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REVIEW

ROLE OF NITROGEN SENSING AND ITS INTEGRATIVE SIGNALING PATHWAYS IN SHAPING ROOT SYSTEM ARCHITECTURE

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KEYWORDS

nitrogen, root system architecture, phytohormone, crosstalk, nitrogen-use efficiency, breeding strategy

HIGHLIGHTS

- The Green Revolution broadened the trade-off between yield and nitrogen-use efficiency.
- Root developmental and metabolic adaptations to nitrogen availability.
- Mechanisms of nitrogen uptake and assimilation have been extensively studied.
- Modulating plant growth-metabolic coordination improves nitrogen-use efficiency in crops.

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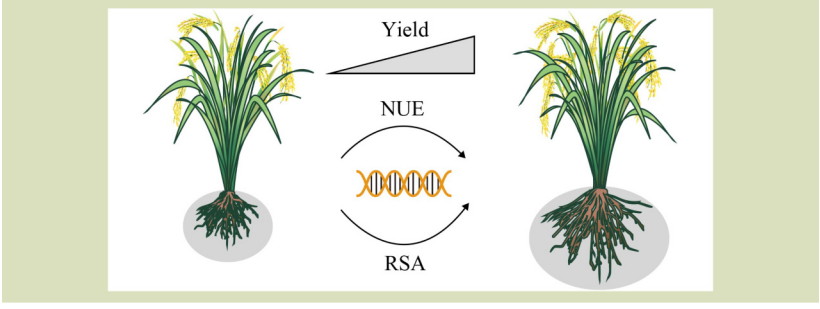
GRAPHICAL ABSTRACT



ABSTRACT

The Green Revolution of the 1960s boosted crop yields in part through widespread production of semidwarf plant cultivars and extensive use of mineral fertilizers. The beneficial semidwarfism of cereal Green Revolution cultivars is due to the accumulation of plant growth-repressing DELLA proteins, which increases lodging resistance but requires a high-nitrogen fertilizer to obtain high yield. Given that environmentally degrading fertilizer use underpins current worldwide crop production, future agricultural sustainability needs a sustainable Green Revolution through reducing N fertilizer use while boosting grain yield above what is currently achievable. Despite a great deal of research efforts, only a few genes have been demonstrated to improve N-use efficiency in crops. The molecular mechanisms underlying the coordination between plant growth and N metabolism is still not fully understood, thus preventing significant improvement. Recent advances of how plants sense, capture and respond to varying N supply in model plants have shed light on how to improve sustainable productivity in agriculture. This review focuses on the current understanding of root developmental and metabolic adaptations to N availability, and discuss the potential approaches to improve N-use efficiency in high-yielding cereal crops.

1 INTRODUCTION

Nitrogen (N) is an essential nutrient for sustaining plant growth and development, and the availability of N in the soil is a major limiting factor for plant performance. How efficiently plants explore the soil for N uptake is largely determined by their root system architecture. As sessile organisms, plant roots are able to absorb and assimilate a variety of N forms through transmembrane transporters or channels, ranging from simple inorganic (e.g., NH_4^+ and NO_3^-) to organic (e.g., amino acids and peptides) N forms. In environments where N is limited, overall plant growth is reduced but root systems are expanded, resulting in biomass allocation to roots at the expense of shoots. However, where N supply is ample, the root-to-shoot biomass allocation is low, allowing resource accumulation and investment in seed production. With low levels of N nutrients in most agricultural soils limiting crop growth and grain productivity, mineral fertilizers are typically applied at high concentrations to increase crop production[1]. A key part of the Green Revolution of the 1960s was the development of semidwarf Green Revolution varieties (GRVs), which exhibit an increased harvest index (the ratio of harvested grain to total shoot dry matter) and reduced risk of yield loss due to the lodging of plants by wind and rain[2]. However, GRVs have a relatively poor N-use efficiency (NUE), and require a large amount of fertilizers to achieve maximum yield potential[3–5]. To support global food production, environmentally degrading levels of N fertilizers have been applied. Therefore, a challenge for sustainable agriculture is to increase NUE with less dependence on mineral N fertilizers can be achieved without yield penalty. Although many efforts have focused on how to improve NUE, the underlying mechanisms of plant growth-metabolic coordination are still elusive. Recently, knowledge of the mechanisms related to how plants sense and respond to changes in N availability has expanded greatly in the model plants[6,7]. The main purpose of this review is to focus on advances in the understanding of N signaling and its crosstalk with phytohormone signaling pathways that shape root system architecture in response to N supply and plant N status. Although these advancements in knowledge can be exploited to improve NUE, more extensive research in crops is still needed.

2 NITROGEN ACQUISITION AND METABOLISM

For most plants, NO_3^- is the main source of soil N supply, but NH_4^+ is the main source of N for plants grown under flooded conditions or in acidic soils[8]. NO_3^- concentration can vary from micromolar to millimolar amounts in soil[9,10]. To cope with such large variation in NO_3^- availability, plants have evolved sophisticated high-affinity (when the external NO_3^- concentration is low, e.g., < 0.5 mmol·L^{-1}) and low-affinity (when the external NO_3^- concentration is high, e.g., > 0.5 mmol·L^{-1}) transport systems[11–13]. After taken up by NO_3^- transporters (NRTs), part of NO_3^- can be reduced in roots immediately, but most of NO_3^- is translocated from roots to shoots, where it is reduced to nitrite by nitrate reductase (NR) in the cytosol of cells, and then translocated to the plastids and chloroplasts, where it is further reduced to NH_4^+ by nitrite reductase (NiR). In contrast, NH_4^+ must be assimilated into glutamine (Gln) in roots rather than being translocated to shoots and leaves because of its toxicity[14]. Recently, the transporters of NO_3^- and NH_4^+ have been identified and functionally characterized, and regulatory mechanisms affecting N uptake, transport and assimilation have been extensively investigated in the model plants[7,15]

