptoms of metabolic disorder, but overexpression of *OsGS1;2* also exhibits growth retardation and loss of grain yield in rice (98). Although many studies have reported increased GS activity by overexpressing *GS1* in crops, attempts to improve grain yield and NUE have not been successful (129).

GS2 plays a dominant role in regulating the reassimilation of photorespiratory NH_4^+ in chloroplasts and assimilation of NH_4^+ deriving from NO_3^- reduction in plastids (139). The rice *abc1-1* mutant caused by a single substitution mutation of *OsFd-GOGAT* exhibits severe developmental defects and typical N-deficient syndromes (164), whereas *are1* (*abc1-1 repressor1*) can partially rescue the N-assimilation-deficiency phenotype of the *osfd-gogat* mutant. Importantly, loss-of-function mutations of *ARE1* increase NUE and grain yield in rice under low-N conditions (141). *OsGS1;1* and *OsNADH-GOGAT2* are key enzymes for N allocation or remobilization processes from sources to sink tissues, and co-overexpression of *OsGS1;1* and *OsGS2* exhibits higher yield under drought and salinity stresses (55). Moreover, the elite *TaGS2-2Ab* allele has been found to increase N utilization and grain yield in wheat GRVs, without affecting the beneficial semidwarfism (49).

Notably, ectopic overexpression of some but not all genes related to N metabolism can coordinate N assimilation and crop production (**Table 1**), suggesting that this will go a long way toward overcoming potentially limiting factors and improving NUE in GRVs. The whole-plant metabolic coordination of N uptake, transport, reduction, assimilation, and remobilization is often affected by internal and external cues (100). Therefore, a more complete understanding of the molecular mechanisms underlying the coordination of N acquisition and utilization and their regulation in the context of a changing environment will be necessary and important for improving sustainable productivity in agriculture. This will require extensive research in both model and crop species.

4. NITRATE SIGNAL-SENSING AND TRANSDUCTION PATHWAYS

Plants optimize N acquisition from the soil through modulating root system architecture, adjusting root–shoot allocation patterns, and coordinating recruitment of root microbiota (100, 174). Under N limitation, overall plant growth is reduced but root systems are expanded, resulting in

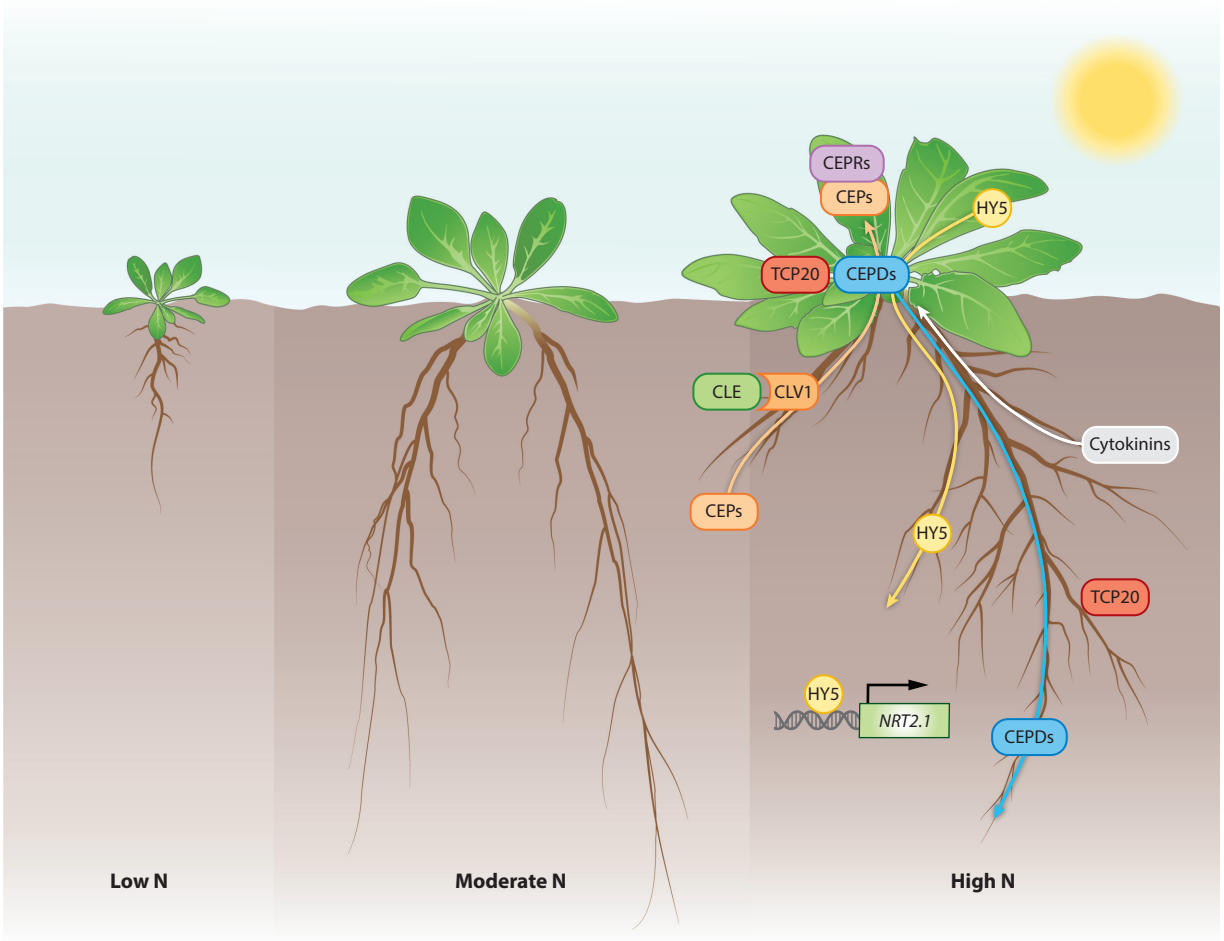


Figure 5

Systemic nitrate-demand signaling and its role in whole-plant response, including diagrams of the root responses of *Arabidopsis* plants grown under three different N levels. Under moderate-N conditions, primary and lateral root growth is promoted, and when N is high, primary and lateral root growth is suppressed. By contrast, plant growth is markedly inhibited under extremely low-N conditions. In response to local N deprivation, both CLE and CEPs are induced in roots. CLE is perceived by CLV1, inhibiting lateral root growth. CEPs are translocated to shoots, where recognition by CEPRs leads to the production of the polypeptides CEPDs, which travel to roots and consequently promote *NRT2.1* expression and N uptake in locations where NO_3^- is ample. TCP20 is also required for the N-foraging response. Shoot-to-root translocated HY5 promotes N uptake through activating *NRT2.1*, and that integrates and coordinates plant growth, photosynthesis, and N metabolism in whole-organismal response to a variable light environment. Abbreviations: CEP, C-terminally encoded peptide; CEPD, CEP DOWNSTREAM; CEPR, CEP RECEPTOR; CLE, CLAVATA3/ENDOSPERM SURROUNDING REGION-related; CLV1, CLAVATA1; HY5, ELONGATED HYPOCOTYL 5; N, nitrate; NRT2.1, NITRATE TRANSPORTER2.1; TCP20, TEOSINTE BRANCHED 1-CYCLOIDEA-PCF 20.

biomass allocation to roots at the expense of shoots (**Figure 5**). When N supply is ample, primary and lateral root growth is suppressed but shoot and leaf growth is promoted, thus increasing the shoot-to-root biomass ratio and allowing resource accumulation and investment in seed production. NO_3^- not only is an essential nutrient for plant growth but also acts as a signaling molecule to trigger changes in downstream signaling pathways (146). The understanding of the mechanisms

involved in developmental and metabolic adaptations to N availability has significantly progressed in model plants (39, 80, 100, 138), and thus there are more opportunities to use this knowledge to optimize N capture and the performance of crops in variable N environments.