2.1 Nitrogen absorption and transport

In higher plants, the NO_3^- transport systems consist of four NO_3^--transporting protein families: NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER (given as NPF below), NITRATE TRANSPORTER 2, CHLORIDE CHANNEL (CLC), and SLOWLY ACTIVATING ANION CHANNEL (SLAC)/SLAC-ASSOCIATED 1 HOMOLOG (SLAH)[6]. NPF is the largest family with 53 members in *Arabidopsis*, most of which function as low-affinity NO_3^- transporters. The *Arabidopsis* CHLORIDE RESISTANT 1 (CHL1, also known as AtNPF6.3 or AtNRT1.1) is the first NO_3^- transporter to be identified by genetic screening for chlorate resistance[16]. Notably, the *chl1-5* mutant caused by a loss-of-function mutation of *AtNRT1.1* exhibits reduced NO_3^- uptake and N-mediated growth responses, whereas the weak mutant allele, *chl1-9*, only exhibits defective NO_3^- uptake, but no changes in response to varying N supply[17], indicating that N uptake and responsiveness are separate processes. Also, AtNRT1.1 functions as a NO_3^- sensor that transduces external signals into the cells and triggers activation of N-responsive gene expression[17,18]. AtNRT1.1 has been shown to be a dual-affinity transporter responsible for both low- and high-affinity NO_3^- uptake, and the two modes of uptake activity are switched by phosphorylation and dephosphorylation of the threonine 101 (T101) residue of AtNRT1.1[17,19]. The T101 residue can be phosphorylated by either calcineurin B-like (CBL) protein-interacting protein kinases (e.g., CIPK8 and CIPK23), or dephosphorylated by ABSCISIC ACID INSENSITIVE 2 (ABI2), which in turn modulates AtNRT1.1 activity, thus triggering N-mediated responses[18,20,21]. In addition to the normal transport of NO_3^-[22,23], the *Arabidopsis*

NPF proteins have been shown to transport various other substrates, including amino acids[24], peptides[25], nitrite[26], glucosinolates[27], auxin[28], abscisic acid (ABA)[29] and gibberellins (GA)[30]. In contrast, NRT2 family proteins act as high-affinity transporters that use NO_3^- as a specific substrate[22]. In environments where N is limited, the high-affinity uptake system is activated and has a key function in the regulation of NO_3^- uptake[22]. The CLC family transporters consist of seven members in *Arabidopsis*, one of which has been shown to be a reverse transporter of NO_3^-/H^+ and is important for regulating the accumulation of NO_3^- in the vacuole[31,32]. The SLAC/SLAH family transporters in *Arabidopsis* have five members, including SLAC1, SLAH1, SLAH2, SLAH3 and SLAH4. It has been demonstrated that SLAC1 and SLAH3 encode S-type anion channels in guard cells and are important in the control of stomatal closure[6]. In addition, *SLAH2* is expressed in stele cells of roots, which may be involved in the transport of NO_3^- from roots to stem[33].

Plant roots possess multiple transport systems for NO_3^- uptake that are driven by the hydrogen ion concentration gradient or protonmotive force generated by plasma membrane H^+-ATPases[34,35]. At least six transporters on the plasma membrane have been shown to be involved in NO_3^- uptake in *Arabidopsis* roots, including AtNRT2.1, AtNRT2.2, AtNRT2.4, AtNRT2.5, AtNRT1.1 and NPF4.6[36,37] (Fig. 1). Under low NO_3^- conditions, AtNRT1.1 acts as high-affinity transporter that is mainly responsible for NO_3^- absorption at the root tips[38], whereas AtNRT2.1 is mainly responsible for NO_3^- absorption and transport in root maturation zone[22,39]. Also, the NRT2 family transporters have complementary functions, for example, lack of the *AtNRT2.1* function (in the *atnrt2.1* mutant) causes the upregulation of *AtNRT2.2* by more than three times[40]. Although the expression of *AtNRT2.1* and *AtNRT2.2* is rapidly induced by N limitation, the transcriptional level of *AtNRT2.4* is upregulated only after long-term N limitation[41]. In addition, the activity of AtNRT2 family transporters requires a critical partner protein AtNAR2.1 (NO_3^- assimilation related protein, also known as AtNRT3.1), and the AtNRT2-AtNAR2.1 interaction promotes AtNRT2 protein stability and its plasma membrane localization, thus enhancing N uptake[40,42,43].

NO_3^- is translocated from roots to shoots through the xylem under the effect of transpiration flow, and then distributed into the vacuoles and protoplasm of the plant stem, leaves and storage organ cells[44]. *NPF7.3* (known as *AtNRT1.5*) is expressed in the pericycle of the root around the primary xylem, which is responsible for the first step to transport NO_3^- from roots to shoots[45]. NPF7.2 (known as AtNRT1.8) and NPF2.9 (known as AtNRT1.9) negatively regulate the process of loading NO_3^- into the xylem[46,47]. *NPF7.2* is expressed in parenchyma cells of root xylem, which is involved in the process of transporting NO_3^- from roots to shoots. In addition, the expression level of *NPF7.2* is induced by Cd^{2+} treatment, and the *Arabidopsis nrt1.8-1* mutant exhibits N-dependent Cd^{2+}-sensitive phenotype[46]. *NPF2.3* is constitutively expressed, and contributes to NO_3^- translocation to shoots under salt stress[48]. *NPF2.9* is expressed in the companion cells of root phloem, and that participates in NO_3^- transport from the xylem to the phloem, and that lack of the *NPF2.9* function results in decreasing the amount of NO_3^- transported from roots to shoots of plants[47]. In contrast, NPF2.7 is found to be a low-affinity NO_3^- excretion transporter located in root plasma membrane[45] (Fig. 1). *NPF6.2* (known as *AtNRT1.4*) is mainly expressed in petioles and veins, and functions as a low-affinity transporter that affects NO_3^- content in petiole and leaf growth[49]. NPF1.1 (known as AtNRT1.12) and NPF1.2 (known as AtNRT1.11) involve in the translocation of NO_3^- from the xylem to the phloem[50]. *NPF2.13* (known as *AtNRT1.7*) is expressed in parenchyma cells of the phloem of leaf veins, and is responsible for transferring NO_3^- from older leaves to newer leaves to ensure the growth of young tissues under N limitation conditions[51]. *AtNRT2.5* and *AtNRT2.4* are also found to be expressed in shoots and leaves, and contribute to phloem loading of NO_3^- and facilitate NO_3^- allocation from source leaves to sink leaves[41,52]. In addition to sucrose, amino acids are usually present in high concentrations in phloem sap, which are transported to the sink during the reproductive phase[53] (Fig. 1). NPF2.12 (known as AtNRT1.6) located on the plasma membrane of vascular tissue of pod is responsible for NO_3^- transport from vegetative organs to embryos to ensure NO_3^- supply of developing seeds[54]. *NRT2.7* is expressed on the vacuolar membrane of seed cells, and is responsible for the accumulation of NO_3^- in seed vacuoles[55]. The *Arabidopsis NPF5.5* gene produces two transcripts (*AtNPF5.5a* and *AtNPF5.5b*) and affects N accumulation of embryos[56] (Fig. 1).