4.1. Mechanisms of Local Perception and Response to Nitrate Availability

NO_3^- availability in the soil often limits plant growth and crop production. Plants have developed a myriad of adaptive mechanisms for NO_3^- uptake and assimilation, as well as the ability to sense NO_3^- fluctuation. DENSE AND ERECT PANICLE1 (DEP1), a plant-specific G protein γ subunit, plays a critical role in N sensing and response in rice (52, 122). In addition, although G protein–coupled receptors (GPCRs) are able to sense nutrient levels and metabolic activity in eukaryotic organisms, there is much to learn about their function in vascular plants. NRT1.1 functions as a NO_3^- sensor that controls responses to NO_3^- availability (44, 102, 123). In *Arabidopsis*, the *chl1-5* null mutant lacking *AtNRT1.1* exhibits a defect in NO_3^- uptake (95), as well as reduced physiological response to NO_3^- availability. In contrast, the *chl1-9* mutant caused by a single nucleotide substitution of *AtNRT1.1* displays a defect in NO_3^- uptake but has no effect on N-responsive regulation of lateral root growth and target gene expression, suggesting that NO_3^- uptake and N responsiveness are each separate processes (94, 111, 133). When the NO_3^- concentration is high (>1 mM), NRT1.1 behaves as a low-affinity transporter. However, when the NO_3^- level falls below 1 mM, NRT1.1 switches to a high-affinity mode. This switch is controlled by phosphorylation through calcium sensor proteins, CBLs, and their interacting partners, CIPKs (77, 79).

The Ca^{2+}-triggered CBL1-mediated activation of CIPK23 and subsequent phosphorylation on an intracellular threonine residue (Thr101) of AtNRT1.1 either induce dimer decoupling or increase structural flexibility, thus modulating NO_3^- transport activity and triggering responses to low-N stress (44, 102, 123). Compared to the wild-type protein, the mutant Thr101Asp variant exhibits increased uptake rates of approximately 2.8-fold; however, the His356Ala variant displays no detectable transport activity, consistent with loss of NO_3^- binding (102, 123). The *abi2-2* mutant lacking the protein phosphatase ABSCISIC ACID INSENSITIVE 2 (ABI2) exhibits delayed N-starvation responses (70). ABI2 interacts with and dephosphorylates CBL1, CBL9, CIPK8, and CIPK23, which in turn inhibits CBL1/CBL9-CIPK23 complex–mediated phosphorylation and the activity of AtNRT1.1 in N sensing (**Figure 4**), representing a mechanism by which plants adjust growth and N capture to preserve cellular energy homeostasis under stress conditions. The phosphomimetic Thr101Asp variant exhibits fast lateral mobility and membrane partitioning that facilitate auxin flux under low-N conditions, but the nonphosphorylatable Thr101Ala variant displays low auxin transport capacity, allowing root growth to finely adjust root system architecture in a variable nutrient environment (178). While the homologs of AtNRT1.1 have been identified in cereal crops (48, 149), the function and roles of the corresponding Thr101 and His365 in sensing NO_3^- status need to be further investigated.

4.2. Gene Regulatory Networks of the Nitrate Response in Plants

Within minutes, NO_3^- perception regulates activities of a subset of transcription factors, thereby triggering rapid transcriptional reprogramming (3, 30, 64, 78, 92). Since the first identification of the ARABIDOPSIS NITRATE REGULATED 1 (ANR1) MADS-box gene involved in NO_3^- signaling (172), several types of transcription factors and cofactors have been identified, including AUXIN RESPONSE FACTOR (ARF); SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 9 (SPL9); TGACG MOTIF-BINDING FACTOR 1 (TGA1)/TGA4; NODULE-INCEPTION-LIKE PROTEIN 7 (NLP7); and NITRATE-INDUCIBLE, GARP-TYPE

TRANSCRIPTIONAL REPRESSOR 1 (NIGT1)/HYPERSENSITIVE TO LOW Pi-ELICITED PRIMARY ROOT SHORTENING 1 (HRS1) (4, 30, 60, 62, 92, 177). The *NLP* gene family is closely related to *NODULE INCEPTION* (*NIN*) genes from leguminous species and directs the majority of NO_3^--responsive gene expression (62, 89). AtNLP7 is localized in the cytosol and excluded from the nucleus under low-N conditions, whereas the NO_3^- signals lead to AtNLP7's rapid accumulation in the nucleus (89). In the presence of NO_3^-, AtNRT1.1 perceives NO_3^- and consequently stimulates Ca^{2+} influx into the cell. Ca^{2+}-dependent protein kinases CPK10/CPK30/CPK32 trigger NO_3^--responsive phosphorylation and nuclear retention of AtNLP7, which in turn activates target gene expression and enhances N assimilation and plant growth (78), thus giving rise to the concept of NO_3^--CPK-NLP7 signaling in the central nutrient-growth network (**Figure 4**). Although several NLPs have been shown to modulate N responses in cereal crops (1, 33, 150), a different mechanism underlies the interaction of OsNRT1.1B and NLP3 (a rice homolog of AtNLP7). A rice SYG1/Pho81/XPR1 family protein, SPX4, has been shown to inhibit the expression of NO_3^--responsive genes by repressing the NO_3^--induced cytoplasmic–nuclear shuttling of NLP3. OsNRT1.1B perceives NO_3^- and induces the formation of a multicomponent complex with SPX4 and E3 ligase NRT1.1B INTERACTING PROTEIN 1 (NBIP1), causing polyubiquitination and degradation of SPX4 (47), thus enabling NLP3 to move into the nucleus (**Figure 4**). Recent studies have shown that increased NLP3 and its homolog OsNLP4 promote the expression of genes involved in N assimilation (e.g., *OsNiR*) and signaling by directly binding to NO_3^--responsive *cis*-elements (NREs) in the promoters, resulting in improved NUE and grain yield in rice (150, 168).

In addition to *NIGT1/HRS1* family genes, the *Arabidopsis* class II LATERAL ORGAN BOUNDARIES DOMAIN (LBD) genes *LBD37/LBD38/LBD39* also act as negative regulators of N-availability signals. The expressions of *LBD37/LBD38/LBD39* are induced by the NO_3^- supply. Loss-of-function mutants and overexpression lines exhibit enhanced and reduced expression of N-responsive genes, respectively (84, 112). In both *Arabidopsis* and rice, the expression of *NIGT1/HRS1* is regulated by N availability via an autoregulatory negative feedback mechanism (116). The transgenic rice plants overexpressing *OsNIGT1/OsHRS1* exhibit NO_3^- response-related phenotypes, whereas loss-of-function mutation results in deregulation of N uptake and accumulation (116). An increasing NO_3^- supply increases NIGT1/HRS1 abundances, which bind to the promoters of N-starvation-responsive genes that are encoded by direct target genes of NLPs, thus repressing the expression of target genes (60, 92). NIGT1/HRS1 family members are considered to be major regulators that integrate N- and phosphorus-starvation responses in both *Arabidopsis* and maize and play an important role in coordinating the utilization of N and phosphorus in a variable nutrient environment (88, 134, 144). NO_3^- signaling networks have been shown to interact with multiple signaling pathways, including potassium, sugar, phytohormones, and G-protein signaling (22, 74, 122, 132, 138, 151). However, the molecular mechanisms underlying the crosstalk between N and other signaling pathways remain to be addressed.