For NH_4^+ uptake, plants use two distinct transport systems: a nonsaturable low-affinity system and a saturable high-affinity system[57,58]. The high-affinity NH_4^+ transporters (AMTs) have been shown to facilitate the movement of NH_4^+ across the membrane. There are six AMT-type NH_4^+ transporters in *Arabidopsis*: AtAMT1;1, AtAMT1;2, AtAMT1;3, AtAMT1;4 and AtAMT1;5 belonging to the AMT1 subclass, whereas AtAMT2;1 is more closely related to bacterial AmtB and yeast MEP proteins[59]. With the exception of *AtAMT1;4*, the five *AtAMT1* genes are highly expressed in roots. *AtAMT1;1* and *AtAMT1;3* are expressed in the epidermal and cortical cells,

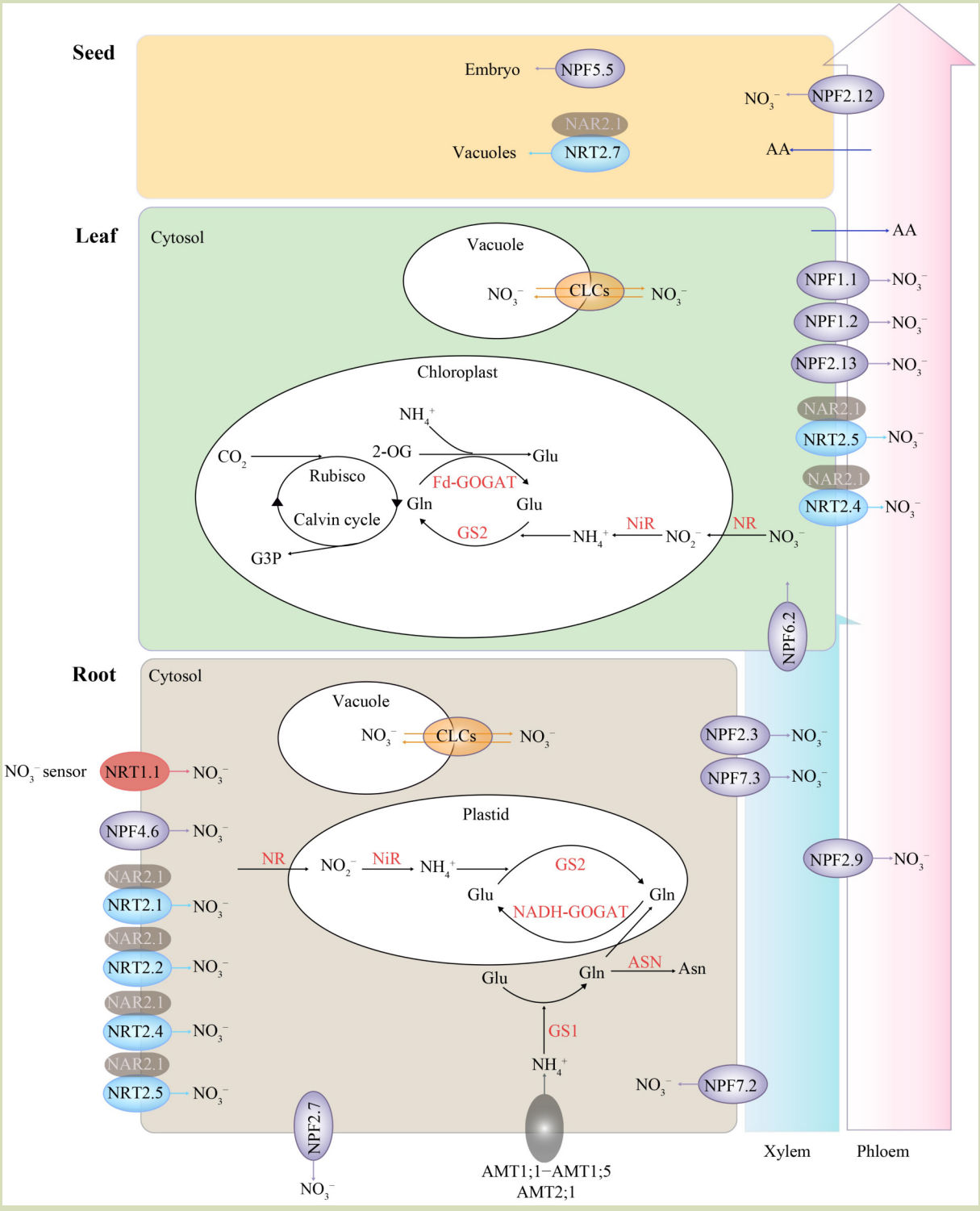


Fig. 1 The absorption, transport and assimilation of inorganic nitrogen in *Arabidopsis*.

which are responsible for high-affinity uptake of NH_4^+ from soil into the root cells and then for symplasmic transport within the roots[60], whereas *AtAMT1;2* is expressed in the endodermal and cortical cells. AtAMT1 family proteins can be phosphorylated in the cytosolic C-terminal region in response to NH_4^+ supply, shutting off their transport activity, thus

consequently inhibiting NH_4^+ uptake to prevent NH_4^+ toxicity[61–63]. In addition, *AtAMT2;1* is expressed in the marginal epidermis, which has an important function in not only NH_4^+ uptake and retrieval from the root apoplast but also NH_4^+ translocation into the vasculature under N limitation conditions[64] (Fig. 1).