4.3. Systemic Signaling Shapes Root Architecture for Efficient Nitrogen Acquisition

Plants modulate the efficiency of root N uptake and assimilation in response to shoot N demand. Decapitated plants lose N-demand and N-supply responses, suggesting the existence of a root-to-shoot-to-root signal relay (113). N supply controls cytokinins (CK) biosynthesis and accumulation in different tissues of plants. Deficiency in CK biosynthesis inhibits the N-foraging response but can be counteracted by the application of CK even in those roots under low-N conditions (113), suggesting that CK plays an important role in interorgan communication in response to changes

in N availability. ATP-BINDING CASSETTE (ABC) SUBFAMILY MEMBER 14 (ABCG14) functions as a transporter that controls root-to-shoot translocation of precursor (*trans*-zeatin) and active (*trans*-zeatin riboside) CK (175). The expressions of *ISOPENTENYL TRANSFERASE* (*IPT*) genes, which regulate a rate-limiting step of CK biosynthesis, are promoted in roots perceiving high NO_3^-, causing a rapid increase of CK level that is subsequently translocated to shoots, thus enhancing shoot meristem activity and shoot branching (7, 66). The *abcg14* and *ipt* mutants not only exhibit decreased levels of CK in the xylem and retardation of shoot growth but also display repression of N uptake and lateral root growth in response to changes in N availability (108, 113, 175), suggesting that CK acts as a systemic signal involved in both N-demand and N-supply signaling (**Figure 5**).

Cell-to-cell communication mediated by small peptides is also involved in N signaling. The transcript abundances of CLAVATA3/ENDOSPERM SURROUNDING REGION-RELATED (CLE) peptide-encoding genes accumulate preferentially in root pericycle cells under N-starvation conditions, the products of which diffuse and bind to the leucine-rich repeat receptor protein kinase CLAVATA1 (CLV1) localized in phloem companion cells, thus inhibiting lateral root emergence and subsequent growth (5). CEPs (C-terminally encoded peptides) are a family of short secreted peptides that have emerged as key regulators of systemic N-demand signaling (125, 127). CEPs are produced in roots under N-starvation conditions and then translocated to shoots through the xylem, where they interact with the leucine-rich repeat receptor kinases CEP RECEPTOR1 (CEPR1) and CEPR2 (125). The CEP-CEPR1/CEPR2 recognition leads to the production of the nonsecreted polypeptide CEP DOWNSTREAM1 (CEPD1) and its homologs in shoots. Resultant CEPDs and CEPD-like polypeptides function as secondary signals and travel to roots through the phloem, thus promoting the expression of NO_3^- transporter genes in roots (99, 101). Long-distance peptide signaling pathways thus enable plants to adapt root morphologic and metabolic responses to the N status of the whole plant (**Figure 5**). However, CLE and CEPs act with opposite effects on lateral organ growth, suggesting that these systemic signals must integrate with other signaling pathways to drive the local response.

In addition, shoot-to-root mobile transcription factors contribute to the adjustment of N assimilation to N availability. The *Arabidopsis* ELONGATED HYPOCOTYL 5 (HY5), a bZIP transcription factor, travels in the phloem between shoots and roots and acts as a reporter for tissue N status (16). TEOSINTE BRANCHED 1-CYCLOIDEA-PCF 20 (TCP20) has a function in the systemic regulation of NO_3^- foraging by *Arabidopsis* roots (37): It interacts directly with AtNLP7 and its homolog, and the resultant TCP20-AtNLP7 heterodimer accumulates in the nucleus and activates the expression of genes related to cell-cycle and N metabolism (36). Compared to the *atnlp7* mutant, the *tcp20* mutant exhibits impaired root foraging on heterogeneous NO_3^- supply in the split-root system (36, 37), suggesting that an unknown TCP20-mediated signal is required for systemic N-demand signaling (**Figure 5**). The miR156-regulated SPLs have been shown to play an important role in regulating NO_3^--responsive gene expression (64). The long-distance translocation of miR156 and miR172 is regulated by HEXOKINASE1 (HXK1)-dependent sugar signaling (163), making the miR156-SPL-miR172 regulatory module an interesting candidate for mediating systemic N-demand signaling. The decoding of local and systemic signaling may contribute to a better understanding of plant developmental and metabolic adaptations to N availability, providing new approaches to improve NUE in crops.

5. MODULATION OF PLANT GROWTH–METABOLIC COORDINATION

A large amount of synthetic fertilizer use underlies current worldwide crop production, but NUE is estimated to be less than 40% in cereal crops. In the past decades, several genes have

been shown to improve NUE (**Table 1**). However, increased expression levels of N uptake and assimilation-associated genes not only boost grain yield but also increase plant height, and the resultant taller plants are more sensitive to lodging than expected. To date, a good deal of effort in improving NUE without loss of yield-enhancing semidwarfism has had limited success. It might be necessary to explore NUE within a plant systems biology context that considers the coregulation of plant growth and nutrient metabolism as a whole, rather than focusing specifically on N assimilation alone.

5.1. Coordinating Nitrogen Assimilation with the Overall Carbon Status

Plants use energy from the sun to convert carbon dioxide (CO_2) into photoassimilates in shoots and leaves, and they acquire mineral nutrients in roots. Although the shoots and roots follow distinct developmental trajectories, the coordination between carbon (C) fixation and N assimilation is key for plant performance and fitness. As mentioned above, HY5, a master regulator of photomorphogenesis, is involved in the C/N balance sensing (16). In shoots, an increasing light fluence increases HY5 abundance, and that binds to and activates subsets of target genes involved in C fixation (e.g., *TREHALOSE-6-PHOSPHATE SYNTHASE1*), sucrose partitioning (e.g., *SWEET11/SWEET12*) and N assimilation (e.g., *NITRATE REDUCTASE1* (*NIA1)/NIA2*); this effect is positively correlated with light fluence–dependent accumulation of biomass production. In roots, phloem-translocated sucrose and HY5 synergistically promote the expression of *AtNRT2.1* and NO_3^- uptake, but the sucrose-induced promotion of *AtNRT2.1* expression and NO_3^- uptake depends on HY5 function (**Figure 5**). Furthermore, the *hy5* mutant abolishes the sucrose-induced promotion of NO_3^- uptake and root growth, and that leads to a change in the C/N content ratio when compared with wild-type plants in a variable light environment (16). Thus, a mobile HY5 protein controls homeostatic coregulation of C and N metabolism in whole-organismal response to ambient environmental conditions.

N assimilation is intrinsically linked to the overall C status, and the upregulation of sugar transporter genes *SUGAR TRANSPORT PROTEIN 13* (*STP13*) and *SWEET16* can increase NUE (61, 117). DNA-BINDING ONE ZINC FINGER 1 (DOF1) transcription factor family genes are shown to enhance C skeleton production; for example, the overexpression of either maize *ZmDof1* or rice *OsDof2* improves grain yield and NUE under low-N conditions (54, 65, 104, 161). Recent studies reveal that photosynthetic capacity and grain yield can be significantly improved by either expressing the C_3-C_4 hybrid Rubisco or engineering photorespiratory bypasses (90, 118). Future research is required to address if NUE can be enhanced by modifying photorespiration under different fertilization conditions. In addition, a major rice QTL responsible for panicle architecture and photosynthetic characteristics has been mapped to *DEP1*, which has been widely used in rice breeding to increase photosynthetic efficiency and grain yield (52). A gain-of-function mutant allele, *dep1-1*, confers an increased level of CK through repressing the expression of *OsCKX2*, a gene encoding CK oxidase/dehydrogenase that plays a principal role in controlling CK levels, leading to improvements in grain number and grain yield. Furthermore, the rice plants carrying the *dep1-1* allele are shorter and more resistant to lodging than wild-type controls. Resultant semidwarf plants exhibit N-insensitive vegetative growth and increased N assimilation, thus improving grain yield at moderate N fertilization levels (122). DEP1 is shown to function as a cofactor of MADS-domain transcription factor OsMADS1, and the interaction of OsMADS1 and the G protein βγ dimer coregulates downstream target genes (**Figure 4**). Manipulation of the DEP1-OsMADS1 regulatory module thus enables enhanced sustainable productivity in rice GRVs (81). It has long been known that ANR1, an *Arabidopsis* homolog of OsMADS1, controls

root developmental adaptations to N availability (172), but it remains to be determined whether NUE can be improved by modulating MADS-box genes in parallel.