2.2 Nitrogen assimilation

N assimilation refers to the process that plant roots absorb NO_3^- or NH_4^+ from the environment, and consequently synthesize N-containing organic compounds through a series of oxidative and reductive reactions[65]. Once NO_3^- is incorporated in root cells, part of it can be stored temporarily in the vacuole, some of it is directly assimilated into amino acids and proteins in roots, but the most is transported to the shoots through the xylem. Given the ready availability of energy from photosynthesis, most of NO_3^- assimilation occurs in shoots and leaves rather than roots[66,67]. In the cytoplasm, NO_3^- is reduced to NO_2^- under the control of NR. NO_2^- is further reduced to NH_4^+ by NiR in the plastids[68]. The *Arabidopsis* has two *NITRATE REDUCTASE* genes (*AtNIA1* and *AtNIA2*) and lack of the *AtNIA2* function (in the *atnia2* mutant) exhibits ~90% reduced NR activation[69], indicating that *AtNIA2* is important in regulating NO_3^- reduction. In contrast, NH_4^+ needs to be locally and rapidly assimilated through the glutamine synthases (GS) and glutamate synthases (GOGAT) pathway, and then transported mainly in the form of Gln[70]. GS exists in plants as a collection of isoenzymes, located either in the cytosol (GS1) or in plastids (GS2)[71]. Cytosolic GS1 is important for primary NH_4^+ assimilation in roots. In addition, NH_4^+ is also produced by both photorespiration and protein turnover in plant shoots, which is mostly assimilated in the leaf chloroplasts by GS2[72]. In higher plants, there are two types of GOGAT, which use either reduced ferredoxin (Fd-GOGAT) or NADH (NADH-GOGAT) as an electron donor[73,74]. Fd-GOGAT is mainly localized in chloroplast[73], whereas NADH-GOGAT is present in roots, companion cells and etiolated leaves[75]. In addition to Gln, asparagine (Asn) is another major translocated amino acid. Given that the N/C ratio of Asn is high, it has been reported to be translocated and stored[76,77]. The concentrations of Asn and Gln increase in the phloem sap during senescence, indicating that both amino acids play important roles in making N available in the senescing leaves for remobilization to the reproductive organs. The glutamate dehydrogenase (GDH), aspartate aminotransferase, and asparagine synthetase are key enzymes that contribute to N assimilation and remobilization, and catalyze the ATP-dependent amido group of glutamine to aspartate and then generate aspartate and asparagine[78]. 2-OG from the tricarboxylic acid cycle serves as the sole C skeleton for N assimilation, making the pivotal role of GS/GOGAT and GDH in N and C metabolic balance[79] (Fig. 1).

3 ROOT DEVELOPMENT ADAPTATIONS TO NITRATE AVAILABILITY

Root plasticity is an important trait for plants to survive and maintain their growth under a variable nutrient environment[80,81]. In addition to being N nutrients, NO_3^- also acts as a signal in the regulation of plants root developmental adaptations to N availability[82,83]. The effects of NO_3^- on root system architecture are mainly in the following aspects: (1) N limitation-induced promotion of the primary and lateral root growth; (2) inhibitory effects of high dose N supply (e.g., > 10 mmol·L^{-1}) on root system growth; (3) stimulatory effects of heterogeneity of NO_3^- on lateral root development in N-rich patches[80,84,85]. Therefore, there might be multiple signaling pathways involved in root developmental adaptations to N availability (Fig. 2 and Fig. 3).

3.1 Nitrate limitation-induced root growth promotion

Early studies of the effects of NO_3^- on root system architecture in *Arabidopsis* were concerned with the ability of a localized NO_3^- treatment to stimulate primary and lateral root growth[86–88]. Recently, multiple pathways (e.g., auxin and GA) have been shown to be involved in directly regulating the lateral root response to varying N supply[28,82,89,90]. TRYPTOPHAN AMINOTRANSFERASE RELATED 2 (TAR2) is responsible for converting L-tryptophan to indole-3-pyruvic acid (IPA), which is the first step in the IPA pathway branching from a Trp-dependent auxin biosynthetic pathway[91,92]. Under low NO_3^- conditions, *TAR2* mRNA abundance is increased, resulting in an increase of IAA content in the developing lateral roots. In contrast, loss-of-function *tar2* mutant has much shorter lengths of the second-order and third-order lateral roots, indicating that N limitation-stimulated lateral root emergence is depended on root-synthesized auxin[93]. In addition, an *Arabidopsis* AGL17-clade MADS-box gene *AGL21* is also found to promote lateral root growth under low N conditions. The expression of *AGL21* is induced by either N deprivation or auxin treatment. *AGL21*-overexpressing lines produce more visible and longer lateral roots, but the *agl21* mutants exhibit a reduction of lateral root growth under N-