5.2. Modulating Growth–Metabolic Coordination Improves Nitrogen Use Efficiency

NUE is inherently complex due to different developmental, physiological, metabolic, and environmental interactions throughout the entire life cycle of plants (39, 80, 138). High-yield GRVs exhibit N-insensitive stem-elongation responses, enhancing plant lodging resistance but leading to inefficient N metabolism (**Figure 2**). Natural variation of *GRF4* confers an elevated abundance of transcription factor GRF4, resulting in simultaneous improvements of C fixation, N assimilation, and grain yield (74). GRF4 directly binds to and activates the transcription of target genes involved in N assimilation, photosynthesis, and cell proliferation. Conversely, DELLAs inhibit GRF4-mediated activation of C- and N-related genes by competitively inhibiting the interaction of GRF4 with its coactivator GIF1 (74), suggesting that the GA-DELLA-GRF4 regulatory system is a homeostatic coregulator of the C/N balance (**Figure 4**). Interestingly, overexpression of *GRF4* and/or GRF4-regulated *TaGS2-2Ab* enhances grain yield and NUE in wheat and rice GRVs, without loss of DELLA-conferred semidwarfism (49, 74). Therefore, an increased abundance of GRF4 partially disconnects the GA-DELLA regulation of plant height from N assimilation and provides a new breeding strategy to improve grain yield and NUE of GRVs at low-N fertilization levels (74, 82).

Unlike DELLA-mediated inhibition of plant height, the high-level accumulation of DELLAs promotes tillering of wheat and rice GRVs. The rice AP2 transcription factor NITROGEN-MEDIATED TILLER GROWTH RESPONSE 5 (NGR5), previously known as SMALL ORGAN SIZE1 (SMOS1) and REDUCED LEAF ANGLE1 (RLA1), is required for N-promoted rice tillering (43, 151). An increased N supply raises NGR5 abundance, which in turn facilitates N-dependent recruitments of polycomb repressive complex 2 (PRC2) to repress the expressions of shoot-branching-inhibitory genes through H3K27me3 modification, thus causing N-induced promotion of rice tillering (151). GA-dependent NGR5–GID1 interaction triggers NGR5 destruction. DELLAs competitively inhibit the NGR5–GID1 interaction, leading to an increase in the stability of NGR5 by reducing GA- and GID1-SCF^{GID2}-promoted proteasomal destruction (**Figure 4**), thus explaining the increased tillering and grain yield of GRVs. More importantly, increased abundances of NGR5 and GRF4 further enhance NUE in high-yield GRVs, reducing N fertilizer use while boosting grain yield above what is currently achievable (151). The rice transcription factor OsTCP19 is also shown to be involved in regulating N-promoted tillering (84). N-regulated OsTCP19 binds to and represses the expression of *DWARF AND LOW-TILLERING* (*DLT*), the product of which can interact directly with NGR5 (43, 130), thus modulating N-responsive tiller growth. Moreover, the introduction of an elite allele from *aus* cultivars into the modern *japonica* rice varieties increases NUE and grain yield under low-N conditions (84). Taken together, although quantitative traits that govern genetic complexity have previously made substantial improvements difficult to achieve, modulating the NGR5-DELLA-GRF4 regulatory system provides a simple route for synergistically improving NUE and grain yield in GRVs (82).