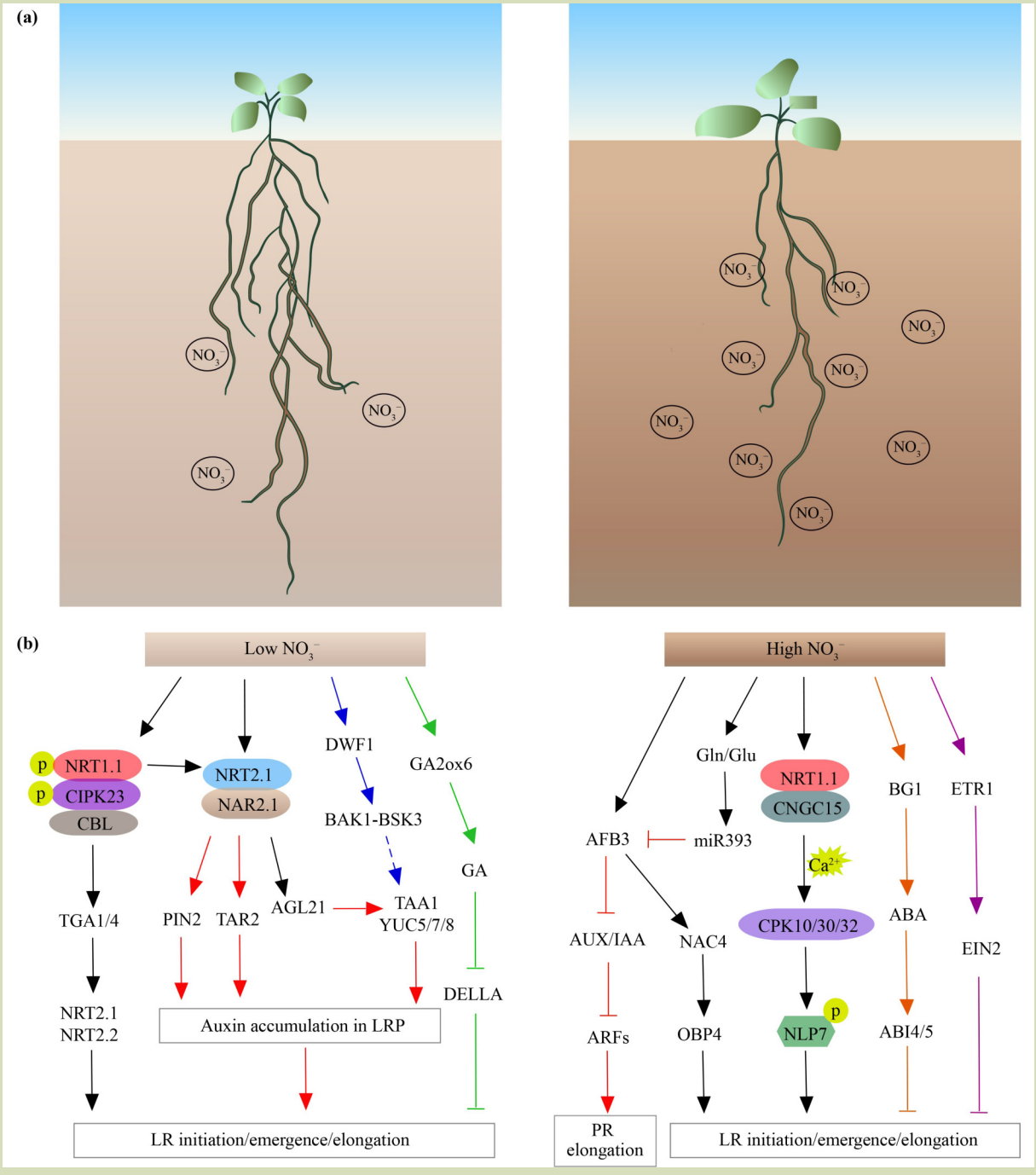


Fig. 2 Root developmental adaptations to nitrogen availability. (a) The diagrams of the root responses of *Arabidopsis* plants under different nitrogen levels. (b) Schematic representation of integrative NO_3^- signaling.

restricted conditions. Also, auxin biosynthesis genes (e.g., *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1* (*TAA1*), *TRYPTOPHAN AMINOTRANSFERASE-RELATED PROTEIN 3* (*TAR3*), *YUCCA5* (*YUC5*) and *YUC8*) are found to be upregulated in *AGL21*-overexpressing lines but downregulated in the *agl21* mutants, indicating that *AGL21* regulates lateral root development by enhancing auxin biosynthesis[94].

Auxin is primarily synthesized in leaf primordium, root tips

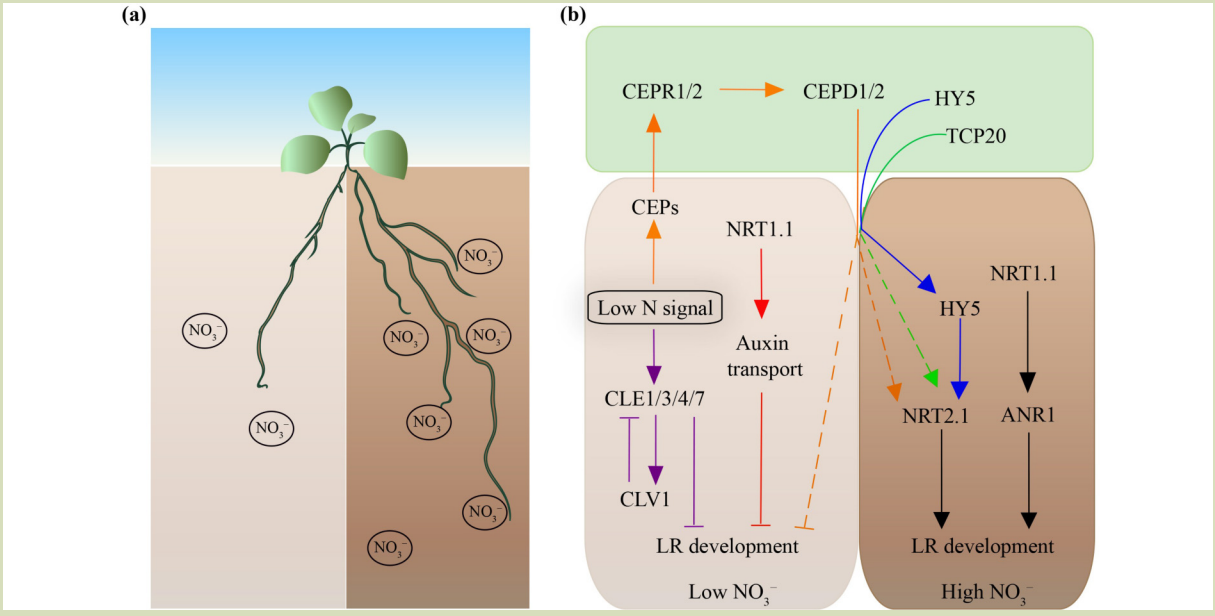


Fig. 3 Systemic nitrate signaling and its role in whole-plant responses in *Arabidopsis* split-root system. (a) Differential responses of primary and lateral roots of *Arabidopsis* plants under nitrate (NO_3^-) heterogeneous conditions. (b) A simplified model of systemic NO_3^- signaling in root foraging responses.

and cambiums. Delivery of auxin in outer tissues including the cortex and the epidermis is largely mediated by the PIN-FORMED2 (PIN2) auxin efflux carrier[95,96]. PIN2-mediated auxin reflux to inner tissues has been shown to be associated with maintenance of root meristem size[97]. Lack of the *PIN2* function (in the *Arabidopsis pin2* mutants) not only interferes with NO_3^--stimulated transport of auxin, but also severely affects adaptive response of roots to N availability. Recent studies have indicated that N-dependent phosphorylation of the S439 of PIN2 has a direct impact on PIN2 localization and protein polarity, thus triggering root developmental adaptations to N availability[98]. In addition, the mutation of the rice *OsNAR2.1* gene causes the inhibition of lateral root formation by reducing the expression of *PINs* in roots under low N conditions[99], indicating that NAR2.1 is important in the regulation of N-responsive lateral root development by affecting auxin polar transport. Genome-wide association studies uncover *BRASSINOSTEROID SIGNALING KINASE 3* (*BSK3*) as a major gene associated with the primary elongation, and *YUC8* as determinant for lateral root response to low N stress[100]. The expression levels of *YUC8*, *YUC3*, *YUC5*, *YUC7* and *TAA1* and auxin accumulation in the root tips are induced by mild N deficiency[101]. In addition, the noncoding variants of brassinosteroid (BR) biosynthesis gene *DWARF1* (*DWF1*) are found to be associated with the changes in abundance of *DWF1* under low N conditions, and that contributes to natural variations of root elongation, indicating that N-responsive BR biosynthesis promotes root growth[102]. Also, N limitation-induced primary and lateral root growth depend on the activation of the leucine-rich repeat receptor-like protein kinase BR-INSENSITIVE1-ASSOCIATED RECEPTOR KINASE 1 (BAK1). The *bsk3 bak1-1* double mutant exhibits blocked cell elongation and primary root growth under low N conditions, supporting the role of the *BAK1-BSK3* regulatory module in controlling root architecture in response to N availability[100,103,104]. N limitation reduces the abundance of DELLA proteins by increasing bioactive GA content through activation of GA metabolism gene expression, thus enhancing cell proliferation and elongation[105]. CALMODULIN-LIKE 38 (CLM38) is found to interact with small peptide receptor protein PEP1 RECEPTOR 2 (PEPR2), which is induced by NO_3^- signal, and that negatively regulates the expression of those genes related to NO_3^- and BR signal to regulate root development in *Arabidopsis*. Notably, the interaction of CLM38 and PEPR2 enhances BR signaling, resulting in increasing the dephosphorylation of BRI1-EMS-SUPPRESSOR 1 (BES1). Therefore, the CLM38-PEPR2 module acts as a fine-switch, which not only promotes lateral root development to absorb soil N under low N conditions, but also inhibits root growth to prevent rapid N consumption under high N conditions, realizing the dynamic balance between root growth and N capture[106,107]. In addition, the basic leucine zipper

(bZIP) transcription factors TGACG MOTIF BINDING FACTOR 1 (TGA1) and TGA4 bind to the promoter regions of *AtNRT2.1* and *AtNRT2.2*, and regulate their expression. The expressions of *TGA1* and *TGA4* are upregulated by NO_3^-, which occur downstream of AtNRT1.1 and calcium signal. The phenotype of the *Arabidopsis tga1 tga4* mutant indicates that TGA1 and TGA4 promote primary root elongation, lateral root development and root hair growth in a NO_3^- dependent manner[108].

3.2 Inhibition of high nitrate concentrations on lateral root growth

Plants optimize N acquisition from the soil through modulating root system architecture, adjusting root-to-shoot allocation patterns. In environments where N is in ample supply, the root-to-shoot biomass allocation is low, with minimal root systems capturing sufficient N supply. Experimentally, primary and lateral root growth is suppressed but shoot growth is promoted when NO_3^- concentration is high. The inhibitory effects have been shown to be involved in multiple signal transduction pathways[80,89]. When the NO_3^- concentration falls below 1 mmol·L^{-1}, AtNRT1.1 behaves as a high-affinity NO_3^- transporter. However, when the NO_3^- concentration is high, AtNRT1.1 switches into a low-affinity system[17,109]. This switch is controlled by phosphorylation of AtNRT1.1 through calcium sensor proteins and their binding partners[19,39,109]. Under N limitation conditions, AtNRT1.1 is phosphorylated by plasma membrane-localized CBL1- and CBL9-CIPK23 complex, which in turn induces dimer decoupling and increases its structural flexibility, modulating NO_3^- transport activity and signal transduction, thus consequently triggering responses to low N stress (Fig. 2).

When N is uniformly high, the expression of *AUXIN BINDING F-BOX PROTEIN3* (*AFB3*), a gene encoding auxin receptor, is found to be upregulated, which promotes the auxin receptors TRANSPORT INHIBITOR RESPONSE1/AFB-mediated ubiquitin-proteasome-dependent degradation of the AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) transcriptional repressors, relieving AUXIN RESPONSE FACTORS from repression and thus allowing them to activate or repress the expression of auxin-responsive genes[110,111], consequently promoting root growth (Fig. 2). The expression levels of *AFB3* are also feedback repressed by N assimilates via miR393 that targets the *AFB3* transcript for degradation[112]. Under adequate NO_3^- supply, *AFB3* is found to regulate the expression of *NAC4* and *OBP4*, the products of which affect the root renovation[110]. When NO_3^- is locally increased in the root environment, it leads to the gradual accumulation of ABA in the root tips, thus stimulating ABA signaling and ultimately regulating NO_3^- uptake and metabolism. Localized stimulation of ABA levels is due to the action of ER-localized β-GLUCOSIDASE 1, which releases bioactive ABA from the inactive ABA-glucose ester[112]. Also, ABA insensitive mutants *abi2-2*, *abi4-1*, *abi4-2* and *abi5-1* exhibit the reduction of either low NO_3^--induced expression of *AtNRT2.1* or high NO_3^--induced inhibition of lateral root growth, indicating that ABI2, ABI4 and ABI5, key regulators of ABA signaling, are required for NO_3^- sensing and signaling pathways[112,113]. ABI2 (perhaps other ABI family proteins) interacts with and dephosphorylate both CBL1 and CIPK23[114], which in turn interferes with activity of the CBL1-CIPK23 complex, thereby modulating AtNRT1.1-mediated NO_3^- signaling. Also, increasing NO_3^- supply increases ethylene production and consequently represses lateral root growth[114]. The *Arabidopsis* mutants defective in ethylene signaling (e.g., *etr1-3* and *ein2-1*) are also found to be insensitive to treatments with high concentrations of NO_3^-, indicating ethylene is also important for regulating systemic repression of lateral root growth under high N conditions[115,116].