6. CONCLUSIONS

N is often a limiting macronutrient for plant productivity in both natural and agricultural ecosystems. The application of inorganic N fertilizer is one of the main approaches to increase crop yields. However, the semidwarf GRVs have relatively poor efficiency of N uptake and assimilation; thus, achieving current worldwide cereal crop yields requires a large input of inorganic

fertilizer, raising concerns about the substantial economic and environmental costs. Agricultural sustainability demands new breeding strategies to cut fertilizer use in high-yield crops. Thus, an in-depth understanding of how plants sense and shape their response to N availability is key for developing new GRVs with improved yield and NUE. In this article, we have provided a comprehensive review of N research spanning molecular genetics, genomics, transcriptomics, metabolomics, and systems biology in both model and crop plants, which have advanced our understanding of the molecular mechanisms regulating N capture, utilization, and signaling. Clearly, the discovery of the NGR5-DELLA-GRF4 regulatory system has shed light on new breeding strategies to overcome the trade-off between grain yield and NUE in crops. New precision breeding practices assisted by multiple allelic combinations and CRISPR/Cas9-mediated modification of key regulators that control N metabolism and signaling pathways of modern high-yield crops have the potential to make it possible to reduce N fertilizer use while boosting grain yield for the sustainable Green Revolution. Plant engagement with microorganisms can modulate root system architecture and enhance N acquisition, and recent research illustrates that *indica*-enriched microbiota are more diverse and contain more genera with N metabolism functions, suggesting that modulating root system architecture coupled with microbial engagement will facilitate N capture and enable us to enhance sustainable Green Revolution yields. Current advances in our knowledge of the underlying mechanisms and designs for improved efficiency of N acquisition and utilization in the model plant systems have mostly been performed under controlled laboratory conditions. The growth–metabolic coordination is strongly affected by varying environmental conditions and metabolic imbalances. Therefore, more refined strategies are needed that exploit gene pyramiding combined with tissue- and cell-specific targeting in crops under field conditions. The identification of the key components that coordinate growth and nutrient metabolism along with the use of precision gene modification will optimize crop breeding strategies and launch a new generation of sustainable Green Revolution.

SUMMARY POINTS

1. The beneficial semidwarfism of the Green Revolution varieties is associated with the trade-off between grain yield and nitrogen use efficiency (NUE) in cereal crops.
2. The enhanced activity of growth-repressing DELLA proteins represses the expression of genes involved in nutrient acquisition and metabolism and explains how reduced plant growth requires less nitrogen (N) assimilation.
3. A dual-affinity nitrate (NO_3^-) transporter, NRT1.1, functions as a NO_3^- sensor that controls plant developmental and metabolic adaptations to NO_3^- availability.
4. Mechanisms of local and systemic NO_3^- sensing and signaling and transcriptional networks underlying NO_3^--controlled responses in the model plant *Arabidopsis* are starting to be unraveled.
5. Over the last three decades, the gene regulatory networks of N uptake, transport, assimilation and remobilization have been extensively studied; pyramiding elite alleles from multiple key genes provides the opportunity for improving NUE in crops.
6. Modulating plant growth–metabolic coordination by tipping the balance of the GRF4-DELLA-NGR5 regulatory module enables enhanced sustainable Green Revolution yields.

FUTURE ISSUES

1. Exploring NUE in the context of global climate change that considers the coordination of elevated CO_2-induced promotion of photosynthesis and inhibition of N capture will enable us to enhance sustainable Green Revolution yields under environmental changes.
2. Converting N signals into hormonal stimuli and environmental signals will be helpful in uncovering the mystery of long-distance communications between shoots and roots, shaping developmental and metabolic adaptations to changes in N availability.
3. Decoding N-signaling diversity and its evolution in both model and crop species assisted by pan-genome-wide association studies will facilitate the discovery of new regulatory networks and use of diverse natural alleles for future design breeding.

DISCLOSURE STATEMENT

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

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