Since the first identification of the *Arabidopsis* MADS-box transcription factor *ARABIDOPSIS* NITRATE REGULATED 1 (ANR1) involved in NO_3^- signaling[117], several transcription factors involved in rapid transcriptional reprogramming of primary NO_3^--response (PNR) genes have been identified[118–121]. Previous studies have shown that NODULE INCEPTION-LIKE PROTEIN (NLP) transcription factors (e.g., NLP6 and NLP7) directs the majority of PNR gene expression. In the absent of NO_3^-, NLP7 is normally localized in the cytosol and excluded from the nucleus. In the presence of NO_3^-, AtNRT1.1 receives NO_3^- signals and consequently stimulates Ca^{2+} influx into the cells, which in turn activates Ca^{2+}-sensor protein kinases CPK10/CPK30/CPK32, causing NO_3^--responsive phosphorylation of NLP7 and rapid accumulation in the nucleus, thus mediating the downstream target gene expression and root growth[122]. Recent studies have revealed that a cyclic nucleotide-gated channel (CNGC) protein, CNGC15, and AtNRT1.1 constitute a molecular switch that controls cytoplasmic Ca^{2+} elevation and root developmental adaptations to N availability. CNGC15 is an active Ca^{2+}-permeable channel that physically interacts with AtNRT1.1. mRNA abundance of *CNGC15* is induced by NO_3^- supply. In contrast, a loss-of-function mutation of *CNGC15* inhibits NO_3^--induced nuclear entry of NLP7. The CNGC15-AtNRT1.1 protein complex dissociates and consequently silences the activity of the calcium channel in response to varying NO_3^- supply, indicating that the dynamic interaction between CNGC15 and AtNRT1.1 enables controlling

AtNRT1.1-dependent Ca^{2+} channel activity in a NO_3^--dependent manner[123,124].

3.3 Systemic nitrate signaling pathways involved in root foraging responses

In environments where N supply is uneven (Fig. 3), the primary and lateral roots proliferate into local patches of high N[82,125,126]. In *Arabidopsis*, *ANR1* is dominantly expressed at lateral root primordia and root tips, and promotes lateral root elongation in the NO_3^--rich patches[117]. AtNRT1.1 also transports auxin[28], which allows preferential root colonization of NO_3^--rich patches by both preventing root growth in response to low NO_3^- stress and stimulating root growth in response to increasing NO_3^- supply[82]. In addition, AtNRT1.1 has been shown to act upstream of ANR1 in the signaling pathway, enhancing mRNA abundance of *ANR1* and promoting lateral root growth[82,127]. The TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR1-20 (TCP20) interacts with NLP6/NLP7[128], and promotes the expression of typical PNR genes, such as *AtNRT1.1*, *AtNRT2.1* and *AtNIA1*[126]. Also, the *Arabidopsis tcp20* mutant exhibits impaired root foraging on heterogeneous NO_3^- media in split-root plates[126], indicating that TCP20-mediated unknown systemic signaling pathway is also required for N-foraging responses (Fig. 3). In addition, the bZIP transcription factor ELONGATED HYPOCOTYL 5, which travels in the phloem between shoots and roots, is important in regulating coordination of light-promoting plant growth, C fixation and N assimilation[129], and that contributes to the adjustment of N uptake and assimilation in response to C availability (Fig. 3).

In a split-root system, primary elongation and lateral root development are promoted when both compartments have low N, but is suppressed in the low N compartment and promoted in the high N compartment in differentially treated roots[80]. The expression levels of *CLE* genes (e.g., *CLE1* and *CLE3*) that encode CLAVATA3/ENDOSPERM SURROUNDING REGION-related peptides are induced in root pericyclic cells of plants grown under low NO_3^- conditions[130], the products of which diffuse into phloem companion cells, and then bind to and activate leucine-rich repeat receptor protein kinase CLAVATA1 (CLV1). The *CLE1*-overexpression lines exhibit the inhibition of lateral root emergence and outgrowth of the new primordia, whereas the *clv1* mutants exhibit an increased lateral root growth[131]. In addition, overexpression of *CLE3* inhibits lateral root development in wild-type plants but not in the *clv1* mutants[132], indicating that local cell-to-cell communication medicated by the CLEs-CLV1 regulatory module is important in regulating lateral root growth in response to changes in NO_3^- availability. It has been shown that N limitation-induced C-TERMINALLY ENCODED PEPTIDES (CEPs) function as systemic signals, which are produced in roots under low N conditions and then translocated to shoots through the xylem, where they recognize and interact with the leucine-rich repeat receptor kinases CEP RECEPTOR1 and RECEPTOR2 in the phloem[133–135], leading to the production of the nonsecreted polypeptide CEP DOWNSTREAM1 (CEPD1) and its homologs[134]. These and related polypeptides function as secondary signals and translocate into roots in NO_3^--rich patches, thus consequently promoting the expression of *NRT2.1*[134–136]. Therefore, long-distance peptide signaling pathways are important in modulating coordination between N acquisition and N demand at the whole-plant level (Fig. 3).

4 IMPROVING NITROGEN-USE EFFICIENCY BY MODULATING PLANT GROWTH-METABOLIC COORDINATION

NUE is a measure of plant ability to capture and use N nutrients, which can be simply defined as yield per unit of N fertilizer applied to crops[137]. Given that environmentally degrading mineral fertilizer use underpins current worldwide crop production, future agricultural sustainability demands the development of the new high-yielding cultivars with improved NUE above what is currently achievable. However, NUE is a complex agronomic trait controlled by quantitative trait loci and influenced by multiple environmental factors. Over recent decades, extensive research efforts to understand how the model plants regulate N uptake, assimilation and utilization have provided ample opportunities to use this knowledge to increase NUE and grain yield in cereal crops. Notably, although introduction of several elite alleles or ectopic overexpression of some but not all genes related to N uptake and assimilation have been shown to improve NUE (Table 1), the upregulation of N metabolism-associated genes not only increase N capture and grain yield, but also increase plant height and delay flowering time, and the resultant taller plants are more sensitive to lodging than expected. To date, a substantial efforts to improve NUE without loss of yield-enhancing semidwarfism have had only limited success.

Recent advances have revealed how to explore NUE within an overall plant systems biology context that considers the co-regulation of plant growth, photosynthesis and N assimilation at the whole-plant level, rather than focusing specifically on N metabolism. The elite *DENSE AND ERECT PANICLE1* allele has been shown to improve NUE and grain yield at low N

Table 1 The genes associated with the improvements of yield and NUE in crops

Gene name	Species	Phenotype
OsAMT1;1[138]	Rice	Enhancing the permeability of NH_4^+ and improving grain yield under low NH_4^+ conditions
OsAMT1;3[139]	Rice	*AMT1;3*-overexpression lines exhibit C and N metabolic imbalance, resulting in a poor growth and reduced grain yield
OsNRT1.1a[140]	Rice	Overexpression of *OsNRT1.1a* enhances N uptake and grain yield with early flowering
OsNRT1.1b[141]	Rice	The upregulation of *OsNRT1.1b* enhances NUE and grain yield in rice
OsNRT2.3b[142]	Rice	Enhancing pH homeostasis, grain yield and NUE
OsNAR2.1[143]	Rice	Promoting NO_3^- absorption and transport, and improving drought resistance
OsNR2[144]	Rice	The upregulation of *OsNR2* increases tiller numbers, grain yield and NUE
OsGS1;1[145]	Rice	Promoting grain filling
OsGS1;2[146]	Rice	Improving uptake and assimilation of NH_4^+
OsNLP4[147]	Rice	Increasing tillering, grain yield and NUE under different N conditions
OsFd-GOGAT[148]	Rice	Involved in N remobilization during leaf senescence
OsTCP19[149]	Rice	N-regulated *OsTCP19* negatively regulates rice tillering
AlaAT[150]	Rice	Promoting biomass accumulation, tiller numbers, N content and grain yield
OsMYB305[151]	Rice	Increasing N assimilation, tiller numbers and shoot dry weight
DEP1[152]	Rice	N-regulated *dep1* allele improves N assimilation, NUE and grain yield
MADS25[153]	Rice	Increasing primary root length, lateral root number and shoot fresh weight
OsBT1/2[154]	Rice	Negative regulator of N uptake and utilization
GRF4[5]	Rice	Integrating and coordinating plant growth, C fixation and N assimilation, reducing N fertilizer use while boosting grain yield without affecting semidwarfism
OsNAP[155]	Rice	Regulating nutrient uptake capacity and affecting plant senescence
NGR5[156]	Rice	N-regulated *NGR5* enhances tillering, NUE and grain yield in rice
DNR1[157]	Rice	Involved in auxin biosynthesis, enhancing N metabolism and NUE in rice
TOND1[158]	Rice	Increasing primary root length, N uptake, shoot dry weight, grain number and yield
OsDRO1[159]	Rice	Modulating root growth angle, enhancing N uptake and grain yield
TaNAC2-5A[160]	Wheat	Promoting root branching and NO_3^- uptake, increasing N harvest index and grain yield
TaNFYA-B1[161]	Wheat	Promoting root branching and NO_3^- uptake, Increasing spike number and grain yield
TaGS2-2Ab[162]	Wheat	Enhancing N uptake and remobilization, grain number, grain weight, and grain yield
TaARE1[163]	Wheat	Increasing N uptake, grain weight and grain yield under low N conditions
TaTAR2.1[164]	Wheat	Enhancing lateral root length, spike number and grain yield
Ms44[165]	Maize	Improving grain yield and NUE

fertilization levels[152,166]. The semidwarf *dep1* allele confers the downregulation of the rice *CYTOKININ OXIDASE 2*, causing increases in grain numbers and rice yield[166]. Remarkably, uncoupling of plant height and panicle branching from N regulation in rice plants with the elite *dep1-1* allele, thus boosting grain yield at a moderate N supply, without affecting beneficial semidwarfism[152]. An allelic variation of GROWTH-REGULATING FACTOR4 (GRF4) has been shown to improve photosynthesis and N assimilation, whereas rice DELLA protein SLR1 inhibits these processes, indicating that the DELLA-GRF4 regulatory module is a coregulator of plant growth, C fixation and N assimilation[5]. More importantly, tipping the DELLA-GRF4 balance toward increased GRF4 abundance significantly increases grain yield and NUE in both wheat and rice GRVs, without loss of DELLA-conferred beneficial semidwarfism. Also, SLR1-interacting NITROGEN-MEDIATED TILLER GROWTH RESPONSE 5 (NGR5) and OsTCP19 have also been shown to regulate N-promoted tillering in rice, introduction of elite alleles into modern rice cultivars increases NUE and grain yield under low N conditions[149,156]. Taken together, modulating the NGR5-DELLA-GRF4 regulatory module provides a simple route for

reducing N fertilizer use while boosting grain yield in high-yielding GRVs[167].

5 CONCLUSIONS

Plants have the remarkable ability to optimize overall plant growth and adapt to survive spatiotemporally variable environments, including altering root system architecture for efficient N uptake and assimilation. Recent advances in the understanding of the molecular mechanisms underlying NO_3^- sensing and its integrative signaling pathways have shed light on root developmental and metabolic adaptations to changes in N availability in model plants. In addition, plant interactions with microorganisms can modulate root system architecture and enhance N acquisition, indicating that the manipulation of the interaction between root system and microorganisms will facilitate N capture and plant growth at low N supply. Knowledge of the underlying mechanisms of N signaling have been mostly performed under controlled laboratory conditions, 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 uptake enables the development of new breeding strategies for future agricultural sustainability and food security. The further identification of the key components involved in N sensing and response along with the use of precision gene modification will launch a sustainable Green Revolution.

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Compliance with ethics guidelines

Hui Liu, Qian Liu, Xiuhua Gao, and Xiangdong Fu declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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